The Plains Area Agricultural Research Service is committed to providing answers to key issues facing the nation’s sorghum breeders and growers. This commitment and collaboration with national and international partners had led to an increased understanding of a variety of issues facing sorghum breeders and growers, ranging from identifying disease resistance to development of germplasms with increased growing ranges. Below is a brief overview of some of the progress made to enhance and protect the nation’s sorghum resources.

**KANSAS**

**Project Number:** 3020-44000-0023-00D  
**Impact of the Environment on Sorghum Grain Composition and Quality Traits.**  
**Lead Scientist:** Scott Bean, Manhattan, Kansas  
**Team Members:** Thomas Herald, Michael Tilley, and Jeff Wilson

*Improved fractionation method for protein complexes in sorghum.* Sorghum lines vary widely in digestibility, but we don’t yet fully understand why. To address this problem, ARS researchers in Manhattan, Kansas developed methods to extract and analyze protein complexes in sorghum. They discovered that sorghum lines with lower protein digestibility have more of a particular protein complex. This information is a step toward developing new sorghum lines with improved digestibility and understanding how the environment impacts nutritional properties of sorghum for both humans and animals.

*Distribution of sorghum protein variants impacts bio-ethanol production.* More and more, sorghum grain is being used to produce ethanol-based bio-fuel. The amount of ethanol that can be produced from a bushel of sorghum depends primarily on the amount of starch in the sorghum kernel. But proteins also influence ethanol production through their structure and interaction with the starch. ARS researchers in Manhattan, Kansas, along with collaborators from Australia and Kansas State University, examined a variety of sorghum samples to determine the effect of protein-starch interactions on sorghum ethanol yield. They found that the amount of starch, protein digestibility and nitrogen were all important factors influencing sorghum-derived ethanol. This information will aid in the development of sorghum lines for biofuel production.

*Properties and composition of sorghum grain are impacted by the environment.* At a basic level, grain hardness and the composition of starch, protein, and fat in the grain influences the end product application of sorghum. Two of the main factors that determine the physical properties and composition of any grain are genetics and the environment. ARS researchers in Manhattan, Kansas along with collaborators at Texas A&M University, determined the impact of environment on the physical grain properties and composition of sorghum grain. They showed protein was more dependent on the genetic influence, whereas the kernel hardness, starch content and fat content were more environmentally influenced. This research will help identify grain quality traits that can be improved at the genetic level.
Factors related to protein digestibility in raw sorghum. Sorghum tends to have lower protein digestibility when compared to other cereal grains. This impacts the utilization of sorghum for both feed and bio-fuel production. Several factors have been suggested to contribute to the low protein digestibility in sorghum including protein structure and composition, grain hardness, and levels of anti-nutritional compounds. ARS researchers in Manhattan, Kansas determined cross-linked protein complexes to be correlated to digestibility. The amounts of these proteins could be used as markers to predict protein digestibility and thus be used to help identify sorghum germplasm with improved protein digestibility.

A sensitive and reproducible high-throughput 96-well method for analyzing tannin in sorghum grain. Tannin containing sorghum grain has high levels of anti-oxidants, which are reported to offer human and animal health benefits. Tannin content in is commonly determined by using the HCl-vanillin assay. But this time consuming method is not efficient for screening large sample sets as seen in association trait mapping panels or breeding nurseries. ARS researchers in Manhattan, Kansas developed the 96-well micro-titer assay to assist breeders in screening these large sample sets. The high-throughput 96-well method is able to perform 224 measurements compared to the 30 measurements using the common HCl-vanillin assay. The 96-well assay may be employed to identify tannin from non-tannin sorghum grain that will assist to mitigate cross contamination of the sorghum grains.

High throughput method for analysis of amylose to amylopectin ratios in sorghum. The composition of sorghum starch is an important factor in determining how well a given sorghum sample may work as an ingredient in human foods, as animal feed and for producing ethanol. However, many methods for determining starch composition are tedious and time-consuming. To overcome this, ARS researchers in Manhattan, KS developed a high throughput method for measuring the two major components of starch, amylose and amylopectin. This method can be used to screen large sorghum populations and assist sorghum breeders and geneticists in identifying sorghum lines with desirable starch traits for specific uses.

NEBRASKA

Project Number: 3042-21220-032-00D
Genetic Improvement of Sorghum for Non-Grain Energy Uses.
Lead Scientist: Scott E. Sattler, Lincoln, Nebraska
Team Member: Deanna Funnell-Harris

Discovery of New brown midrib (bmr) Mutants in Sorghum.
In the U.S., sorghum stalks and leaves serve as an important forage crop for livestock. Additionally, sorghum is being developed as a bioenergy crop for cellulosic biofuels. Cellulosic biofuels are made from breaking down the biomass cell wall components into sugars and converting these sugars into fuels. A third cell wall component, lignin, make cell walls resistant to breakdown into sugars. In sorghum, brown midrib (bmr) mutants are a class of mutants that have reduced lignin content, improved bioenergy conversion and increased livestock digestibility. ARS scientists at Lincoln, Nebraska, discovered six new bmr mutants. These mutants may help scientists to identify genes that control lignin synthesis and thereby improve sorghum biomass for bioenergy and forage uses.
Waxy sorghum grain no more susceptible to pathogens than normal grain. Starch is normally composed of two types of molecules, amylose and amylopectin. Waxy sorghum starch is almost entirely composed of amylopectin, which changes the physical properties of the starch making it more useful for the food and ethanol industries. The waxy grain is also more digestible than normal grain. Therefore, there was a concern that waxy grain would be more susceptible to grain molds than normal grain. ARS scientists at Lincoln, Nebraska, screened field-grown normal and waxy sorghum lines for grain mold infections. Waxy lines were determined not to be more susceptible to these diseases compared to normal sorghum lines. In a second experiment, grain samples from nearly identical lines that only differed for the waxy trait (normal or waxy) were infected with different grain molds and the disease responses were measured. Again, the waxy lines were not more susceptible than normal lines. These studies demonstrated that waxy sorghum grain is not more susceptible to pathogens than normal grain, which is valuable information for developing waxy sorghum hybrids with resistance to grain molds.

Improvement of Waxy Hybrid Sorghum for Use in the Food and Ethanol Industries. Waxy sorghum alters the starch composition of grain, which is useful for the food and ethanol industries. Previously, waxy lines performed poorly compared to normal lines in food and biofuel applications, which has prevented commercialization of the trait. However, a set of waxy hybrids were bred and tested by ARS scientists at Lincoln, Nebraska, and the grain yield of waxy hybrids was not reduced compared to the normal hybrids. These lines are some of the first publicly available germplasm that can produce high-yielding waxy hybrids.

This project has developed grain sorghum germplasm with white grain, which serves the target audience of gluten-free food producers for consumers with celiac disease or other forms of gluten intolerance. These highly valued grain and plant color traits do not increase susceptibility to plant pathogens. The project also developed grain sorghum germplasm with altered starch composition, which will benefit the target audiences of ethanol and food producers. Small farmers could also benefit as production will require identity preservation.

Structure and function of an essential enzyme in lignin synthesis. Understanding lignin synthesis is critically important for developing plants with altered biomass composition to be used with emerging bioenergy conversion technologies. The sorghum caffeic O-methyltransferase (SbCOMT) and hydroxycinnamoyltransferase (SbHCT) are key enzymes that participate in the last steps and early steps of lignin synthesis respectively. The structure of these enzymes was determined to understand how the enzyme functions. The structure of these enzymes is similar to the structure of other lignin synthesis enzymes found in plants. Our observations explain how these sorghum enzymes and other enzymes that share similar structural features can participate in different biochemical pathways in different plant species. Knowledge of this protein structure will direct future research on modifying lignin content and composition of sorghum for bioenergy.

Low-lignin (bmr12) grain sorghum leaves and stalks are valuable cattle feed. Grain sorghum is an important crop for rain fed production systems in the U.S. Sorghum plants with the brown midrib (bmr) trait have less lignin in their leaves and stalks, which makes them easier to digest, but can also result in reduced yields. Sorghum bmr12 plants yielded approximately 6% less grain than sorghum without this mutation, but the steers grazing on bmr residue for two months had increased body weight relative to their counterparts. This increase in body weight translated to an additional $53 in net return per acre compared to normal sorghum. These results indicate that
bmr12 sorghum hybrid is an effective dual purpose sorghum crop for both grain production and cattle grazing.

*Reducing lignin content of bioenergy sorghum does not increase its susceptibility to stalk rot.* Sorghum brown midrib (bmr) lines have reduced lignin content, which has been shown to improve bioenergy conversion to ethanol. A critical question is whether decreased lignin content in sorghum affects disease susceptibility. ARS scientists from Lincoln, Nebraska, screened a series of bmr mutant lines and the normal counterparts for susceptibility to the fungal pathogen Fusarium, which causes stalk rot disease. Stalk rot can significantly decrease biomass yields because it contributes to lodging. None of the lines showed increased susceptibility to the disease, and two bmr mutant lines were actually more resistant to this disease than their normal counterparts. These results indicate that bmr traits do not increase the susceptibility of sorghum to stalk rot diseases. In addition, the increase resistance observed in some bmr lines is driving research toward the identification of biochemical compounds that inhibit these fungal diseases.

**OKLAHOMA**

**Project Number:** 3072-21000-008-00D  
**Identification, Characterization, and Development of Insect-Resistant Wheat, Barley, and Sorghum Germplasm**  
**Lead Scientist:** Yinghua Huang, Stillwater, Oklahoma

*Screening and developing genetic sources for resistance to sugarcane aphids.* A core collection of sorghum germplasm were developed, which was selected from the approximately 40,000 germplasm accessions. Then, the core collection (~280 sorghum lines) were evaluated for their responses to sugarcane aphids (SCA), as a result and led to identify >10 sources with various levels of resistance (from strong to medium) to SCA. In order to study the genetics of SCA resistance and to clone the resistance genes, several mapping population were developed with the resistance lines. Genetic mapping with one of the mapping populations is progressing well towards the placement of the QTLs on sorghum chromosomes. Furthermore, sorghum germplasm improvement focused on transfer the newly identified resistance to the elite sorghum lines, and 10 crosses were made during the past two years. Advanced resistant lines and/or varieties will be released next year. At the same time, the DNA markers developed in this study will be useful in screening germplasm for resistance to SCA and in conducting marker-assisted selection for breeding sorghum to resist to the aphids more efficiently.

**Fine-mapping of candidate genes associated with Greenbug resistance in sorghum.** Greenbug is also a serious constraint to cereal crop production, particularly in the Midwest and Plain areas, and it causes significant economic loss to U.S. sorghum producers. During the past years, we conducted a fine-mapping of the resistance QTL using near-isogenic lines (NILs) derived from a cross between a highly resistant variety, and a highly susceptible variety, BTx623, using SSR markers. This allowed us to narrow down the target genes in a small region (59-kb) of sorghum chromosome harboring 8 candidate genes. Putative function of these candidate genes has been assigned based on the genetic analysis. Thus, our results provide the baseline information for cloning greenbug resistance genes of sorghum, and the markers developed in this study will be useful for molecular breeding of greenbug resistant sorghum varieties. If greenbug resistance genes are successfully used to control greenbug damage on
Identification of expression profiles in sorghum using NGS technology. Greenbug (Schizaphis graminum) is the most important insect pest of grain and forage sorghums in the Midwestern United States. ARS Stillwater, OK utilized next generation sequencing (NGS) technology to examine the gene expression profiles of sorghum challenged by virulent greenbug in order to discover the resistant mechanisms and to improve the understanding of host plant and aphid pest interactions. The RNA-sequencing results revealed transcriptional activities of 27,130 unique genes from the sorghum plants challenged by greenbug. Out of these actively expressed genes, 897 were differentially expressed between the greenbug resistant and susceptible lines. Furthermore we successfully characterized many of the newly-identified genes that were already assigned as plant defense factors as well as regulatory sequences. This study generated a substantial amount of sorghum transcript (i.e. genes) sequences and the results facilitated the identification of candidate genes responsible for insect resistance in sorghum, which led to new insights into the molecular basis of the host defense against greenbugs and the new tools for insect resistance breeding in sorghum.

Gene identification in preferred lignocellulosic biomass sorghum to improve feedstock quality. Sorghum is one of the most competitive bioenergy crops for biomass production. However, converting biomass to ethanol is currently far too expensive to be commercially competitive because removing lignin from biomass is very costly step in the ethanol conversion process. Our recent study identified differentially expressed genes in brown mid-rib (bmr) sorghum mutants using a complementary approach combining suppression subtractive cDNA library and cDNA microarray expression profiling. Then expression of the genes associated with the reduction of lignin production in bmr sorghum was characterized at the molecular level, which contribute a better knowledge on which genes are involved in lignin biosynthesis and how these genes are regulated. Furthermore, these newly identified candidate genes offer the new resources for genetic improvement of biomass digestibility, leading to effective conversion of biomass to bioethanol at a low cost as well as better feeding value of forage sorghum.

Evaluation and development of sweet sorghum as bioenergy feedstocks. Sweet sorghum has the potential to be used as a renewable energy crop and has become a viable candidate for ethanol production. However, characterization of the sweet sorghum germplasm and their genetic improvement are behind the current demand. Thus, we collected 687 sweet sorghum varieties and conducted field studies to evaluate certain essential traits that could contribute to the ethanol yield potential. These characteristics and traits included plant height, sugar content, biomass yield, phenology of flowering, resistance to diseases and insects, and tolerant to alkaline or acidic soils. Based on the phenotypic data, substantially genetic variations were observed among the germplasm originating from a wide range of geographic locations worldwide. The resultant data represent the first document for the 687 sweet sorghum lines on their biological characteristics and agronomic traits. These untapped genetic resources harbor an excellent gene pool for crop improvement. Furthermore, a group of selected germplasm lines are valuable materials for breeders to do further manipulation toward the production of new cultivars or hybrids.
Evaluation of the entire National sorghum germplasm collection for greenbug resistance. Now we have completed screening of approx. 40,000 sorghum accessions for their reaction to greenbug (biotype I) and have found over 30 resistant lines. Among the newly identified resistance sources, eight lines have very good levels of genetic resistance to greenbug. This complete set of evaluation data for reaction to greenbugs are being processed for submitting to the National Plant Germplasm System (GRIN), which is valuable information for both crop breeders and sorghum producers. In addition, genetic diversity of these resistance accessions was studied using DNA (AFLP) markers. A high level of genetic variation was observed among these genetic sources and there is a broader genetic base in the germplasm collection than those resistance sources currently used. This information is very important for selecting resistant parent lines to develop durable resistance in sorghum.

Project Number: 3072-22000-015-00D
Biologically Based Management of Cereal Aphids
Lead Scientist: Gary Puterka
Team Members: Scott Armstrong, Norman Elliott

Identification of sorghum germplasm resistant to a new invasive aphid pest of sorghum, sugarcane aphid. In 2013, the sugarcane aphid appeared in the southern U.S. and severely damaged sorghum crops. Within months, Scientists at USDA-ARS in Stillwater assisted sorghum breeders at Texas A&M University to screen and identify sorghum that was resistant to this new sorghum pest and develop new germplasm. The scientists were able to piggyback on resistance sources that also offer protection against another sorghum pest, the greenbug. Sorghum breeders now have germplasm with resistance to not just one, but two significant aphid pests of sorghum.

Efficient Sampling and Monitoring Methods for Sugarcane Aphid. Sampling methods are needed to determine if and when sorghum fields require insecticidal treatment to suppress sugarcane aphid infestations. With collaborators we are developing sampling methodology for sugarcane aphid in sorghum. In 2015 we collected data from 55 sugarcane aphid infested sorghum fields in Oklahoma and Kansas according to a detailed protocol, and are analyzing these data to develop an efficient sampling methodology. We also obtained airborne multispectral remote sensing data from sugarcane aphid infested fields for the purpose of developing methodology to detect and monitor sugarcane aphid infestations via airborne multispectral remote sensing.

Sorghum Headworm Sequential Sampling and Decision Support System. We developed an efficient sequential sampling method for sorghum headworm in grain sorghum, and a computer-based expert system for sorghum headworm pest management. The system significantly improves the reliability and speed of control decisions for sorghum headworm, and is freely accessible by the public via the internet. It is used by extension specialists in Oklahoma and Texas, especially during sorghum headworm outbreak years, and as a training tool for crop consultants and growers.
TEXAS

**Project Number:** 3091-21000-034-00D  
Innovative Genetic Approaches to Sorghum Germplasm Improvement and Analysis of Traits Critical to Hybrid Development.  
**Lead Scientist:** Robert R. Klein, College Station, Texas

*Tropical germplasm adapted for temperate-zone sorghum growers.* Sorghum is an important grain crop in many areas of the U.S. and other temperate regions worldwide. However, much valuable sorghum germplasm originated in the tropics and does not successfully flower and produce seed in temperate environments. Until recently, tropical sources of genetic variability were unavailable to many of the world's sorghum producing regions. However, ARS researchers at College Station, Texas, recently teamed up with industry scientists to address this issue. They used molecular tools along with classical plant breeding techniques to convert tropical sorghums to short stature, early flowering versions. More than two dozen converted germplasm lines were released to public and private breeders to ensure the development of higher-producing sorghum varieties for farmers in the U.S. and throughout the world.

**Project Number:** 3091-22000-030-00D  
Characterization and Identification of Resistance in Sorghum to Fungal Pathogens.  
**Lead Scientist:** Louis K. Prom, College Station, Texas

*New anthracnose, head smut, and long smut resistant sorghum.* Sorghum diseases annually cause economic losses in both yield and seed quality amounting to tens if not hundreds of millions of dollars. The most effective and economical solution to major sorghum disease issues is the development of new disease-resistant sorghum varieties. ARS scientists at College Station, Texas; Lincoln, Nebraska; and Mayaguez, PR, working with collaborators from Texas A&M University and the Centre National de la Recherche Agronomique, Senegal, West Africa, evaluated an extensive number of sorghum lines drawn from a variety of sources for their inherent resistance to anthracnose, grain mold, head smut, and long smut. Through this work, the researchers were able to establish a number of sorghum varieties that were highly resistant to one or more of these diseases. The identification of these varieties is a significant advancement in preventing sorghum disease. The results of this research provides sorghum breeders and growers with critical new germplasm that is disease-resistant that will increase worldwide production and eliminate the application of chemical controls.

*New pathotypes or "races" of the sorghum anthracnose pathogen.* Effective control of sorghum anthracnose hinges upon a thorough understanding of the distribution and characteristics of the various pathotypes or "races" of the anthracnose fungus. Since genetic resistance is key to control of the anthracnose fungus, this knowledge is vital to the breeding and deployment of resistant sorghum lines. ARS researcher’s at College Station, Texas, collected
totally new pathotypes of the anthracnose fungus from Cairo, Georgia and Puerto Rico. This discovery will provide breeders and commercial seed companies with the opportunity to evaluate their sorghum lines (anthracnose-susceptible or –resistant) against previously unknown pathotypes.

**New sources of downy mildew resistance in sorghum.** Sorghum downy mildew causes yield losses of up to 78% in mildew-susceptible sorghum lines throughout the world. For decades, seed treatment with the fungicide, metalaxyl, prevented major downy mildew outbreaks in the U.S. However, in 2001, a new race (P6) of the downy mildew pathogen emerged, causing major sorghum losses in south Texas. ARS scientists at College Station, Texas, working with collaborators at Texas A&M University, evaluated many (hundreds, scores?) sorghum lines and hybrids drawn from a number of sources for inherent resistance to P6. They found several lines to be highly resistant. This research provides sorghum breeders and growers with critical new germplasm that is essential to developing new, downy mildew-resistant sorghum varieties for use in the U.S. and abroad.

**New sorghums resistant to grain mold.** Globally, grain mold is the most important sorghum disease. The development of new sorghum types that are resistant to grain mold offers the most promising, environmentally friendly approach to successfully controlling the disease. ARS scientists at College Station, Texas, evaluated 38 sorghum accessions from Ethiopia and Mali, along with resistant and susceptible controls, for resistance against the grain mold fungus, *Fusarium thapsinum*. Three accessions showed lower grain mold severities and higher germination rates when compared with the resistant controls Sureno and SC719. This work identifies critical new germplasm that sorghum breeders can use to develop new sorghum varieties that will be resistant to one of the world’s most important grain molding diseases. New resistant varieties will increase sorghum production efficiency and profitability in the U.S. and in many other areas of the world.

**Project Number:** 3096-21000-020-00D  
**Genetic Enhancement of Sorghum as a Versatile Crop.**  
**Lead Scientist:** Zhanguo Xin, Lubbock, Texas  
**Team Members:** Gloria Burow, John Burke

**Novel multi-seeded sorghum mutants with potential to double sorghum grain yield identified.** Scientists at the Plant Stress and Germplasm Development Unit at Lubbock, Texas, identified a large class of novel sorghum mutants that have the potential to increase sorghum grain yield. The multi-seeded mutants have increased number and size of primary and secondary flower branches and both types of florets can develop into seeds. Compared to the wild type sorghum, the mutants tripled the seed number and doubled the seed weight on individual panicle basis. Scientists are evaluating the mutants for yield increases and have filed a patent to pursue this promising means of enhancing grain yield.

**Two genes increasing seed number in sorghum identified.** ARS researchers at Lubbock, Texas, identified two genes that can double the seed number and significantly enhance the seed
weight per panicle (head) in sorghum. From the sorghum mutant library established at the Plant Stress and Germplasm Research Unit, several multiseeded (msd) mutants that have potential to increase grain yield were isolated previously, and in collaboration with ARS researchers at Cold Spring Harbor, NY, two genes underlying the msd trait were identified through next-generation sequencing. Two patents detailing the inventions were filed. The msd mutants have been distributed to eight breeding companies to develop high yield sorghum hybrids.

**Perfect molecular markers to select multiseeded traits.** ARS researchers in Lubbock, Texas, developed precise molecular markers that can double the seed number per panicle. Most markers based on other genetic approaches are usually too far away to be effective selection markers, but molecular markers developed according to the cloned genes are right on the gene and can select the trait at early seedling stage with 100% accuracy. In this way, marker-assisted selection can dramatically accelerate the breeding process. The genes and markers discovered are now being utilized by the seed industry to rapidly incorporate the multiseeded trait into their proprietary lines.

**Mapping population for cold tolerance registered.** The United States is the leading sorghum producer in the world, but stand establishment is hampered by cold soil and ambient temperature in the U.S. sorghum belt during early season. Planting at early season is needed to fully use the early season soil moisture reserve and to realize higher yield with longer growing season cultivars. ARS researchers at Lubbock, Texas, developed and registered a new genetic mapping population, RTx430 x PI610727 recombinant inbred population. This population exhibits an overall increased germinability at cold soil and ambient temperatures between the range of 55-58ºF (14-16ºC). Selected lines from this population were distributed to three sorghum seed companies and two universities for evaluation and use as parent for breeding early season cold tolerance.

**Dhurrin content was evaluated in 700 diverse sorghum lines as a trait for drought tolerance.** Dhurrin is usually considered as an undesirable metabolite because it can produce prussic acid (HCN) that is toxic to cattle. However, dhurrin also contributes toward post-flowering drought tolerance in a number of sorghum lines. ARS researchers from Lubbock, Texas, in collaboration with University cooperators at University of Illinois, evaluated the dhurrin content in 700 unique sorghum lines and associated dhurrin levels with genome-wide DNA sequence variation in these lines with a technique known as “genome wide association analysis”. The study discovered a total of 19 genetic markers associated with dhurrin accumulation. The results firmly established dhurrin as an attribute to post-flowering drought tolerance and provide molecular markers for selection of either high or low dhurrin accessions based on breeding objectives.

**New drought-tolerant lines identified from Sudanese sorghum germplasm collection.** Post-flowering drought tolerance was evaluated in 367 Sudanese sorghum lines using a fluorescence bioassay developed by scientists in the USDA Plant Stress and Germplasm Development Unit in Lubbock, Texas. Eight lines were initially selected for their stress tolerance. Additional studies led to the selection of three lines to move into the germplasm pipeline within the Cropping Systems Research Laboratory for future public release. These lines will provide new genetic resources for improved drought stress tolerance during grain fill.

**New post-flower drought-tolerant sorghum lines identified.** Scientists at the Plant Stress and Germplasm Development Unit at Lubbock, Texas, developed a method that can quantitatively determine the degree of post-flower drought tolerance, commonly known as the "stay-green" trait. Using this method, they evaluated 376 sorghum lines from the Sudan for the stay-green trait and selected three lines for further study. Identification of these lines validates that the stay-green trait is a valuable tool for quickly screening sorghum lines for drought tolerance.