ACCOMPLISHMENTS REPORT
Western Regional Biomass Research Center (WRBRC)
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Submitted by: Hussein Abdel-Haleem, Coordinator

Locations Reporting:
Aberdeen, Idaho (National Small Grains and Potato Germplasm Research Unit)
Albany, California (Western Regional Research Center)
    Crop Improvement and Genetics Research Unit
    Bioproduct Research Unit
Maricopa, Arizona (U.S. Arid Land Agricultural Research Center)
    Plant Physiology and Genetics Research Unit
    Water Management and Conservation Research
Riverside, California (Agricultural Water Efficiency and Salinity Research Unit)

Research Focus:
1. **Feedstock Development**
2. **Feedstock Production**
3. **Conversion and Co-product Utilization**

(1) Feedstock Development:

A. Accomplishments

Acyl-lipid metabolism candidate genes controlling the natural variation for seed fatty acid traits in Rapeseed: Finding environmentally responsible solutions to produce hydrotreated renewable fuels, also known as "renewable diesel fuel," is an alternative path for securing sustainable, carbon-based energy production. However, to meet market and user demands, current bioenergy feed stocks, such as vegetable oil produced from rapeseed (*Brassica napus*), must be optimized with respect to end-product composition and quality before these biofuels can be utilized for large-scale energy production. ARS scientists at Maricopa, Arizona, Peoria, Illinois, Morris, Minnesota, Sidney, Montana, Mandan, North Dakota, Temple, Texas, Ames, Iowa, Akron, Colorado, Pendleton, Oregon, and scientists from Idaho State University, university of Arizona and Cornell University, evaluated B. napus diversity panel planted in four different environments across multiple years to identify candidate genes, via Genome Wide Association Studies (GWAS), within the acyl lipid biosynthetic pathway responsible for fatty acid (FA) synthesis. These candidate genes, which control the various enzymatic steps in FA synthesis, could serve as precise targets for genomics-assisted breeding to directly alter seed oil composition and quality to meet market criteria. The outcomes from this research provide information on how genomics can be leveraged to enhance the speed and effectiveness of rapeseed cultivar development for biofuel production.

A high-oil castor cultivar developed through recurrent selection: Castor (*Ricinus communis*) seed oil is the only commercial source of hydroxy fatty acid (HFA) which is used in industrial products such as lubricants, coatings, plastics and cosmetics. Researchers at ARS lab, Albany, CA and University of California screened and selected high-oil seeds from a base population of castor cultivar Impala. Through two cycles of recurrent selection of seeds, the average oil content of the Impala cv. increased from 50.33% dry weight (Cycle 0) to 54.47% dry weight (Cycle 2). The results confirm that recurrent selection for high-oil content seeds is an effective approach to increase the mean oil content of a castor population.

Characterize the structures of these tetraacylglycerols in lesquerella oil: Castor oil has many industrial uses such as the manufacture of biodegradable and renewable plastics, plasticizers, lubricants, cosmetics, paints and surfactants. Lesquerella oil can also be used industrially in similar applications as castor oil. Both oils are mainly triacylglycerols containing hydroxy fatty acids, although tetraacylglycerols have also been identified in lesquerella. Tetraacylglycerols can be used in industry, providing different physical properties from those of triacylglycerols e.g., viscosity and pour point modifier. ARS researchers at Albany, CA identified and quantified the molecular species of tetraacylglycerols in lesquerella oil.

Fructan reduction by downregulation of Sucrose:sucrose 1-fructosyltransferase gene in guayule: guayule is under development in the US as a source of natural rubber, organic resins, and biofuel feedstock. Increases in rubber yield for the crop could contribute to its economic sustainability. The guayule shrub produces most of its rubber in the winter, at the same time it also stores carbohydrates (fructans). A possible strategy to increasing rubber is to divert some of the some of the carbon from carbohydrate. ARS researchers at Albany, CA successful in reducing carbohydrate production by downregulation of a key pathway gene. Even though more sucrose was made available, in greenhouse plants that did not translate to more rubber. This suggests the mechanism that controls rubber production is not induced by higher concentration of carbon (in sucrose form).

Field evaluation of genetically modified guayule: Under greenhouse conditions, increased guayule rubber yield was correlated with downregulated allene oxide synthase (AOS). ARS researchers in Albany, CA, evaluated natural rubber production in guayule plants with varying levels of AOS gene expression grown under field conditions. Four plant genotypes: wildtype plants, vector controls, and transgenic plants with overexpressed and downregulated AOS (total of 960 plants) were grown at the Bridgestone Guayule Research Farm in Eloy, AZ for 2 years. Downregulated AOS genotypes showed significant higher photosynthetic activity (net assimilation rate), total number of branches per plant, and plant stembark thickness. Moreover, the rubber particles were smaller and had higher activity (radiolabeled isoprenyl pyrophosphate (IPP) incorporation per g of rubber). The downregulation of AOS also led to significant changes in phytohormone levels, which may have influenced the plants' morphology and physiology. However, in contrast to laboratory and greenhouse studies, natural rubber concentration and yield were normal in the transgenic plants.

Phenotypic diversity of USDA guayule germplasm collection grown under different irrigation conditions: Natural rubber (NR) is a critical industrial natural resource. Its outstanding properties such as elasticity, resilience, heat dispersion and abrasion resistance cannot be completely replaced by petroleum-derived synthetic rubber. However, the current production of NR, mainly harvested from *Hevea brasiliensis*, is faced with many obstacles, including the shortage of supply due to increased demands, the risks of fatal diseases, changes in economic and social behaviors in rubber-producing regions. Guayule is considered as a domestic source for NR as well as hypoallergenic latex that is suitable for semi-arid and arid sustainable agricultural systems for rubber, resin and latex production. The study conducted by ARS researchers from ARS lab, Maricopa, AZ is the first to explore phenotypic diversity in important agronomic traits in a global USDA guayule germplasm that include improved germplasm as well as wild accessions collected from natural habitats at Mexico and United States. The results summarized their responses and stability grown under different irrigation regimes, in which water-stressed condition increased resin and rubber accumulation while well-watered condition increased dry weight biomass. The results indicated significant correlations between ploidy level and resin (or rubber) content indicated the possibility of polyploidy breeding to achieve specific breeding goals. This study lays the foundation for guayule breeding efforts to select parental candidates in breeding programs to grow guayule under different growing conditions and to achieve different production goals.

Genetic diversity and population structure of a Camelina sativa spring panel: *Camelina sativa* (L. Crantz), a crop originated from southeastern Europe and southwestern Asia, is showing renewed public interest due to its exceptional level of omega-3 essential fatty acids and high seed oil content. These oil qualities, combined with positive agronomic attributes such as early maturity, low-input requirements for water, nutrients and pesticides, broader adaptability to diverse environments and resistance against insects and pathogens, make C. sativa an ideal alternative resource for biofuel and animal feedstock in the development of sustainable agriculture. Studies on genetic diversity and population structure are important for characterizing the natural selection history and genetic relationships among C. sativa accessions and these will facilitate the faster genetic enhancement and efficient breeding progresses such as marker-assisted selection. In this study a research team consists of scientists form ARS lab, Maricopa, AZ, University of Nebraska and Danforth Plant Science Center used high-throughput genotyping-by-sequencing technology to explore genetic diversity and adaptation among *Camelina sativa* accessions and the possibility of utilizing single nucleotide polymorphic markers for genomic analyses in Camelina genetic enhancement. Results showed that the panel was genetically diverse. This level of genetic diversity could be the basis for developing new Camelina cultivars with desirable characteristics such as high yield potential, high oil production and tolerance to abiotic stress while being adapted to diverse environments. Knowledge of population structure and genetic diversity of Camelina accessions is important for future genetic studies such as genomic selection, marker-assisted selection and genome-wide association studies.

Phenotypic variations in leaf cuticular wax classes and constituents in a spring Camelina sativa: Increasing the accumulation of leaf cuticular wax in Camelina could be one of the strategies to reduce nonstomatal water loss and thus resist abiotic stresses. Collaborated work between ARS researchers from Maricopa, AZ and scientist from West Virginia University showed a wide range of phenotypic variation in leaf total wax, wax classes and constituents in a Camelina sativa diversity panel collected from different geographical regions. The detection of wide variations in leaf wax traits is the first step to understand wax biosynthetic pathways in Camelina, dissect its genetic network elements, identify candidate genes controlling these traits, and develop molecular markers for molecular breeding and genomic selection programs to increase drought resistance in Camelina. Results indicated that among the wax constituents detected, primary alcohol homologues were dominant in all Camelina accessions, and were highly heritable traits. Secondly were the alkanes, which were also highly heritable in nature. Abundance of these wax constituents identifies them as good biomarkers for selection and breeding for drought resistance in Camelina through modifying cuticle composition and properties.

Identification of genes controlling leaf cuticular wax components in Camelina sativa: To expand Camelina production areas into arid regions, there is a need to breed for new drought-tolerant cultivars. Leaf cuticular wax is known to facilitate plant development and growth under water-limited conditions. Dissecting the genetic loci underlying leaf cuticular waxes is important to breed for cultivars with improved drought tolerance. ARS researchers at Maricopa lab, AZ and researcher from Danforth Plant Science Center collaborated to combine phenotypic data and single nucleotide polymorphism (SNP) data from a spring C. sativa diversity panel using genotyping-by-sequencing (GBS) technology, to perform a large-scale genome-wide association study (GWAS) on leaf wax compositions. A total of 42 SNP markers were significantly associated with 15 leaf wax traits including major wax components such as total primary alcohols, total alkanes, and total wax esters as well as their constituents. The vast majority of significant SNPs were associated with long-chain carbon monomers, indicating the important effects of long-chain carbon monomers on leaf total wax biosynthesis. These SNP markers are located on genes directly or indirectly related to wax biosynthesis. These loci could potentially serve as candidates for the genetic control involved in intracellular wax transport that might directly or indirectly facilitate leaf wax accumulation in C. sativa and can be used in future marker-assisted selection (MAS) to breed for the cultivars with high wax content to improve drought tolerance.

Predictive accuracy of switchgrass genotypic data is inflated in the presence of population structure: The cost of genotyping switchgrass individuals is cheaper and faster than acquiring phenotypic data necessary for breeding. By using predictive data modeling along with dense genotypic data higher yielding perennial energy crops such as switchgrass can be developed more economically. ARS researchers in Albany, California and Lincoln, Nebraska analyzed the predictive ability of genotypic data for cell-wall estimations of sugars, theoretical ethanol yields, and forage digestibility from near infrared spectroscopy data in five switchgrass sub-populations. When population structure was not accounted for, predictive accuracy was falsely inflated by up to 68% over methods that correctly accounted for population structure. These findings will encourage breeders to use more realistic prediction models that will not bias their selection methods and will lead to stable increases in switchgrass yields and feedstock quality.

Drought stress in switchgrass impacts crown gene expression and physiology: Switchgrass has been subject to breeding to improve its yield and composition for bioenergy. Increased tolerance of environmental stress is another factor that can improve yields over the life span of a field plot. Natural ploidy differences in switchgrass act as somewhat distinct gene pools that maintain differences with respect to tolerance of environmental stress and which can only be bridged through whole genome reduction or duplication events. In order to document potential wide-ranging effects of whole genome duplication ARS researchers in Albany, California and Lincoln, Nebraska examined the effects of water stress on growth, physiology, and gene expression in related tetraploid and octoploid switchgrass. The octoploids behaved similarly to tetraploids under water stress and recovery conditions: Water-stressed plants had approximately 50% or greater reduction of biomass, plant height, and node numbers. Tiller number and photosynthesis rates of stressed plants were reduced by up to 50%, while proline levels were significantly higher in stressed plants than well-watered plants. A total of 6134 differentially expressed genes were detected under water-deficit stress relative to well-watered treatments. Only 187 genes were identified as being differentially expressed between genetically related tetraploids and octoploids. This study indicates genome duplication is a potentially viable approach to broaden the gene pool with respect to environmental stress traits in switchgrass.

Cuticular leaf wax variants in a population of switchgrass: Switchgrass is a perennial warm-season grass that is being studied extensively for development as a biofuel and biomass crop in the US. Like most other plants, switchgrass is susceptible to environmental abiotic stresses, including heat and drought. Efforts to improve and stabilize crop yields through breeding will be dependent in part on determining the phenotypic variation present in important stress-related traits within a diverse switchgrass population. Scientists at the University of Arizona, West Virginia University, and ARS lab in Maricopa, AZ collaborated to evaluate the phenotypic diversity of cuticle wax content and composition present in a diverse panel of switchgrass varieties. The cuticle forms a hydrophobic protective barrier on the surface of plants and is known to be important for tolerance to abiotic stresses, particularly for drought. The scientists demonstrated that there was significant phenotypic variation in wax content and composition, suggesting that there are important genetic differences in the population that might contribute to this variation. Identification of certain plant lines with distinct and variable wax loads will provide useful tools to breeders for future efforts to improve switchgrass tolerance to abiotic stresses through directed modification of the plant cuticle.

Genetic loci controlling leaf surface wax in switchgrass: Aerial plant surfaces are covered nearly entirely by a surface cuticle and an epicuticular layer that provide a protective extracellular coating covering the outer epidermal cell wall. This cuticle contains a matrix of cuticular waxes derived from very long chain fatty acids which functions primarily as a protective water barrier which also lowers leaf temperature through increasing light scattering. ARS scientists in Albany, CA and collaborators used a switchgrass population planted at field locations in Texas, Missouri, and Michigan to identify three genetic loci controlling overall levels of epicuticular wax and their interactions with location. A locus on Chromosome 7K was found to have the largest effect on wax levels and explained approximately 17% of overall variation. This work represents the first example of a trait relevant to environmental stress being mapped in switchgrass and should allow marker assisted selection approaches to breeding for increased stress tolerance.

The genome of jojoba species: Jojoba is a desert shrub cultivated for its unique seed oil, which is enriched in wax esters that can be used in high-value applications such as cosmetics and industrial lubricants. Researchers from ARS lab at Maricopa, AZ collaborated with researchers from University of North Texas, Huazhong Agricultural University, China, Heinrich-Heine University, Germany, Leibniz Institute of Plant Genetics and Crop Plant Research, Germany and University of Gottingen, Germany to sequence the genome of this important industrial crop and provides a detailed analysis of the genes and enzymes involved in wax production in developing seeds. The genome contains 26 chromosomes, with 23,491 protein-encoding genes. Novel mass spectrometry-based methods were used to "visualize" where waxes were stored in specific regions of jojoba seed, providing new insight to the spatial organization of wax production. Collectively, these results provide rich genomic and metabolomic information that will underpin future efforts to further improve crop performance and yield, and also provide new opportunities for producing high-value waxes in transgenically modified crops.

An RK/ST C-terminal motif is required for targeting of OEP7.2 protein to the plastid outer envelope membrane: The cells of all living organisms are in a constant state of renewal, continuously replacing parts that have broken down or building new structures to perform needed biological functions. One of the greatest challenges of modern cell biology is to figure out how the various organelles in a cell are put together. Much like the construction jobs in modern cities, the necessary building materials must first be transported to the correct job site where they are subsequently assembled into the final form. In the current manuscript, scientists at the ARS lab in Maricopa, the University of Guelph, Wilfrid Laurier University, and the University of Toronto describe a polypeptide sequence that serves as a "zip code" for delivery of proteins specifically to the outer surface of chloroplasts. Once the zip code sequence was defined, it was used to identify other, previously unknown proteins that also contained this sequence, and were also located on the chloroplast outer membrane. Collectively, these results define a new "targeting sequence" in plant cells required for the delivery of proteins to the chloroplast surface, and increase the number of proteins known to reside at that location. These results will be of greatest interest to other scientists interested in the fundamental mechanisms of chloroplast formation and function, activities that are essential for overall crop performance and yield.

Mouse Fat-Specific Protein expressed in plant cells localizes to lipid droplets and promotes lipid droplet accumulation and fusion: Plant oils are important commodities used for a variety of purposes including food, feed, cooking, industrial applications, and biofuels. Given their high value, there is significant interest in finding ways to increase oil content in plants. While much is known about the enzymes that synthesize plant oils, little is known about the proteins involved in "packaging" these oils into subcellular organelles called "lipid droplets". In recent years, several proteins have been identified that are involved in this process, and surprisingly, manipulation of these proteins has proven to be an effective means to increase oil content in plants. In the current study, ARS scientists at Maricopa, AZ, the University of North Texas, the University of Guelph, and Ohio University characterized the function of a mammalian lipid droplet protein called FSP27 when expressed in plant cells. Notably, plants lack any apparent FSP27-like genes, so it was unclear how FSP27 would function when expressed in plants. Like in mammalian cells, FSP27 targeted to lipid droplets in plants, induced their clustering, and ultimately lead to their fusion. The end-result was an increase in both the number and size of lipid droplets in plants, as well as an increase in seed oil content. These results suggest that expression of FSP27 in plants represents a potential strategy for increasing oil content and energy density in bioenergy and/or non-food oilseed crops.

Castor patatin-like phospholipase gene facilitates removal of hydroxy fatty acids from phosphatidylcholine in transgenic Arabidopsis seeds: Many non-domesticated plant species produce high amounts of "unusual" fatty acids in their seed oil, many of which have desirable properties for use in high-value industrial applications. Several examples include the hydroxy fatty acids present in the seed oils of castor bean or *Physaria fendleri*, which have potential usage in formulations of varnishes, cosmetics, surfactants, and engine oil lubricants. Castor bean and *P. fendleri*, however, have undesirable agronomic traits that limit their usage in the large-scale production of these valuable feedstocks for industry. Metabolic engineering offers an attractive approach for resolving this problem by transferring genes for hydroxy fatty acid synthesis to other crops that have better agronomic traits. Experiments to date have indicated, however, that relatively low amounts of hydroxy fatty acids are produced in engineered crops due to inefficiencies in transfer of these fatty acids into the oil of developing seeds. In the current study, scientists from the University of Alberta, Agriculture and Agri-Food Canada, and the ARS labs in Maricopa, AZ, and Albany, CA, identified a gene in castor bean that helps relieve this bottleneck, which shows promise for future studies aimed at further increasing hydroxy fatty acid content in engineered crops. These findings will be of greatest interest to other scientists interested in understanding the genes and enzymes required for production of high value oils in crop plants, as well as companies involved in the production and distribution of these oils for industrial partners and other end users.

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(2) Feedstock Production

A. Accomplishments:

A near-infrared (NIR) spectroscopy quantification for resin and rubber contents in Guayule: Rapid screening tools are needed to identify guayule cultivars with optimal levels of rubber and resin. ARS scientists at ARS at Maricopa, AZ have successfully constructed reliable high-throughput prediction models for the determination of resin and rubber in dry-ground guayule biomass samples using near-Infrared spectroscopy. The prediction power of the models for resin content were better than rubber content and the increased spectral resolution of data. Samples collected from different growing conditions are suggested to be separated for independent model establishment. In general, the established models might be used in the future to form a simple, low-cost and efficient pipeline to maximize the phenotyping efficiency in determining guayule rubber content. The established models could enable guayule breeders to efficiently screen large populations for individuals with superior traits of interests.

Phenotypic variations in dry biomass, rubber and resin production among guayule germplasm in planted at different geographical zones: Guayule is originated in the Southern Texas and northern Mexico deserts, which suggests it as a good candidate for arid and semi-arid sustainable agricultural systems to produce natural rubber and industrial byproducts. Continued improvement of guayule for higher biomass, rubber and resin production, and high resistance to environmental stresses, are necessary to meet the demand of the guayule industry. To establish agronomic practices suitable for different production zones of guayule, a team of researchers from ARS lab at Maricopa, AZ, and scientists from University of Arizona and Texas A&M AgriLife Research evaluated guayule varieties in Arizona and Texas. The results indicated that improved guayule germplasm showed different responses in dry biomass, rubber and resin production between states and locations. Positive correlation coefficients among these traits suggest the possibility of selection for more than one trait at the same time, which could reduce the guayule breeding time and effort to produce superior germplasm for each production zone.

Irrigation scheduling for guayule: In recent years, U.S. tire companies have invested significantly in development and commercialization of guayule in the arid U.S. Southwest to supplement natural rubber supplies from Asia. Some of the critical issues in making guayule both profitable and sustainable in the area are improving the irrigation management and water use efficiency for this native crop. ARS researchers in Maricopa, Arizona, developed irrigation scheduling guidelines for achieving economic guayule rubber yields with reduced water use. This guayule irrigation research modernizes limited information that was provided over 30 years ago and will significantly help improve the domestic guayule production efforts of the U.S. tire industry and other rubber industry partners.

Tolerance of transplanted guayule seedlings to post-emergence herbicides: Continued improvement of guayule for higher biomass and rubber and resin production, should be combined with improving the agronomics of the crop. Researchers at ARS lab at Maricopa, AZ showed the possibility of using post-emergence herbicides to control weeds growing in guayule fields. Gramoxone herbicide was able to suppress weed growth and mortality with minimal effect on guayule seedlings. When using new herbicide application, caution should be paid to eliminate weed growth with minimum damage of guayule plants to keep the plant number/planted area as high as possible for maximal rubber production.

Nitrogen fertilizer and irrigation effects on seed yield and oil in camelina: Interest in the production of camelina (*Camelina sativa* L. Crantz) for biofuel feedstock has been growing, including for arid and semiarid regions. A research team of researchers at ARS lab at Maricopa, AZ conducted a two-year field study under a linear-move overhead sprinkler system to assess the effect of 10 water levels and five Nitrogen fertilizer rates on seed yield, seed oil, and N use efficiency. Camelina seed yields were maximum at 93 and 83 % base irrigation, for 2013 and 2014 respectively. In 2014, the optimal N rate was 113 kg N ha^{-1} at all irrigation levels. Oil content (maximum at 40%) decreased with N rate but increased with water level. Seed N increased with N rate but decreased with irrigation level. Percent recovery of N fertilizer by camelina ranged from 12% to 72% for the highest and lowest N rates, respectively. The results indicate that reasonable camelina seed yields can be produced in the Southwest US with modest N and irrigation inputs.

Simulation of efficient irrigation management strategies for grain sorghum production over different climate variability classes: Crop simulation models are increasingly being used as research tools to address a variety of agricultural issues, including assessments of irrigation management strategies for sorghum in semi-arid regions of the western U.S. Scientists at the ARS center in Maricopa, Arizona, in collaboration with scientists at Texas A&M University, College Station evaluated crop simulation models for sorghum and cotton using nine years of data collected at a research site near Halfway, Texas. The evaluated sorghum model was then used to identify optimal levels of soil moisture at planting and irrigation management strategies that optimized sorghum yield and water use. Results showed that initial water content of 75%, triggering irrigation at 50% soil water, and irrigating to 85% soil water was sufficient for sorghum production in cold, wet years. However, in warm, dry years, triggering irrigation at 60% soil water and irrigating to 100% soil water was necessary to prevent sorghum yield loss. The study will be most useful for scientists, researchers, and producers who require irrigation management recommendations for sorghum production systems.

Foliar fertilizers impact on growth, and biochemical responses of *Thymus vulgaris* to salinity stress: Salinity stress reduces plant growth, biomass, yield, and may lead to death when severe. Different species have diverse responses to salinity stress. These responses may be biochemical, physiological, or expressed through a mineral imbalance in the plant. In this work, we hypothesized that, spraying salt-stressed thyme leaves with solutions containing potassium or calcium could mitigate the negative effects of salinity on plant growth and metabolism. To test this hypothesis, ARS scientist at salinity lab, Riverside, CA collaborated with researchers from University of Gabes, Tunisia, Jazan University, Saudi Arabia, Jouf University, Saudi Arabia, King Abdulaziz University, Saudi Arabia, University of Antwerp, Belgium and evaluated thyme plants that cultivated under salt stress. After salinity stress, thyme plants had their leaf fresh weight reduced. Salinity decreased the relative water content, water and osmotic potentials and led to ion imbalances and nutrient deficiencies. Salinity also altered some essential oil components, but leaf antioxidants remained fairly stable, except for a significant increase for plants under salt stress, and sprayed with potassium two weeks after treatment. Results indicated that stressed plants accumulated significantly more soluble sugars and amino acids. Overall, the effects of salinity stress and/or potassium and calcium treatments were also observed after the two-week recovery period.

<u>Grape rootstock response to salinity, water and combined salinity and water stress</u>: There is increasing concern about the sustainability of irrigation in arid regions of the world because available fresh water resources are being depleted and salinized. Producers with limited water fresh water face the option of either under-irrigating, using available saline ground water, or a combination of both. It is important to develop and evaluate new salt tolerant crops as well as to understand the interaction of water and salt stress on crop production. Researchers at ARS salinity lab, Riverside, CA evaluated three purported salt tolerant grape rootstocks for fruit and vegetative production in a 4 years field experiment, with salt stress, water stress and combined water and salt stress. across all treatments Ruggerio was the top producer. There were significant differences in fruit yield under saline conditions, with Ruggerio cv being the top performer and most salt tolerant (relative yield) There were no significant rootstock differences to water stress, suggesting that grape salt tolerance is more associated with toxic ion accumulation (sodium and chloride) rather than osmotic stress. Researchers determined reduced water consumption under salinity, thus under combined stress, yield was reduced but there was no actual water stress at the highest salinity levels. This research is of interest to wine grape producers in areas where salinity and drought is of concern, as well as extension specialists, farm advisors and researchers evaluating crop response to combined stresses and developing new grape varieties.

B. Citations

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(3) Conversion and Co-Product Utilization

A. Accomplishments

Massaranduba Sawdust as source of charcoal and activated carbon: The Amazon is one of the world's major tropical timber producing regions of the world. Industrial wood processing in the Amazon is the main economic activity, therefore, producing a value-added product that uses the wood residue would provide economic advantages, contribute to sustainability and resource utilization. ARS researchers at Albany, CA in collaboration with researchers from Universidade Federal De Lavras, Brazil, Federal Rural University of Rio de Janeiro, Brazil, State University of Pará, Brazil, and the French Agricultural Research Centre for International Development (CIRAD) evaluated the microstructural, thermal and physical characteristics of activated carbon produced from sawdust residues of massaranduba (*Manilkara huberi*) wood. Activated carbon was obtained and their properties were compared with the starting wood and charcoal (before activation). Microstrutural, thermal and physical properties of these sample treatments were evaluated. The activated carbon had higher reactivity with moisture, lower crystallinity and higher thermal stability compared to its wood and charcoal precursors. Scanning electron microscopy images showed the formation of nanoscale pores after the activation treatment of charcoal. Differential scanning calorimetry was effective in detecting the changes in the moisture adsorption and heat of dehydration. The present study contributed important information about the production and characterization of activated carbons from Amazonian commercial wood residues.

Green composites based on polypropylene and corncob agricultural residue: Green Composites Containing Corn Cob. Plastics are a major source of landscape and marine pollution yet they are still used widely due to the lack of viable alternatives. One way to reduce the amount of plastic in everyday products is to blend it with renewable agricultural residue. Scientists from Brazil and ARS lab at Albany, CA found that corncob residue could be successfully blended with polypropylene to make materials that had sufficient tensile strength for many applications and were heat stable. This research could result in wider use of green composites containing agricultural residue.

Lactic acid production from almond hulls: Lactic acid used in making the corn plastic, poly (lactic acid), is typically made from the fermentation of corn starch, which requires large amounts of land and water. Almond hulls are a cheap agricultural byproduct that have high sugar content and could be used as a carbon source in the fermentation of lactic acid. As global food reserves become more limited, it is important to find more sustainable feedstocks for making non-food products. Scientists from ARS scientists at Albany, CA found that almond hulls contain enough fermentable sugars to make high quality lactic acid. Almond hulls were fermented using a mixed culture from primary sludge and a mono-culture from *Lactobacillus rhamnosus* and compared the production of lactic acid from almond hulls against that of other alternative feedstocks. This research could help develop new uses for agricultural wastes such as almond hulls and help minimize the use of food reserves for making non-food products.

Cellulose made from fibers of bamboo, jute, and eucalyptus cellulose pulps: Cellulose is a natural, abundant, renewable polymer that has many uses and can reduce or replace non-renewable resources made from petrochemicals. Particle-size reduction of cellulose fibers improves functionality compared with original fibers. However, environmentally friendly and cost-effective methods for production particle-size reduction need to be perfected in order to take full advantage of cellulose for use in advanced materials. Researchers form ARS lab at Albany, CA, Federal University of Lavras, Brazil, Universidade Federal De Minas Gerais, Brazil and University of Toronto, Canada investigated the properties of cellulose sheets made from fibrillated micro/nanofibrils of bamboo, jute and eucalyptus. Structural properties of high strength cellulose sheets made from micro/nanofibrils derived from bamboo, jute and eucalyptus using mechanical defibrillation were characterized and compared. Jute degraded to short fibrils rapidly at high temperatures. Cellulose sheets made from bamboo and jute micro/nanofibrils had higher flexural strength and energy to break than those made from eucalyptus. Eucalyptus sheets had poor performance (strength and water-resistance) probably due to incomplete defibrillation resulting in greater particle size distribution. Further development of the mechanical defibrillation process could lower the production costs of micro/nanofibrils and improve the performance of engineered cellulose-based materials.

Torrefied almond shells as a plastic filler. Plastics often contain fillers and colorants and compatibilizers that are not renewable, may be costly, and generally do not improve the properties of the plastics. ARS researchers in Albany, CA have demonstrated that torrefied almond shells not only act as a natural pigment for plastics but also function as a renewable filler that can improve the properties of the plastics without the need of a compatibilizer. The results of these findings could extend the commercial value of almond shells and increase the renewable content of plastics.

Amazonian palm fibers for using as potential reinforcing materials: The use of environmentally sustainable materials is necessary to replace fossil fuels, find value-added uses of agricultural residues and find new uses of fiber-based plants. Researcher from ARS lab at Albany and Federal University Of Amazonas, Brazil, Embrapa National Research Center, Brazil, Universidade De Sao Paulo, Brazil and Universidade Federal De Lavras, Brazil studied the morphological, chemical, structural, thermal, and tensile properties of cellulose, lignin and hemicelluloses constitute natural fibers from three Amazonian palm (*Leopoldinia piassaba*, *Desmoncus polyacanthos*, and *Astrocaryum* sp.). Methods to predict the optimal uses of such natural fibers using three palm species were investigated and developed. Results show the potential for widespread use of underexploited Amazonian palm fibers as a source of raw material that can be engineered into cellulose micro/nanofibrils, composites, and new materials in a wide range of applications.

Activated carbons prepared by physical activation from different pretreatments of amazon piassava fibers: ARS researchers at Albany, CA in collaboration with researchers from Universidade Federal De Lavras, Brazil, and the French Agricultural Research Centre for International Development (CIRAD) evaluated the properties of activated carbons (ACs) produced from different pre-treated piassava (*Leopoldina piassaba*) fibers found in the Amazon. The ACs were produced by physical activation using CO2 at 800°C, and of fibers that experienced different pre-treatments (mercerization, corona discharge and removal of extractives). Microstructural, thermal and physical properties of these samples were evaluated. The activated carbon had higher reactivity with moisture, lower crystallinity and higher thermal stability than the raw sample.

Scanning electron microscopy images showed the formation of nanoscale pores after the activation treatment of the charcoal. Differential scanning calorimetry was effective in detecting moisture adsorption and heat of dehydration. ACs from Amazon piassava fibers were a potential adsorbent alternative for the removal of dyes from wastewater. The present study contributed important information about the production and characterization of ACs from piassava for comparison to AC from other biomass sources.

Solution blow spun nanocomposites of poly lactic acid /cellulose nanocrystals from Eucalyptus pulp: Cellulose nanocrystals help improve poly lactic acid nanofibers. poly lactic acid nanofibers have been used in membranes or as adsorbent materials but the nanofibers need greater stability. ARS scientists in Albany, CA collaborated with scientists at EMBRAPA in Brazil to incorporate cellulose nanocrystals in poly lactic acid nanofibers which improved the stability of the nanofibers and increased their ability to wet. These improved composite materials could lead to applications in filters and absorbents.

Development of novel processes for aqueous extraction of natural rubber from Taraxacum kok-saghyz: Alternative rubber-producing crops can supply a strategic raw material while providing benefit to American farmers. One of the crops under development as a US source of rubber and inulin (a carbohydrate) is Kazakh dandelion (TK). TK stores rubber and inulin in root tissues, and both are closely associated with root biomass. Therefore, efficient and effective extraction processes are needed. ARS researchers at Albany, CA in collaboration with Ohio Agricultural Research & Development Center and Atlantic Biomass Conversions, Inc. conducted series of water-based extraction processes to evaluate the separation of rubber and inulin from root tissues. The best results included alkaline (basic) pretreatments, and enzyme digestion of root tissues. This process resulted in TK rubber recovery at high yield and quality. Thus, water extraction of inulin followed by alkaline pretreatment and/or enzymatic digestion form the basis for aqueous processes that could be used for large scale, efficient extraction of TK rubber of purity and quality sufficient to meet industry standards.

Thermostable hydrolytic enzymes for novel application in extraction of high-quality natural rubber from Taraxacum kok-saghyz: *Taraxacum kok-saghyz* (TKS, Kazakh dandelion) is under development in the US as a source of domestic natural rubber. Rubber in TKS is isolated from root tissues, usually by grinding in aqueous solvent. Researchers form ARS lab, Albany, CA, the Ohio State University and Quaid-I-university Islamabad, Pakistan found that pre-treatment of root tissues with *Thermomyces* (natural fungus) enzymes effectively soften the root tissue to allow more rubber to be extracted. Importantly, this newly-isolated fungus lives naturally in warm environments (isolated from self-heating garden compost) so performs well at higher temperatures (55 'C). In addition, their study showed the *Thermomyces* fungus can also break down wheat straw and guayule bagasse, so provides alternatives to chemical pretreatment when these materials are used for biochemical or biofuel production.

Molecular species of triacylglycerols in the rubber particles of guayule and rubber tree: Natural rubbers are obtained after latex coagulation. Hevea latex is mostly from South-east Asia and its rubber is mostly (~70%) used to make tires. Guayule rubber, from plants grown in the US, can be used to make medical devices and has the potential to replace imported Hevea rubber and/or synthetic rubber from petroleum. The type and amount of rubber particle lipids are important because they affect the biosynthesis of rubber and the physical properties of the rubber. ARS scientists at Albany, CA identified and quantified many intact molecular species of triacylglycerols in the rubber particles of Hevea and guayule. This is the first report of the intact molecular species of TAG containing furan FA.

Sugar yield and composition of tubers from Jerusalem Artichoke (*Helianthus tuberosus*) irrigated with saline waters: Currently, major biofuel crops are also food crops that demand fertile soils and good-quality water for biomass production. Jerusalem artichoke produces high tonnage of tubers that are rich in inulin sugars. Researchers from ARS salinity lab, Riverside, CA in collaboration with researches from University of California and Federal Rural University of the Semi-Arid, Brazil evaluated Jerusalem Artichoke grown under five salinity levels. This plant can be classified as moderately salt tolerant if the classification is based on tuber production. Results indicated that Jerusalem artichoke is a biofuel crop that yields more sugars than corn and sugar cane, and that can be cultivated in marginal lands and with saline recycled waters, thus not competing with food crops. Jerusalem artichoke cultivation under moderately saline conditions can result in low-cost production of sugars that can be easily converted to ethanol for transportation fuels. This research is of interest to researchers working on renewable fuels and chemicals, food, bioproducts industry, land managers, and organizations evaluating feasibility of biofuel production on marginal lands or with recycled waters.

Algae ash characterization: Algae are a valuable source of both protein and lipid for farmed fish. Yet algae are known for having high ash content (some as high as 70% of dry matter). ARS researchers in Aberdeen, Idaho, recently conducted a study to characterize the ash component in algae. The study is among the very few to document that silica-containing materials are important contributors of the ash component for algae, particularly those with high ash content. Three types of silica materials were identified in algae: cellular structures of non-diatoms, diatom cell walls, and sandy particles of geologic origin. Contamination by diatoms and sandy particulates are the two major contributors to high ash content of algal samples. Several measures were proposed to produce algae with reduced ash content, which will be more suitable for fish feed.

Standardization of ash measurement for all biomass: In another development relating to biomass characterization, many laboratories nowadays routinely measure ash content as a part of analysis for nutritional or compositional evaluation of biomass. An accurate and reliable measurement of ash content is critical in documenting quality of a biomass, whether it is either a food, feed, industrial material, or renewable fuel feedstock. The ash content in algae and other biomass is commonly measured by burning samples in a muffle furnace at a high temperature for a specified duration. The process is known as dry ashing. However, there has been large variation in ashing temperature and duration, as well as sample size (load), among reported methods. This variation makes it difficult to compare results among studies. ARS researchers in Aberdeen, Idaho, recently investigated several factors affecting ash analysis for algae and non-algae biomass. It was found that for most biomass, both ashing temperature and duration affect ash contents measured. For algae with high ash content, sample size is another determining factor. It was proposed that ashing 1 to 4 g samples at 600°C overnight be a standard method for measuring ash content in all biomass.

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