

SICNA 2015

SORGHUM IMPROVEMENT CONFERENCE OF NORTH AMERICA

Proceedings of the 2015 Sorghum Improvement Conference of North America (SICNA)

“TACKLING TODAY’S GRAND CHALLENGES”

September 1-3, 2015

*Hilton Garden Inn
Manhattan, KS*

Organized by:



**KANSAS STATE
UNIVERSITY**
Department of Agronomy



INTRODUCTION

The 2015 Sorghum Improvement Conference of North America (SICNA) meeting was held at the Hilton Garden Inn, Manhattan, KS from September 1-3, 2015. The meeting was attended by nearly 200 participants representing a diverse cross section of the sorghum industry including sorghum research community from both public and private sectors, producer stakeholders and commodity group leaders. SICNA 2015 was organized by the Kansas State University Department of Agronomy, the Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet, the KSU Center for Sorghum Improvement, and with support from the Plant Stress & Germplasm Development Unit, USDA-ARS in Lubbock, TX.

SICNA 2015 was a resounding success and offered opportunities for investigators and faculties in various fields of the sorghum research to exchange information and promote collaborative efforts to address the challenges facing the sorghum industry. Simultaneously, SICNA served as forum for graduate students to showcase their research work through oral research competitions and poster presentations. There were over than 50 poster presentations on topics ranging from sorghum genomics to weed control management.

The opening keynote address was given by Dr. Graeme Hammer, Professor and Director of the Center for Plant Sciences, Queensland Alliance for Agriculture and Food Innovation (QAAFI), Australia, and provided a stimulating tone for the conference as he discussed findings on field and whole plant scale agronomic and physiological bases of sorghum's resilience to abiotic stresses and how they are being harnessed for Australia's sorghum industry.

The first day of the meeting involved presentations of research findings on (1) *Challenges on sorghum trait identification to technology transfer* and (2) *Novel phenotyping approaches to accelerate plant breeding and gene discovery*. Also featured was a Sorghum industry expo, "Taking Sorghum Further," that saw the participation of researchers, producers and companies focused on sorghum improvement and utilization.

The meeting banquet was highlighted by keynote addresses from Dr. Tim Dalton, Director of the Sorghum and Millet Innovation Lab, KSU, and Mr. Florentino Lopez, Executive Director of U.S. Sorghum Checkoff Program. Director Lopez discussed the forward looking presentation on "Sorghum 2025 - Visions for the U.S. Sorghum Industry. The presentation covered visions and projections on the way forward for the US sorghum industry to grow in the coming 10 years. Dr. Tim Dalton presentation on "The Global Sorghum Economy" covered the worldwide outlook for the sorghum industry and its vital role in world agriculture and sustainable food production. During the banquet, the winners of the 2015 SICNA graduate student competition were recognized as: Vuyiswa Bushula of the Department of Plant Pathology & Agronomy at Kansas State University (1st place); Moriah Massafaro of the Department of Agronomy at Purdue University (2nd place) and Baily McHenry of the Department of Agronomy at Kansas State University, tied with Patrick Ongom of the Department of Agronomy at Purdue University (3rd Place).

The second day of the meeting involved sessions on (1) *The challenge of sugarcane aphid and sooty mold in sorghum production* and (2) *Grain sorghum: more than just an alternative to corn*

thanks to unique chemical components and unique applications. Panel discussions were conducted for all the sessions and provided excellent forum for exchange of information and interactions that will assist the sorghum industry to move forward.

You will find here the **Proceedings of the 2015 SICNA Conference**, which include the abstracts of oral and poster presentations in addition to access to many of the Power Point slides presented by the invited speakers in each session at the meeting. You will also find the agenda and list of sponsors in Section II.

Please refer to any of the content herein judiciously by appropriate citation of each of the work in your research documents, manuscripts, articles or presentations. An example of a citation from this proceeding as an example (this will vary based on journal format/requirements):

Jugulam, M. 2015. Investigating opportunities and addressing challenges to improve options for weed control in sorghum. Proceedings of the 2015 SICNA Meeting, Manhattan, KS, Sept 1-3, 2015 (eds., G. B. Burow, M. Jugulam, and K. E. Valentin), pp.12-13.

We hope you will find the 2015 SICNA Proceedings as a valuable resource for your research, extension and worthy sorghum related activities.

Sincerely,

Editors

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December, 2015

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Section I: Abstracts from Scientific Sessions, Posters, and Student Oral Competitions

Session 1: Challenges from sorghum trait identification to technology transfer (Agronomy and Physiology)

Paper#: SICNA2015-AP1

Challenges for sorghum technology transfer: a view from the public sector

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Relevancy in the technology transfer and adoption process is one of the main challenges faced by the Public sector in the foreseeable future. Extension methods and communication outlets should evolve in order to fulfill the increasing demand of information from end users (e.g. producers, consultants, and agronomists). In the past years, challenges encountered on funding support for applied research and extension jeopardizes the level of information (both quantity and quality) provided to end users. Potential new collaborations with Private- and Public sector are expected to occur in the next coming years. Public sector is currently seeking innovative communication outlets for increasing relevancy (e.g. social media, electronic publications, and apps for smartphones and other electronic devices). Following this rationale, in recent years several efforts were focused on: 1) use of Twitter, Facebook, YouTube and blogs; 2) preparation of electronic publications (for smartphones and tablets); and 3) development of smart phone and tablet applications. For example the K-State Crop Production Lab (KSUCROPSlab, Dr. Ciampitti) is currently developing an app for estimating sorghum yields before harvest, the app collects imagery of sorghum heads with the goal of predicting seed number trait and estimate on-farm yield. Thus, implementation of this app at larger-scale would allow to get rapid and accurate estimations of sorghum yields. Overall, these applied research projects are aimed at development of effective and relevant technology transfer mechanisms for facilitating selection of management practices and, ultimately, for increasing grain sorghum yields in a sustainable approach.

Paper#: SICNA2015-AP2

Physiological response of grain sorghum to temperature and drought stress: Opportunities and challenges for yield improvement (Panel Discussion)

Prasad, P.V.V.

Department of Agronomy, Kansas State University, Manhattan, KS

Paper#: SICNA2015-AP3

Investigating opportunities and addressing challenges to improve options for weed control in sorghum

Jugulam, M.

Department of Agronomy, Kansas State University, Manhattan, KS

Weed control in grain sorghum is a major challenge for farmers across the US including Kansas. Although effective weed management programs are available for sorghum, often times they are either expensive, or provide inadequate crop safety and pose crop rotational restrictions. Additionally, increasing occurrence of herbicide-resistant weeds complicates weed management and limits weed control options. On the other hand, temperature stress can influence herbicide efficacy, which can directly impact weed control. Evolution of multiple herbicide resistance in kochia and Palmer amaranth, two problem weeds of Kansas can pose a challenge for grain sorghum production. Research programs of Weed Physiology laboratory at Kansas State University focus on a holistic approach for weed management in sorghum, including 1) understanding the evolution and spread of herbicide resistance in weeds; 2) improving effectiveness of currently available herbicide options, and 3) comprehensive search for herbicide-tolerant traits in sorghum germplasm representing diverse backgrounds. Results indicate that both target site and non-target site-based mechanisms determine multiple herbicide resistance in kochia and Palmer amaranth. We also found that efficacy of mesotrione on Palmer amaranth control can be greatly improved when applied under cooler temperature. Thus, the fluctuations in temperature may be exploited for greater efficacy of herbicides and may help avoid weed control failures. Screening of sorghum germplasm representing diverse background to identify genotypes with tolerance to herbicides (used for post emergence control of weeds) identified promising genotypes of sorghum with tolerance to hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitors. This is the first step towards development of HPPD-inhibitor-tolerant technology in sorghum. Overall, these research activities are aimed at development of better weed management strategies for grain sorghum to increase crop yields.

Session 2: Novel phenotyping approaches to accelerate plant breeding and gene discovery (Breeding and Genetics)

Paper#: SICNA2015-BG1

Novel phenotyping approaches to accelerate plant breeding and gene discovery

Vermerris, W.

Department of Microbiology & Cell Science and UF Genetics Institute, University of Florida, Gainesville, FL 32610, USA

In the past 25 years we have witnessed the rapid development of high-throughput genotyping technologies that have taken us from isozymes to genotyping by sequencing, and that have made it possible to efficiently identify individual plants with specific combinations of alleles within breeding populations. These technological advances have also spurred the development of computational models to predict plant performance based on genotype, such as genome-wide association studies and genomic selection. The utility of these models depends heavily on the ability to obtain reliable and high-quality phenotypic data on a diverse set of traits, ranging from the subcellular to the whole-plant level. While our ability to obtain detailed phenotypic measurements has improved tremendously with the development of metabolite profiling,

proteomics, and a variety of portable devices to measure plant performance *in situ*, there are still substantial practical hurdles as a result of intrinsic environmental variation, throughput, and operator effects. This presentation will illustrate some of these practical limitations and outline novel approaches that are being explored to overcome them.

Paper#: SICNA2015-BG2

Phenotyping approaches in commercial breeding

Mayor, L.

Dupont Pioneer

The sorghum research department at Dupont Pioneer has been providing elite commercial products to farmers for more than 40 years. Over the last few years the department has been through a transitional period with the implementation of new technologies in its breeding programs. These include different approaches to improve phenotype characterization and molecular breeding techniques to better predict hybrid performance. Since accurately characterizing the phenotype is such a crucial component in effectively utilizing newer technologies; it has become a major focus of our research efforts, both in specialty traits as well as improvement in the quality of the data collected throughout the season. An overview on Sorghum research and current efforts in different phenotyping approaches will be presented.

Paper#: SICNA2015-BG3

Identification of physiological, morphological and metabolic traits to enhance biomass accumulation and drought response in sorghum

Jahn, C.E.¹, Turner, M.F.¹, Miller, S.B.¹, Kirkwood, J.S.^{1,2}, Heuberger, A.L.³, Wolfe, L.M.², Wolfrum, E.J.⁴, Broeckling, C.D.², and Prenni, J.E.²

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⁴National Renewable Energy Laboratory

Sorghum bicolor is a globally important, multipurpose crop used in food, forage and biofuels markets. This low-input species displays substantial variation in traits related to plant size and architecture as well as drought-tolerance, and thus exhibits a high degree of variability in traits which contribute favorably to its use as an energy crop. However, little is known about the morphological, physiological and metabolic phenotypes which contribute to final biomass yield and quality. Changes in plant metabolism are instrumental to the plant developmental processes, and thus underpin many of the ways in which plants respond to the environment. Therefore, comprehensive study of plant metabolism is valuable in identifying phenotypic effects of abiotic stresses on plants and may uncover potential breeding targets to improve sorghum for multiple uses in both benign and stressful environments. We evaluated sorghum lines varying in biomass yield and resistance to drought for above and belowground morphological and physiological traits. Additionally, a multi-omics approach using proteomics and targeted and non-targeted metabolomics was used to identify differences in protein, lipid, and primary and secondary metabolite content. Here, we report bioenergy-related parameters including cell-wall composition and yield, and a putative model to predict final biomass from metabolite data.

Further, we report the trade-offs between these quality traits and pre-flowering drought stress. Taken together, these results provide a systems-level view of biomass accumulation and drought response, and provide candidate phenotypic and molecular markers to facilitate breeding for enhanced biomass through the modification of primary and secondary metabolism.

Session 3: The challenge of sugarcane aphid and sooty mold in sorghum production (Entomology & Plant Pathology)

Paper#: SICNA2015-E1

Research challenges in sooty mold and the sugarcane aphid. (Panel Discussion)

Funnell-Harris, D. and Armstrong, S.

USDA-ARS

Paper#: SICNA2015-E2

Genetic studies on sugarcane aphid resistance in sorghum

Harris-Shultz, K., Ni, X., Knoll, J., and Anderson, W.

USDA-ARS, Crop Genetics and Breeding Research Unit, 115 Coastal Way, Tifton, GA 31793

In the southern United States, the white sugarcane aphid (*Melanaphis sacchari*) has recently become a major pest of sorghum. The aphid population can build up rapidly on the undersides of sorghum leaves causing leaf damage, leaf death, stunting, delayed flowering, and plant death. Furthermore, the overwhelming numbers of sugarcane aphids excrete honeydew, a sticky sugary liquid waste, which clogs harvest equipment, and causes further yield and grain quality loss. The identification and incorporation of multiple resistance genes into sorghum hybrids is needed if sorghum is to be grown in areas with high sugarcane aphid infestation. A previous study identified a dominant gene, *RMESI* on chromosome 6, which confers resistance to the sugarcane aphid in a grain sorghum line. August of 2014 was the first year of sugarcane aphid detection in Tifton, GA. A sweet sorghum line was identified, Entry 22, that displayed resistance to the white sugarcane aphid. This line was crossed with susceptible line AN109, and analysis of the F₁ and F₂ population revealed that the resistance is likely conferred by a major recessive gene. Furthermore, no linkage exists between the previously reported dominant *RMESI* locus and this newly identified recessive gene. The F₃ population is currently being evaluated this season in which the aphid infestation started at the seedling stage, instead of at flowering time as last year.

Paper#: SICNA2015-E3

Genetic characterization of an emerging aphid pest in sorghum

Medina, R.F. and Armstrong, S.

Texas A & M University

On July 2013 a new aphid in sorghum was observed in Texas. By the end of November the area of influence of this emergent pest included Texas, Oklahoma, Louisiana and Florida. Sorghum fields in these States sustained considerable losses. In some locations, yield losses of 33% to 50% were observed despite having multiple insecticide applications. So far this aphid has been reported on grain sorghum, forage sorghum, sweet sorghum, energycane, and Johnson grass. Morphological and molecular work has identified this aphid as *Melanaphis sacchari* (Zehntner). The speed, at which this aphid spread in 2013, raises serious concerns about future infestations.

We hypothesized that a biotype or host-associated population pre-adapted to sorghum but rare in sugarcane, may have shifted to sorghum and dramatically increased its population numbers on this crop. To test this hypothesis, we genetically characterized this pest in sorghum, sugarcane and wild vegetation in Texas, Louisiana, Oklahoma and Florida to determine its genetic diversity and to identify potential sources of this novel pest in sorghum. The present study provides the population genetic profile of the sugarcane aphid in the US on several of its host-plants. Our data provide an overarching evolutionary framework that will contribute to explain the recent emergence of the sugarcane aphid as a sorghum pest in Texas, Oklahoma, Louisiana and Florida.

Session 4: Grain sorghum: More than just an alternative to corn thanks to unique chemical components and unique applications (Chemistry and Utilization)

Paper#: SICNA2015-CU1

Introduction to grain sorghum: More than just an alternative to corn

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Grain sorghum (milo) is an ancient grain that has long been recognized as an excellent source of nutritious food. Recent research has identified several classes of unique chemical components in sorghum which have promising nutritional and industrial applications. Several types of health-promoting antioxidants (including tannins, anthocyanins, and other phenolics) have been reported to occur in very high levels in certain cultivars and breeding lines of sorghum. Grain sorghum was also designated to be one of the first “Advanced Biofuels” by the US Environmental Protection Agency based on its reduced levels of greenhouse gas emissions. Sorghum kernels have also been shown to contain unique waxes which are being investigated for their industrial applications. In addition, the quest for identification and utilization of unique chemical components in sorghum is aided by the fact that among the grains, sorghum contains one of the largest numbers of germplasm samples (the USDA GRIN Database contains about 46,000 sorghum accessions, exceeding the 30,000 accessions of corn), which are available for breeding programs to enhance the levels of desirable chemical components.

Paper#: SICNA2015-CU2

Sorghum polyphenols as bioactive and functional food ingredients

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Sorghum contains an array of polyphenols not commonly found in other cereal grains and most plant foods. The composition and content of the polyphenols vary depending on the genetic background of the sorghum. Among these compounds, the most uniquely interesting in terms of functionality and bioactivity are the monomeric 3-deoxyflavonoids and the polymeric condensed tannins. Unlike most other natural sources, the 3-deoxyflavonoids are rarely glycosylated in sorghum. This, along with their higher partition coefficients, are an advantage in terms of enhancing their stability, bioavailability, and bioactivity compared to other natural flavonoids. However, this also presents interesting challenges in terms of their functionality as food ingredients, e.g., the high hydrolytic but low hydrophilic stability of the rare sorghum pigments. Even though their antioxidant properties have been most prominently reported, recent evidence

indicates that the composition of specific polyphenols in sorghum is a better predictor of bioactivity than ‘total antioxidant’ content. For example, extracts from white food type sorghums low in antioxidants demonstrate stronger chemoprotective properties (*in vitro* and *in vivo*) against estrogen-beta dependent carcinogenesis compared to higher antioxidant red and black sorghums. We have also found that the polymeric tannins, but not monomeric 3-deoxyflavonoids, have specific interactions with starch that can slow amylolytic enzyme action on starch, and thus slow the rate of dietary carbohydrate digestion. This has important implications on reducing caloric impact of starchy foods. The presentation will highlight the latest advances in our understanding of bioactive and functional properties of sorghum polyphenols and propose strategies to capture these benefits to improve human health.

Paper#: SICNA2015-CU3

Challenges Facing Food Product Development with Sorghum

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Food product development is an unavoidable risky endeavor undertaken by companies to stay competitive in the market place. Usual challenges include creativity, changing consumer preferences, technological challenges and fierce competition. These challenges are magnified when dealing with new applications of a particular commodity such as sorghum grain. The gluten-free market has opened the door for use of sorghum flour in food products targeted to a specific consumer group. Technical and sensory properties still hinder wider use of sorghum. Innovative concepts and products can and have been produced as prototypes, but commercialization efforts remain scarce.

Posters (P) and Student Oral Competitions (SOC)

Paper#: SICNA2015-P1

Corn and grain sorghum comparison

Assefa, Y., Roozeboom, K., Thompson, C., Schlegel, A., Stone, L., and Lingenfelter, J.

Corn (*Zea mays* L.) and grain sorghum (*Sorghum bicolor subsp. bicolor* L) are among the top cereal crops worldwide, and both are key for global food security. The similarities between corn and grain sorghum, particularly the fact that they are warm-season cereals with a C4 pathway adapted for summer season cropping in the United States, has made them a topic for comparison by many authors. In places where rainfall is dependable or irrigation systems are available, corn is the dominant summer crop due to yield superiority. In places where rainfall amount and timing is not dependable in parts of Oklahoma, Kansas, Colorado, Texas, and Nebraska, for example sorghum has been historically recommended. The main objective of this analysis was to provide a comprehensive review of the comparison of corn and grain sorghum from different aspects, i.e., key morphological, physiological, and developmental, historical yield and harvest area trends, yield distribution and yield variability, resource use efficiencies, and their fit in crop rotations common to the Great Plains. Results suggested that grain sorghum and corn are morphologically similar, at least aboveground and in the vegetative growth stages. Their physiology and developmental stages also are similar in resource-rich environments. Notable differences reported for the two crops in morphology, physiology, phenology, and resource use

were related to their adaptation to different levels of stress conditions. The mean and the maximum possible yields of corn were greater than for grain sorghum. On the other hand, the variation in dryland yield was less for sorghum than for corn. Even though corn and sorghum appear to compete for summer cropping area, they take advantage of different parts of the season, and thus should be considered alternative crops to utilize different environmental conditions. Breeding should focus on increasing maximum yield level for sorghum and decreasing corn yield sensitivity for variations in environmental conditions to increase competitiveness of these crops.

Paper#: SICNA2015-P2

Dhurrin content relates to sorghum [*sorghum bicolor* (L.) Moench] seedling growth in marginal soils

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Dhurrin content in leaves of mature sorghum plant is a quantitative measure of the level of pre- and postflowering drought tolerance (Burke et al., 2013). Postflowering drought tolerance in sorghum is linked to the staygreen (delayed senescence) trait (Howarth, 2000; Rosenow et al., 1977) which has been associated with decreased lodging, less susceptibility to charcoal rot, increased stem carbohydrates during and after grain filling, and improved grain filling and grain yield under stress. Thus, early enhanced screening of grain sorghum lines for high dhurrin levels will be an important component of breeding for improved post-flowering drought tolerance.

Sorghum genotypes of known dhurrin levels, determined from mature leaves at the late vegetative stage, were grown in four soil-irrigation combinations: potting soil Mix 1 plus nutrient irrigation (GM1), play sand plus nutrient irrigation (GM2), potting soil Mix 1 plus water irrigation (GM3), and play sand plus water irrigation (GM4). Fresh seedling weight (Fsw), seedling length (Fsl), dhurrin and sugars (Sucrose, Glucose, and Fructose) contents were determined at 5 and 10 days after emergence (DAE). Genotypes with high dhurrin (HD) levels show higher fresh seedling weights and lengths than low dhurrin (LD) levels genotypes only at 10 DAE, grown under play sand and water irrigation. Low dhurrin genotypes show significant negative correlations for seedling dhurrin content to Fsw and Fsl, and significant positive correlations of monosaccharide sugars to Fsw and Fsl, at 10 days after emergence under no nitrogen supply (sand and water). Genotypic variations in seedling Fsw and Fsl at 10DAE, grown under sand and water, showed similar trends (high to low) as known dhurrin levels of genotypes. No such variations were observed for dhurrin levels using either seedling dhurrin or sugar contents irrespective of soil-irrigation combinations and days after emergence. This study concludes that, at 10 days after emergence and under N-deficient soils, seedlings of sorghum genotypes with staygreen and thus post-flowering drought tolerance potentials (high dhurrin levels) will outperform genotypes with senescence or pre-flowering drought tolerance potential (low dhurrin levels).

Paper#: SICNA2015-P3 (SOC4)

Evaluating the effectiveness of iron chelates in managing iron deficiency chlorosis in grain sorghum

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Soil conditions such as high pH, high free calcium carbonate (lime), and low organic matter favor development of Fe deficiency chlorosis (IDC) in grain sorghum, which delay crop maturity and reduce yields. Two field experiments were conducted in the summer of 2014 to determine the effectiveness of Fe chelate application in alleviating IDC in grain sorghum. In the first study, four Fe chelate application rates (0, 3, 6, and 9 lb product/a) were applied either in-furrow with the seed at the time of planting or 2 weeks after planting. A split treatment of 3 lb/a applied at planting and another 3 lb/a applied 2 weeks after planting was included. The second study was a split-plot design with two Fe chelate products as main plots and sorghum hybrids (Golden Acres 5613 and Sorghum partner hybrid NK5418) as the subplot factor. Results showed IDC scores among the treatments were significant only in the early stages of growth. Severity of IDC tends to decrease throughout the growing season, confirming the ability of sorghum hybrids to recover from IDC under favorable environmental conditions. Iron chelate application improve sorghum yield; the highest yield occurring when Fe chelate was split-applied at 6 lb/a. GA5613 showed greater tolerance to IDC than NK5418. Application of Fe chelate to GA5613 had no effect on grain yield; however, Fe chelate application significantly improved grain yield in NK5418. Our preliminary findings suggest the first 30 days of growth may be the critical period to control IDC in grain sorghum.

Paper#: SICNA2015-P4

Physiological and anatomical characterization of sorghum nam founder lines under water-deficit stress

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Changing climate presents new challenges to agricultural production and global climate models project greater intensity and magnitude in water-deficit stress conditions in future. This is especially challenging for the arid and semi-arid regions of the world, where sorghum forms an important component of the cropping systems. Eleven sorghum Nested Association Mapping (NAM) founder lines have been used to develop 10 different mapping populations (with RTx430 as the common line crossed with other 10 genetic diverse lines). Our major objective was to characterize the NAM founder lines for both shoot related physiological parameters and root morphology and anatomical variation. A lysimeter based experiment was set up and water-deficit stress (50% water saturation) was imposed for 15-days starting 30-days after emergence. Gravimetric pot weighing was followed daily to determine cumulative water transpired for the 15-days period. Impact of stress was captured at both the shoot and root level: data collected include leaf area, photosynthetic rate, stomatal conductance, and total biomass – used to determine whole-plant water use efficiency. Investigations will be aimed to capture variation in water usage and association between physiological and agronomic traits. To highlight how water-deficit stress conditions affects rooting behavior, both morphological and anatomical complexity (late metaxylem number, root and stele diameter) will be assessed. In view of sorghum's adaptation to challenging environmental conditions, significant genotypic variation is expected for both physiological and anatomical characteristics. Using already developed and

genotyped NAM populations, our findings will provide a means to map genomic regions responsible for increased drought stress resilience in sorghum.

Paper#: SICNA2015-P5 (SOC5)

Influences of previous cover crop and nitrogen rate on sorghum nitrogen uptake and use efficiency in a no tillage cropping system

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Nitrogen (N) is the most yield-limiting nutrient in sorghum (*Sorghum bicolor* L. Moench) production and its efficient management is necessary for improving sustainability and profitability. Growing cover crops (CCs) in rotation with cash crops can influence N fertilizer requirement for succeeding crops. This study aimed to evaluate the effects of different types of CCs and N rate on sorghum N uptake and N use efficiency (NUE). This experiment was conducted in a three-year wheat/cover crop-sorghum-soybean rotation containing six different CCs and check treatments (chemical fallow, CF; double-crop soybeans, DSB; summer legume, SL; summer non-legume, SNL, winter legume, WL; winter non-legume, WNL) and three N rates (0, 90 and 180 kg ha⁻¹). In-season plant-N uptake was determined using GreenSeeker NDVI. Plant tissue samples to determine total N uptake were collected after sorghum physiological maturity. Both in-season and total plant-N uptake were significantly affected by CCs and N treatment. In-season N uptake was greater for sorghum planted after DSB and SL and was positively correlated with total N at harvest ($r^2=0.45$). Within CCs treatment, total N uptake ranged from 112.2 (after SNL) to 164.6 kg ha⁻¹ (after SL) and was significantly correlated with grain yield ($r^2=0.57$). After eight years of management in no-till, total plant-N uptake was greatest in the SL treatment and was not different from the DSB treatment. The additional available N from legume CCs led to an increase in N uptake and grain yield but with no significant effects on NUE.

Paper#: SICNA2015-P6

Functional genomics of seedling cold tolerance in *Sorghum bicolor*

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Sorghum originated in the tropical and sub-tropical regions of Africa. Owing to its origin, sorghum adapted to marginal soils, drought and high temperatures. However, susceptibility to cold (below 10 °C) at the seedling stage restricted sorghum cultivation to few states in the United States. For developing cold tolerant sorghum, knowledge of the mechanistic basis of seedling cold tolerance is vital. Therefore, we took advantage of functional genomic approaches, transcriptomics and lipidomics, to decipher seedling cold tolerance in sorghum. To evaluate the transcriptome, two-week-old seedlings of cold tolerant Chinese landrace NuiSheng Zui (NSZ) and cold susceptible BTx623 inbred were subjected to 36 h cold (10/8 °C day/night). While NSZ and BTx623 seedlings maintained at 28/25 °C were used as the controls. Thirty million paired-end 100-bp reads were sequenced per sample on an Illumina HiSeq 2500. iPlant DNA Subway pipeline using Ensembl Sorbi1 Release 22 as the reference genome was used for analyzing the transcriptome. 93 and 88 % of BTx623 and NSZ reads, respectively, were aligned uniquely to the genome. We identified 10,195 genes differentially regulated in NSZ compared to BTx623 under control conditions. Under cold stress, 12,311 genes were differentially regulated in NSZ in

comparison with BTx623. Gene Ontology analysis demonstrated that cold tolerance in sorghum is possibly conferred by a series of complex regulatory mechanisms. In addition to transcriptome, lipid profile of NSZ and BTx623 under control and cold is currently under progress. The functional genomics data obtained can aid in developing seedling cold tolerance in sorghum.

Paper#: SICNA2015-P7

Evaluation of sorghum germplasm for tolerance to HPPD-inhibitors

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Weed control in grain sorghum is a major challenge for producers across the US. Because of limited number of herbicide options, POST emergence management of weeds, especially grasses is a challenge in grain sorghum production. HPPD-inhibitors are effective in controlling broadleaf and grass weeds in many crops. The overall goal of this research is to screen and identify HPPD-inhibitor (e.g. mesotrione or tembotrione) tolerant sorghum genotypes from diverse sources. We have screened a total of 317, 70 and 25 genotypes representing sorghum diversity panel (US), minicore (ICRISAT), and EMS mutant lines (USDA-Texas), respectively for tolerance to mesotrione or tembotrione. Initial *in vitro* screening was performed in culture vessels containing solidified agar supplemented with 0.6x of mesotrione (1x is 105 g ai ha⁻¹) or 0.025x of tembotrione (1x is 92 g ai ha⁻¹). Herbicide doses were selected based on the concentration that discriminated a known sensitive vs tolerant plant *in vitro*. On the basis of visual injury, 35 sorghum genotypes from diversity panel and one genotype from mutant lines exhibited tolerance to mesotrione and/or tembotrione. Preliminary evaluation of tolerant genotypes under greenhouse (2x mesotrione and/or 0.5x tembotrione) and field conditions (1x mesotrione and/or tembotrione) revealed 2 genotypes with ≤30% injury to mesotrione and 5 genotypes with ≤40% injury to tembotrione, 2 weeks after treatment. Further evaluation of tolerant genotypes is in progress. Successful completion of this research will enable use of tolerant lines in breeding programs to develop HPPD-inhibitor tolerant sorghum technology to enable POST emergent grass weed control.

Paper#: SICNA2015-P8

Temperature effect on the post-herbicide efficacy of palmer amaranth (*Amaranthus palmeri*) control in sorghum

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Grain sorghum yields are significantly influenced by weeds, especially Palmer amaranth infestations, and POST herbicide application is a major method for Palmer amaranth control in sorghum. POST herbicide efficacy is known to be influenced by environmental factors, including temperature. Many studies have reported decreased POST herbicide efficacy at higher temperatures, but some have reported the opposite trend. Short episodes of high temperature during sorghum production are common in the central Great Plains. The objective of this study was to evaluate the efficacy of POST applied herbicides on Palmer amaranth when exposed to different temperatures. Experiments were conducted under controlled growth chamber and field conditions. In growth chambers, Palmer amaranth plants were grown under low (25/10 °C) or high (35/25°C) d/n temperatures. When plants reached 10-12 cm tall, they were treated with 0,

0.25, 0.5, 1, 2 and 4X rates of Atrazine (1X: 2240 g ai ha⁻¹), Huskie (1X: 35.3 g ai pyrasulfotole and 199 g ai bromoxynil ha⁻¹), or dicamba (1X: 560 g ae ha⁻¹). In the field, Huskie (1X) was applied at 8:00 am (cooler) or 4:00 pm (hotter) on V3 and V8 stages of sorghum. Visual control of weeds relative to untreated plots was recorded at 1, 2 and 4 weeks after treatment. Preliminary results indicate a decline in Palmer amaranth control under high temperatures, suggesting a decrease in POST herbicide efficacy. Understanding temperature effects on herbicide efficacy is important for recommending application times for POST herbicides that improve weed control in grain sorghum.

Paper#: SICNA2015-P9 (SOC11)

Balanced nutrition and crop production practices for closing grain sorghum yield gaps

McHenry, B

Mid-west grain sorghum (*Sorghum bicolor* (L.) Moench) producers currently face low attainable yields, therefore, closing yield gaps will increase productivity. Yield gaps are the difference between maximum yield and attainable “farmer” yield. Maximum yield can be achieved through the optimization of utilizing the best genotypes and management practices for the specific site-environment (soil-weather) combination. This project examines several management factors in order to quantify complex farming interactions in nutrient partitioning and for maximizing sorghum yields. The factors that were tested include narrow row-spacing (37.5 cm) vs. standard row-spacing (75 cm), high (197,600 pl/ha) and low (98,800 pl/ha) seeding rates, balanced nutrient management practices including applications of NPK and micronutrients (Fe and Zn), crop protection with fungicide and insecticide, plant growth regulator effects, and the use of precision Ag technology (precision N application). This project was implemented at four locations in Kansas during 2014 (Rossville, Scandia, Ottawa, and Hutchinson). Results from Rossville show that when water is not a limiting factor (e.g. irrigated), yield variability was minimized. The greatest yield difference (1318 kg/ha) was documented between ‘farmer practice’ (low input treatment) and ‘kitchen sink’ (high input treatment). Similarly, grain sorghum yields in Scandia under dryland conditions varied from 6465 to 7219 kg/ha for the low and high input treatments, respectively; although the treatment difference was not statistically significant. The Ottawa site experienced severe drought-stress during reproductive stages of plant growth, which resulted in low yields (averaging 4300 kg/ha) and was not influenced by the cropping system approach. When water was not limiting sorghum yields, a balanced nutrient application and optimization of production practices did increase grain sorghum yields (‘kitchen sink’ vs. ‘farmer practice’). Further evaluation of nutrient uptake and partitioning in different plant fractions should be investigated to better understand if high-yielding grain sorghum can be achieved via balancing nutrient applications and optimizing production intensity.

Paper#: SICNA2015-P10

Investigating physiological and yield potential of bloomless (*bm*) sorghum under water deficit

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Bloomlessness (*bm*), considered a recessive trait in *Sorghum*, has been associated with low abiotic stress tolerance compared to their bloom (*Bm*) counterparts. But this rather too generalized conclusion is amenable to the historically insufficient data that might hinder the full exploitation of the bloomless trait. In this study, we tested the hypothesis that photosynthetic efficiency (PnE) during primordial germinal cells differentiation and development defines the difference in reproductive potential between *Bm* and *bm* under water deficit. We used ethylmethanesulfonate (EMS) mutagenized TX623 to produce BC2F2 and BC1F5 progeny segregating for *Bm* and *bm*. Water deficit treatments were imposed from 40 days after germination (DAG) till physiological maturity. Each treatment was set up in three reps and two separate greenhouse experiments. Intrinsic responses were evaluated for the relationship between PnE and canopy temperature (TD), stomatal conductance (Gs), transpiration (E), vapor pressure deficit (VPD), net photosynthesis (Pn), leaf cuticular wax load (WL) and biomass and grain yield parameters, from flag leaf emergence (FLE) to 5days after pollination (DAP). Compared to *Bm* type, bloomless types showed exponential and stronger correlation between PnE and seed number in both well-watered and water deficit conditions, even though *bm* had generally fewer seeds under drought. Both *Bm* and *bm* showed transgressive segregation for WL, with the higher wax variants showing slightly reduced canopy temperature, elevated PnE and slight increase in Pn and on average, higher seed number in water deficit treatment compared to lower WL variants. Surprisingly, some of the higher WL *bm* types showed lower Gs, higher PnE, and greater biomass weight and total and single grain weights under water deficit conditions than the *Bm* sibs. Taken together, these observations suggest that improving drought resistance among some *bm* types could potentially have more dramatic increase in seed number and biomass than their *Bm* counterparts.

Paper#: SICNA2015-P11 (SOC17)

Genome wide mapping of flavonoid pigmentation in sorghum seedlings

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Sorghum harbors substantial genetic variation for seedling color, ranging from green to red or purple, due different levels and types of flavonoid compounds. In some plant species, seedling flavonoid pigmentation is thought to confer tolerance to abiotic or biotic stressors, but the role of seedling pigmentation in sorghum is not known. As a first step towards understanding the role of flavonoids in sorghum seedlings, we are mapping the genes controlling variation in seedling pigmentation. A genome wide association study (GWAS) will be performed on a diverse *Sorghum* Association Panel (SAP) and a Nested Association Mapping (NAM) population. Genetic variants in the NAM and the SAP have been identified using genotyping by sequencing (GBS). The seedling pigmentation phenotyping is being carried out using visual scoring, with color classified as either green, yellow, tan or red, and quantitatively with a spectrophotometer, to increase accuracy and compensate for the effects of ambient light. The spectrophotometer measures the percentage of reflected light at the across the spectrum from 300nm to 1000nm. Association analysis of pigmentation phenotypes and genetic variants will identify candidate genes for seedling pigmentation. By determining the genes underlying variation in seedling pigmentation we can better understand the effects of flavonoid pigmentation and assess the potential value of pigmentation for improved stress tolerance.

Paper#: SICNA2015-P12 (SOC16)

Selection for drought tolerance in sorghum using desiccants to simulate post-anthesis drought stress

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Field screening for late season drought in sorghum is difficult as the stress is unpredictable and often confounded with diseases and lodging. We evaluated the effectiveness of desiccants in simulating post-anthesis drought, for potential usage in selection for drought tolerance. First, the efficacies of three desiccants KI, NaClO₃ and KClO₃, were tested, each desiccant applied at three concentrations: 0.4% w/v, 0.6% w/v and 1% w/v, on three genotypes. Yield and seed weight of the genotypes differed significantly under each desiccant. Genotype P898012, known to be tolerant to post-anthesis drought, had the highest yield and seed weight after treatment with the desiccants. A known post-anthesis drought susceptible genotype, TX7078, had the lowest yield. The effect of desiccants were significantly different, with NaClO₃ having the most severe effect while KI had the least effect. The second experiment assessed the sensitivity to KI (0.6% w/v) of 18 diverse sorghum genotypes. Known drought tolerant genotypes: P898012, SRN39, B35 and P9405 had lower but non-significant yield reduction than the susceptible ones: TX7078, P721N, P721Q and K159. A high stress tolerance and low susceptibility indices were observed for the known drought resistant genotypes. These genotypes also showed significant remobilization of, stem soluble sugars and starch. Grain yield under stress was significantly correlated with remobilized sugar ($r = 0.91^{***}$) and starch ($r = 0.75^{**}$). Results from this study, suggested that the desiccants simulated the effect of drought stress. This technique could be used in sorghum to rapidly screen for post-anthesis drought tolerance.

Paper#: SICNA2015-P13

“Virus Induced Flowering (VIF)” to deliver florigen and promote flowering in photoperiodic crops: Applications for sorghum breeding and productivity

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Sorghum flowering ranges from day neutral in grain lines to photoperiodic in high-biomass and wild accessions. These differences create barriers for breeders, especially those hoping to exploit the diversity of landraces and wild accessions. “Virus-Induced Flowering (VIF)” was developed to transiently induce flowering in photoperiodic cotton and bypass environmental cues that otherwise delay flowering. The goal of this presentation is to highlight our accomplishments in cotton and stimulate interest and feedback from the sorghum community on our efforts to adapt this technology to sorghum. Like sorghum, domesticated cotton is inbred while wild accessions are a tremendous reserve for desirable traits. Wild cottons are short-day photoperiodic while domesticated lines are day neutral. These differences in the onset of flowering complicate breeding and increase costs. Using a disarmed Geminivirus as a transient expression system, we delivered the gene *FLOWERING LOCUS T (FT)*, encoding the universal flowering hormone, florigen, to both photoperiodic and day-neutral cotton. Ectopic *FT* expression in domesticated lines promoted synchronized fruiting and a compact structure preferred by producers. In wild accessions, *FT* promoted flowering under non-inductive conditions to provided fertile blooms simultaneously with domesticated varieties, demonstrating that transient *FT* expression from a disarmed virus is an effective and efficient mechanism to increase genetic diversity in a crop species. Significantly, virus was not detected in the F₁

progeny, indicating that crosses made by this approach do not harbor recombinant DNA molecules and may be suitable for combining natural and beneficial traits in markets where GMOs are undesirable.

Paper#: SICNA2015-P14

Occurrence of feral sorghum (*Sorghum bicolor* L.) in roadside habitats in southern Texas: implications for gene flow

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Feral sorghum is found along roadsides in southern Texas and their sympatry with johnsongrass is a concern because of the likelihood for pollen-mediated gene flow between these two species and potential transfer of novel traits (present in sorghum) to the progeny. However, little is known on the prevalence and distribution of feral sorghum populations in the region. A survey was conducted in fall 2014 to document 1) the occurrence of feral sorghum along roadside habitats in three important sorghum producing areas in southern Texas (Rio Grande Valley, Coastal Bend and Gulf Coast) and 2) the proximity of feral sorghum to johnsongrass. A semi-stratified survey methodology was followed. Site characteristics, population density and adjacent land use were noted. Feral sorghum was frequently found in all the three survey regions, with a total of 366 feral sorghum sites in the survey route. The majority (67%) of feral sorghum populations were found adjacent to crop fields, particularly near sorghum and cotton fields. About 56% of the feral populations fell under a density category of 6 to 25 plants and 19% under 26 to 50 plants within a 25 m transect along the roadside. About 10% of the feral sorghum sites (38 out of the 366 feral sites) had johnsongrass within the site (<5 m between sorghum and johnsongrass), whereas about 20% (78 out of 366) had johnsongrass within a distance of 25 m. The survey revealed that feral sorghum is commonly present along the roadsides in southern Texas within distances that favor outcrossing between sorghum and johnsongrass. More research is necessary to understand the levels of outcrossing between feral sorghum and johnsongrass in natural environments.

Paper#: SICNA2015-P15 (SOC18)

Mapping and identification of increased protein digestibility in sorghum

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Sorghum (*Sorghum bicolor* [L.] Moench) is a dietary staple for approximately 500 million people in over 30 countries of the Semi-Arid Tropics (Wang et al., 2014). Unlike maize, sorghum becomes much less digestible after it is cooked, often providing as little as 20% of the available protein. Digestibility appears to be under genetic control, although the specific details remain unclear. The goal of these studies is to identify sorghum cultivars that do not show this large decrease in digestibility after cooking. A high lysine sorghum mutant, P721Q, identified 30 years ago, has recently been shown to have a defect in a gene for one of its prolamins (kafirins) (Wu et al., 2013), similar to maize opaque2 mutants, which are deficient in prolamins (zein) production in the seed. In addition to high lysine, P721Q also exhibits a 3- to 4-fold increase in protein digestibility after cooking, which makes it even more valuable as a food staple. However, it is not clear that the kafirin mutation also causes the improved protein digestibility of this

mutant. Interesting preliminary data have suggested it may not; rather, this improvement may be caused by a mutation in a separate gene that has not yet been linked to protein digestibility. We will present QTL mapping data from a P721Q x BTx623 mapping population to to characterize the high digestibility trait in P721Q. We will also present data on two additional highly digestible sorghum mutants we have identified and characterized from an EMS mutagenized population of BTx623.

Paper#: SICNA2015-P16

Translational genomics for abiotic stress in sorghum: Transcriptional profiling and validation of SNP markers between germplasm with differential cold tolerance

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One focus of the Sorghum Translational Genomics Lab (part of sorghum CRIS, PSGD, CSRL, USDA-ARS, Lubbock TX) is to utilize nucleotide variation between sorghum germplasm such as those derived from RNA seq for translation and validation of Single Nucleotide Polymorphism (SNP) into easy access DNA markers for molecular and marker assisted breeding. In this report, we performed transcriptome profiling between cold sensitive and tolerant sorghum lines using RNA sequencing technology under control and cold stress treatments. We identified differentially expressed genes between contrasting germplasm under each temperature stress and validated bi-allelic, single nucleotide polymorphic variants between the susceptible and tolerant lines. Expression analysis showed that in response to cold, a total of 1,903 differentially expressed genes were detected during cold stress treatment in both genotypes. A number of transcription factors were differentially expressed under stress between the sensitive and tolerant genotypes including Dehydration-responsive element-binding factors, C-repeat binding factors, and Ethylene responsive transcription factors. A set of gene transcripts including; 27 Plant Cytochromes, 7 Glutathione S-Transferases, and 20 Heat Shock proteins displayed prominent differential expression under cold stress. A total of 41,603 SNPs were identified between the sensitive and tolerant genotypes with minimum read of 4 using Alpheus pipeline. A total 100 SNP markers between the lines were validated thru endpoint genotyping method. These results provide an insight into the complex mechanisms involved in cold tolerance in sorghum, and provide genomic foundation for future studies on cold tolerance in sorghum and other cereal crops. Here the SNPs identified and validated between the tolerant and sensitive lines demonstrated translation of variation from RNA seq analysis into easily usable DNA marker format for downstream applications of genetic mapping and molecular breeding.

Paper#: SICNA2015-P17

Translational genomics and marker assisted selection in sorghum: case study using brown midrib (*bmr*) trait

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Translational genomics is a critical phase in harnessing the rich genomic data available for sorghum. There is a need to transform nucleotide variation data between sorghum germplasm such as that derived from RNA seq, genotype by sequencing or whole genome resequencing through translation and validation of Single Nucleotide Polymorphism (SNP) into easy access DNA markers for molecular and marker assisted breeding. Here, we use brown mid-rib an important trait in energy sorghums and is known to be associated with reduced lignin content as a case study for translational genomics. This case study applied previous work on mutations in genes involved in monolignol biosynthetic pathway that affect the lignin content, development of SNP markers and coupling of the molecular markers with the phenotype. There are three known genes - caffeic acid O-methyltransferase, cinnamyl alcohol dehydrogenase and 4-coumaroyl CoA that affect the lignin content based on both natural and induced mutations. Twenty mutations are known in these three genes and nineteen of them were converted into SNP molecular markers that can easily be assayed using endpoint genotyping technology. These markers were assayed in a pool of lines which consisted of commercial varieties, bmr-6 and bmr-12 standards and 5 new bmr mutants from EMS populations. The SNP molecular markers for bmr described here provide a platform to screen for the variations present in diverse lines and provide an opportunity to identify new alleles for crop improvement.

Paper#: SICNA2015-P18 (SOC15)

Evaluation of diversified sorghum lines for grain mold complex and its impact on physical and quality kernel traits

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An experiment was conducted using 227 lines of the sorghum association panel in 2014 using a split-plot design with two replications. At 50% flowering, nine plants/line/replication were selected and three plants each were assigned: (i) panicles inoculated with *Fusarium thapsinum* and bagged for seven days, (ii) panicles non-inoculated and bagged for seven days, and (iii) panicles non-inoculated and non-bagged. All panicles were scored for panicle grain mold rating (PGMR) at physiological maturity and for threshed grain mold rating (TGMR) after harvesting on a scale 1 to 5 (1 = < 1% and 5 = > 50% threshed seeds molded). Based on PGMR and TGMR mean scores, 46 lines were selected for laboratory analyses. Harvested seed samples were analyzed for quality traits by using near-infrared reflectance (NIR) spectroscopy and for physical grain traits. Fungal colonies growing from bisected kernels (i.e., grain mold) and from the kernel surface (i.e., grain weathering; non-inoculated, non-bagged treatment) were counted and identified via sequencing using ITS and genus-specific primers. Correlation studies showed that PGMR and TGMR had significant negative correlations with seed viability, protein and fat content, tannins, phenolic acids, seed hardness index, and with fungal species including *F. verticillioides* and *Alternaria*. PGMR and TGMR scores showed significant positive correlations with the grain mold pathogen, *F. thapsinum*. Results indicated that *Alternaria* was the main genus isolated from both the seed surface and internal portions of the seed followed by *F.*

verticillioides, *F. thapsinum*, and *F. incarnatum* in the environment tested. *F. oxysporum*, *Curvularia*, and *Aspergillus* were present in traces.

Paper#: SICNA2015-P19 (SOC20)

Remote sensing screening tools for sorghum breeding programs

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Sorghum breeders typically screen up to 10,000 experimental lines in a growing season. When selecting for desirable traits, time required for observation and notes can become the factor limiting discovery of advances germplasm. However, advances in small unmanned aerial systems (sUAS) and camera imaging technologies provide opportunity to develop new screening tools, accelerate the screening process and expand the range of traits for which breeders can rapidly screen selections. An objective of this study was to utilize ground-based measurements to develop remote sensing techniques, suitable for sUAS application that quantify advanced canopy traits including upright leaf orientation, limited transpiration, heat tolerance, green leaf duration and above-ground biomass productivity. Advanced canopy traits were represented by 24 parental lines of the Nested Association Mapping populations (Kansas State and Texas A&M sorghum breeding programs) and six additional lines. These lines were grown in irrigated (Colby, KS) and non-irrigated (Ashland Bottoms, KS) plot (5' x 15') with four replicates in randomized complete block designs. Leaf orientation was represented by the initial slope of leaf blades from the stalk. Green leaf duration was evaluated by sequential measure of the uppermost mature leaves using a SPAD 502 Plus Chlorophyll Meter. Images were acquired using a Canon s100 converted to be sensitive to blue, green and near infrared wavelengths (Ashland Bottoms) and a Tetracam ADC (green, red and near infrared wavelengths, Colby). These channels were used to create the Normalized Vegetation Index (NDVI). Leaf fluorescent yield was evaluated using an OS5p fluorometer following heat stress events. Above-ground biomass and grain yield components were determined at maturity. Three lines had leaf trajectories less than 35° from vertical (upright orientation). Three lines had leaf trajectories greater than 85° from vertical (horizontal orientation). SPAD values differed among lines for each of the six sampling periods. Seasonal changes in SPAD values, from mid-grain fill through late-grain fill appeared to differ among lines. For two lines, SPAD values increased ~7 SPAD units from mid- to late-grain fill. For two lines, SPAD values decreased ~7 SPAD units during same time interval. More than 1600 vegetative index images and 3600 thermal images were collected at Colby using a truck-mounted boom; similar quantities of images were acquired at Ashland Bottoms. Image analysis is focused on developing semi-automated processing procedures, which are required for high throughput phenotyping.

Paper#: SICNA2015-P20

Identification of a Dhurrin QTL (Dhu1) that aligns with a novel stay-green QTL (Stg5) in *Sorghum Bicolor*

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Dhurrin is a cyanogenic glucoside produced by (*Sorghum bicolor* L. Moench) and is generally considered a natural defense compound capable of producing the toxin hydrogen cyanide (HCN) to deter animal herbivory. Recently, high levels of leaf dhurrin have been found in grain sorghum lines that also exhibit stay-green during post-anthesis water deficit. Post-flowering drought tolerance (stay-green) in sorghum is an economically important trait in production regions where drought stress post-anthesis is common. Stay-green is associated with reduced lodging, charcoal rot resistance, increased grain fill, and increased grain yield. The genetic control of stay-green is complex, with multiple QTL being identified in affecting expression of stay-green. Here we report the discovery of a dhurrin QTL (Dhu1) on SBI01 using a RIL mapping population derived from BTx642/Tx7000. Dhu1 is highly heritable and explains a large percentage of the variation in leaf dhurrin in the population. The QTL is aligned with genes involved in dhurrin biosynthesis on SBI01. Dhu1 is also aligned with a previously un-identified stay-green QTL (Stg5) on SBI01, consistent with prior studies showing an association between high leaf dhurrin levels, this region of SBI01, and expression of the stay-green trait.

Paper#: SICNA2015-P21

Characterization of genetic diversity for high temperature tolerance in sorghum

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As global warming becomes inevitable, the sustainability of agricultural production in US and worldwide faces serious threat from extreme weather conditions, such as drought and elevated extreme temperatures (heat waves). Among cereal crops, sorghum is considered a versatile crop for semiarid area and marginal land for its' high tolerance levels for heat and drought. Nevertheless, our field observation show great genetic variation in heat tolerance among sorghum accession. In this study, we evaluated vegetative stage heat tolerance traits of 377 sorghum breeding panel under 4 environments in field study. Preliminary genome-wide association analyses revealed several chromosome regions that are associated with observed heat tolerance traits. Detained analysis in the identification of genetic loci contributing to heat tolerance in sorghum is underway. The outcome of this study will further our understanding about the genetic mechanisms of heat tolerance in sorghum and help in the genetic improvement of heat tolerance in crop varieties/hybrid.

Paper#: SICNA2015-P22

Breeding for resistance to the sugarcane aphid [*Melanaphis sacchari* (Zehntner)]

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The sugarcane aphid [*Melanaphis sacchari*] (SCA) was first reported to damage sorghum [*Sorghum bicolor* (L.) Moench] in the United States in Louisiana and Texas in 2013, and was

subsequently detected in Oklahoma and the Mississippi Delta. In 2014 the aphid spread and was eventually reported in states representing over 90% of U.S. sorghum production, including grain and forage. The aphid is a variant of the sugarcane aphid identified in Florida in 1977. Screening of sorghum lines for resistance resulted in identification of numerous putative sources of resistance. Among resistance sources Tx2783 expresses a high level of resistance in the U.S. and Southern Africa. Tx2783 was developed for resistance to biotype C and E greenbug [*Schizaphis graminum* (L.) Rondani] and in development was not screened for reaction to sugarcane aphid. To identify lines resistant to sugarcane aphid approximately 290 advanced breeding lines, converted exotic sorghums, or exotic introductions were evaluated in greenhouse juvenile plant trials in 2014. Breeding lines were evaluated in adult plant trials at three locations in 2014. From previous releases of lines developed for biotype C and/or E greenbug resistance, seven lines were identified as resistant. Thirty-three advanced breeding lines, 26 R-lines and 7 B-lines, were identified with putative resistance. Additionally, exotic introductions and converted exotic sorghum lines were evaluated for resistance. The R-lines will be evaluated as hybrid parents for yield potential and resistance and the B-lines are being sterilized.

Paper#: SICNA2015-P23 (SOC19)

Agronomic characterization of inbred lines and hybrids of sorghum introgressed with QTL for cold tolerance

Xu, X.

Cold temperatures occurring in the early growing season are a major limitation for sorghum (*Sorghum bicolor* (L.) Moench) cultivation in the northern and central United States and other temperate regions. Chinese kaoliang sorghum landraces, known to have excellent seedling cold tolerance, were used as a source of genes to improve early-season cold tolerance in sorghum at Purdue University. In the last few years, two QTL were identified and introgressed into five inbred lines generating five pairs of isogenic lines with and without these markers. Selection for the cold tolerant alleles from the donor kaoliang parent was based on SSR markers in the two QTL regions. A major objective of this study was to compare performance differences between the original inbred and the new cold converted (CC) sorghum sister lines. The iso-lines were grouped into male and female parents and used to generate contrasting hybrids. Four cold converted inbred lines and four cold converted hybrids were evaluated in the growth chamber and field for early-season traits under cold stress conditions. Flowering time, mature plant height and final grain yield were also evaluated in the field trials. A germination test was also conducted in the growth chamber to compare the converted and unconverted lines. The effect of the QTL varied with inbred lines. K1597-CC4 and Tx2737-CC showed improved cold tolerance over their unconverted counterparts in terms of seedling emergence and germination under cold stress conditions in the field trial, growth chamber experiment and germination tests; whereas in the inbred line Wheatland, both converted and unconverted iso-lines showed good seedling cold tolerance. Hybrids, generally showed superior performance to the lines in both seedling emergence and seedling vigor, probably due to the effect of heterosis. Though the R-line Tx2737 showed relatively poor seedling emergence, Tx2737 hybrids showed the best seedling emergence and germination under cold stress conditions in this study.

Paper#: SICNA2015-P24 (SOC7)

Transcriptional regulation in ALS herbicide resistant sorghums with interveinal chlorosis

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Sorghum (*Sorghum bicolor* (L.) Moench) acreage in the US has shown a sharp decline in the past few decades. Among the many factors responsible were better weed control options that competing crops have offered. Sorghum farmers craved for years for effective post-emergence weed control option. The discovery of the ALS (Acetolactate synthase) resistance trait in wild sorghum was received with much optimism for improving management of grass weeds in sorghum. The trait was incorporated in to cultivated sorghum and released to the industry. While the trait offers resistance to a wide range of ALS herbicide chemistries, most resistant plants tend to show interveinal chlorosis and reduced seedling vigor. While this phenotype persists only for short duration, it may be of concern to some growers and industries may like to deploy resistant hybrids without such awkward phenotype. The objective of this study was to understand the genetic basis of this bizarre phenotype. Two ALS resistant genotypes expressing yellow and normal phenotypes were field grown and leaf tissues were harvested at four time points until yellow genotype re-greened. RNA extracted from leaf tissue samples were subjected to RNA sequencing. Differential gene expression analysis was performed using DESeq2 and genes with similar expression patterns were clustered using mclust. Gene Ontology enrichment and SorghumCyc pathway analysis revealed significant regulatory activity in genes related to chloroplast, plant defense responses and hormonal networks which provided insight on mechanisms that help re-greening process in yellow genotypes.

Paper#: SICNA2015-P25

Photosynthesis, chlorophyll fluorescence and leaf temperature variation in sorghum under drought

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Drought tolerance is a major target for breeding sorghum (*Sorghum bicolor* (L) Moench) as a bioenergy crop. It is important to determine the optimal growing conditions to evaluate photosynthesis and chlorophyll fluorescence in a large number of genotypes to exploit the genetic diversity and discover genes associated with stress tolerance in sorghum. The objectives of this study were: 1) to evaluate changes in leaf temperature, chlorophyll fluorescence and photosynthesis of sorghum genotypes subjected to soil drying conditions and their subsequent recovery; and 2) to develop an accurate protocol for phenotyping of sorghum plants to be used in linkage disequilibrium studies. Twelve sorghum genotypes were grown in growth chambers with a 16 hour photoperiod, photosynthetically active radiation of 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, and day and night temperatures of 28°C and 24°C, respectively. Plants were fully irrigated during 35 days, after which drought was imposed. Plants were differentially watered during fourteen days to achieve a final water level of 7% volumetric water content (VWC), after which they were re-watered. Carbon assimilation and fluorescence parameters were measured using a Li-6400 gas analyzer (Li-Cor Biosciences, Lincoln, NE) and leaf temperature was monitored with a portable thermocouple in a fully expanded illuminated leaf. A wide range of responses were obtained for fluorescence parameters (non-photochemical quenching, PhiPS2, Fv/Fm), gas exchange (photosynthesis, stomatal conductance, transpiration) and leaf temperature. A general linear response was observed in most parameters when plants achieved values lower than 18% in VWC.

Paper#: SICNA2015-P26

Identification and mapping of QTL for bioenergy-related traits in sweet sorghum

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Sweet sorghum is a potential energy crop. To be an efficient energy crop, sweet sorghum needs to be genetically improved. Marker-assisted breeding will be important in genetic improvement of sweet sorghum. The objective of the present study was to identify quantitative trait loci (QTLs) associated with bioenergy-related traits in sweet sorghum. We used 188 F₇ recombinant inbred lines (RILs) derived from a cross between sweet sorghum (Wray) and grain sorghum (Macia). The RILs and their parental lines were grown at two locations in 2012 and 2013. Genotyping-by-sequencing analysis of the RILs allowed the construction of a high-density genetic map with 912 single nucleotide polymorphisms. Using the inclusive composite interval mapping of additive QTLs, a total of 27 major QTLs for bioenergy-related traits in sorghum were identified, including anthesis date, plant height, biomass yield, stem diameter, brix, head moisture content, and grain yield. A major QTL for anthesis and head moisture content co-localized on chromosome 6, and explained 28.41% and 20.96% of the phenotypic variances (PVE), respectively. A major QTL for plant height (17.56% PVE) on chromosome 7 also co-localized with a major QTL for total biomass yield (20.96% PVE). A major QTL for brix (32.85% PVE), grain yield (29.88% PVE), and stem moisture content (15.78% PVE) co-localized on chromosome 3. A major QTL for stem diameter was detected on chromosome 6 (14.23% PVE). The identified QTLs in this study could help to further understand the underlying genes associated with bioenergy related traits and could be used for development of molecular markers for marker-assisted selection.

Paper#: SICNA2015-P27

Development of genomic-enabled breeding resources for sorghum improvement in Senegal

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In Senegal, sorghum [*Sorghum bicolor* (L) Moench] is a staple crop cultivated for human consumption and forage production in multiple agro-ecological regions. The improvement of sorghum in Senegal since 1970s has led to the creation of varieties with short cycle (that mature in 90-105 days after planting), higher quality grain, and improved yield. However, most of these varieties are susceptible to drought and grain mold, which reduce productivity and grain quality. In this context, the Sorghum and Millet Innovation Lab (SMIL) project 'Improving sorghum adaptation in West Africa with genomics enabled breeding' is focused on developing genomics resources for breeding of grain mold and drought tolerance into local sorghum varieties. As part of the project framework, a phenotyping platform and Nested Association Mapping population are being developed in Senegal for mold resistance and drought tolerance screening. In addition, Genotyping-By-Sequencing is being carried out to characterize Senegalese germplasm, identify beneficial alleles and better understand the molecular basis of mold and drought resistance. The

genetic resources being utilized in this project are made up of a panel of 500 breeding lines from Senegal, Mali, and Togo, 553 Senegalese accessions from the United States gene bank (GRIN), and 100 local landraces from CERAAS (Centre d'étude regional pour l'Amélioration de l'Adaptation à la Sécheresse). The identified alleles will be used in marker-assisted breeding and incorporated into the Senegalese sorghum varieties to develop high yielding varieties adapted to the different agro-ecological regions of Senegal.

Paper#: SICNA2015-P28 (SOC10)

Genomic dissection of leaf and panicle architecture traits in sorghum using nested association mapping

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Sorghum is an important crop in many agroclimatic regions worldwide, and has adapted to a wide range of conditions. Accordingly, global sorghum germplasm exhibits substantial variation in agroclimatic traits—traits that differ in germplasm from different agroclimatic zones—including many aspects of leaf and panicle architecture. Currently, our ability to improve sorghum adaptation and transfer useful alleles across different genetic backgrounds is constrained by our limited knowledge of the genomic regions that underlie agroclimatic traits. Nested Association Mapping (NAM), which uses multiple biparental families linked by a common parent, can improve dissection of agroclimatic traits by reducing the confounding effects of population structure and increasing the frequency of rare alleles. A sorghum NAM population comprised of 10 families and almost 2,500 recombinant inbred lines (RILs) has been developed and genotyped at approximately 100,000 SNPs with Genotyping-by-Sequencing (GBS). The population was phenotyped for leaf angle, leaf width, panicle compactness and primary rachis branch length in two contrasting environments (locations) in Kansas, semi arid (Hays) and humid continental (Manhattan). Significant genotypic variation for these traits was observed. Association mapping confirmed several previously identified quantitative trait loci (QTL) and revealed many new QTL for leaf and panicle architecture. QTLs were found for leaf angle around the *Dw3* region in chromosome 7. Likewise, for lower rachis branch length, we identified Sb07g023640 a flavin monooxygenase gene close to the *Dw3* region on chromosome 7. The QTL identified will be helpful in marker-assisted selection for better adaptation and yield.

Paper#: SICNA2015-P29 (SOC9)

Genome-wide analysis of genetic structure and linkage disequilibrium in sorghum [*Sorghum bicolor* (L.) Moench] public inbred lines

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Recent advances in genotyping with high-density markers have enabled genome-wide analyses in crops. Knowledge of genetic structure and linkage disequilibrium (LD) is important for the application of genomic approaches and knowledge-based breeding. The objectives of this study were (1) to investigate the genetic structure of the sorghum public inbred lines, and (2) to assess the extent and pattern of linkage disequilibrium and determine the effect of minor allele frequency (MAF) and sample sizes on LD in the collection. A total of 279 sorghum inbred lines from public sorghum breeding programs were genotyped using genotyping-by-sequencing (GBS). The method generated 282,536 single-nucleotide polymorphism (SNP) markers distributed across the entire genome with minor allele frequency (MAF) greater than 1% and less

than 20% missing data. More than half of the SNPs (216,271) are rare (MAF<0.05). The genetic structure of the collection was estimated using the entire set of SNPs. Linkage disequilibrium (LD) as measured by mean r^2 was estimated in the whole genome and within chromosomes, allelic frequency groups and subgroups of different sample sizes using 66,265 SNPs (MAF \geq 0.05). Population structure, principal component and neighbor-joining (NJ) tree analyses identified three major subgroups that correlated well with pedigree information. Genome-wide linkage disequilibrium, r^2 estimates varied among chromosomes, allelic frequency groups and subgroups of different sample sizes. The LD decay distance varied among chromosomes, and increased with increase in minor allele frequency (MAF) and with smaller sample sizes. These results provide valuable information about the genome structure of the sorghum inbred lines for further genome-wide studies.

Paper#: SICNA2015-P30

Power and resolution of QTL mapping in sorghum using a nested association mapping population and diversity panels

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The efficiency of Marker-Assisted-Selection relies on the power and resolution of QTL mapping. To maximize recombination, the plant scientific community can take advantage of both diversity panels covering most of historical recombinations and controlled crosses. Multi-parental designs are a good compromise. KSU developed a Nested Association Mapping (NAM) populations of ten families, using RTx430 as a common parent. In total, 2200 RILs were produced by single seed descent for 8 generations without selection. This is the largest sorghum composite map, consisting of 90,441 GBS (Genotyping By Sequencing) markers, 44% being specific to one family and 19% being polymorphic in at least 5 families. Polygenic variances are 65% for flowering time (FT) and 74% for plant height (PTHT). We confirm that *Ma1*, *SbFL9.1* and *SbCN12* are major QTLs for FT, *dw2*, *dw1* and *Dw3* for PTHT. We discuss the distribution of QTLs in regards of signatures of selection, ie hotspots of recombination, regions of low recombination or segregation distortion, in the *Ma1-dw2* region in particular. We demonstrate that this NAM population increases the power of QTL detection compared to association panels, using increasing sample sizes, for FT, PTHT and simulated traits of different heritabilities. We discuss the possibility to reduce the population size for future phenotyping without decreasing power. Those results are promising for deciphering the architecture of complex adaptive, quality and yield traits, for both forage and grain sorghum, thanks to high-throughput phenotyping and genotyping.

Paper#: SICNA2015-P31

Grain quality traits in a sorghum association mapping panel

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Grain quality traits were analyzed in a diverse sorghum sample set which consisted of 174 sorghum lines (110 non-tannin lines and 64 tannin lines). These samples were previously grouped into five distinct genetic populations which made it possible to compare grain quality traits across the genetic groups. For the non-tannin samples, in-vitro protein digestibility (IPVD) was highest in the zerazera-caudatum (60.3%) and caudatum (60.1%) groups, though there was wide variability across all the groups. However, nine of the 20 lines with the highest IPVD were

from the caudatum group (with the highest line ~80%). Within the non-tannin samples total phenolics were similar across genetic groups though average ORAC values varied. The durra genetic group had the highest ORAC (61.4 μM Trolox equivalents (TE) /g) with the guinea-caudatum having the lowest (46.0 μM TE/g). Starch granule size distribution also varied across the non-tannin genetic groups with the guinea-caudatum group having the highest proportion of A-granules (53.9%) and the caudatum group the lowest (45.9%). Within the tannin samples, IVPD varied from 37.5% (kafir group) to 55.5% (zerazera-caudatum) and tannin content highest in the durra group (14.1 g catechin equivalents (CE)/kg) and lowest in the zerazera-caudatum group (4.49g CE/kg). Interestingly, ORAC values were highest in the kafir group (118.4 μM TE/g) with the lowest in the zerazera-caudatum (70.4 μM TE/g). Tannin content and ORAC value were only loosely correlated ($r=0.55$) suggesting that tannin chemistry, not just content, is important in governing anti-oxidant capacity in tannin sorghum lines. The variation of grain quality traits across genetic groups provides information needed to identify sources of new genetic material for improving the end-use quality of sorghum.

Paper#: SICNA2015-P32

Sorghum alters lipoprotein subfractions supporting the cardioprotective role of Sorghum in the diet

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Cardiovascular disease (CVD) is the leading cause of death in the United States. Elevated total cholesterol and low-density lipoprotein (LDL), and reduced high-density lipoprotein (HDL) are major risk factors for CVD. HDL and LDL density subfractions provide a better estimate of CVD risk. Sorghum polyphenolic compounds may alter lipoprotein density profiles (LPDP) to reduce CVD risk. In a random crossover design, overweight human subjects consumed 50 or 100 g of a sumac sorghum cereal daily for 11 weeks. Plasma was collected for LPDP analysis using isopycnic ultracentrifugation and to determine apoprotein and adiponectin concentrations. Plasma from rats consuming diets containing either cellulose, or bran from sumac, black, or high-tannin-black sorghums for 10 weeks was also collected for LPDP. Consumption of sumac cereal by overweight subjects reduced LDL-5 ($p=0.014$) and HDL-3b ($p=0.014$), and increased less dense, more cardioprotective, LDL-2 ($p=0.006$) and LDL-3 ($p=0.007$); ApoB ($p=0.008$) and ApoB/ApoA1 ($p=0.046$) ratios were increased. Consistent with the human data, rat HDL-2b ($p=0.0043$) and LDL-3 ($p=0.0134$) increased with sumac bran. These results suggest that consumption of sorghum, specifically sumac, may be protective against CVD by altering lipoprotein profiles.

Paper#: SICNA2015-P33

***In vivo* antioxidant status in diet-induced obese rats consuming sorghum flour fractions**

Moraes, E.A.^a, Lenquiste, S.A.^a, Marineli, R.S.^a, Queiroz, V.A.V.^b, Turner, N.D.^c, and Júnior, M.R.M.^a

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This work aimed to evaluate antioxidant activity (AA) of whole sorghum flour (WF), decorticated sorghum flour (DF) and sorghum bran (SB) of the genotype SC 21 and their effects on antioxidant capacity in diet-induced obese rats. AA was determined by DPPH, ABST, FRAP and ORAC assays. Thirty *Wistar* rats were divided into five groups receiving an: 1) AIN-93M diet, 2) high-fat and high-fructose diet (HFF), or HFF added with 3) WF, 4) DF or 5) SB for 12 weeks. Plasma and liver samples were used to determine antioxidant enzyme activities, GSH levels, and for FRAP and TBARS assays. SB produced the highest AA. Sorghum flour fractions did not improve plasma GPx, GR, SOD activities or FRAP status. GSH levels of HFF-WF group and plasma TBARS of all sorghum diets were similar to AIN-93M group. Liver GPx activity was improved in sorghum diets, while liver CAT activity and GR were higher in HFF-WF and HFF-SB. Thus, sorghum bran had the highest AA and sorghum flour fractions improved liver CAT, GR and GPx activities and decreased plasma lipid peroxidation.

Paper#: SICNA2015-P34

Sorghum bran down-regulates cox-2 and bcl-2 expression in rats induced to develop colon cancer

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We have previously demonstrated that sorghum brans suppress colon carcinogenesis and that the inhibition was related to their phenolic compounds. This study aimed to evaluate the effects of sorghum brans containing different phenolic compounds on expression of inflammatory and apoptotic proteins in colon mucosa. We used 24 Sprague-Dawley rats consuming diets containing 6% fiber from either cellulose (CC), or bran from white (WS), brown (BRS) or black (BLS) sorghum (n=6). Diets were fed for 10 wk, during which two azoxymethane (AOM) injections (15 mg/kg body weight) were administered in wk 3 and 4. Fifty micrograms of protein was used to determine cyclooxygenase-2 (Cox-2), Bcl-2 and Bax expression by Western blot assay. β -actin was used as housekeeping protein. The pro-inflammatory protein Cox-2 was down-regulated in rats consuming the sorghum diets ($p \leq 0.05$). Equally, Bcl-2, an anti-apoptotic protein, was expressed at lower levels in sorghum diet groups ($p \leq 0.05$). There was no difference in Bax expression, a pro-apoptotic protein, among experimental groups ($p > 0.05$). We concluded that sorghum brans containing phenolic compounds can decrease the inflammatory process and may increase cell apoptosis due to the down-regulation of Cox-2 and Bcl-2, respectively, in rats induced to develop colon cancer.

Paper#: SICNA2015-P35

Sugarcane aphid in Oklahoma: Responding to a new pest

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The sugarcane aphid (SCA) was first found in Oklahoma in 2013, and quickly became a major threat to grain sorghum production. Scientists at Oklahoma State University and the USDA's Wheat, Peanut and Other Field Crops Research Lab in Stillwater, working with cooperators in other sorghum producing states, have developed a multi-pronged research/extension approach to

assist producers in managing this pest. OSU researchers, with support from the Sorghum Checkoff program, are evaluating season-long systems for management of SCA in grain sorghum, field screening and quantifying SCA resistant sorghum hybrids, comparing damage and calculating plant-stage specific economic thresholds in a SCA resistant and susceptible hybrid at two growth stages. OSU researchers are also conducting standardized insecticide efficacy trials with and without spray adjuvants. USDA scientists are screening for SCA resistance in hybrids and germplasm, developing sampling protocols, studying sugarcane aphid biology and studying the dynamics of natural enemy interactions with the aphid. This presentation will provide a brief summary of completed work as well as future goals. Much of the work is financially supported by the Sorghum Checkoff program and the National Institute for Food and Agriculture.

Paper#: SICNA2015-P36 (SOC3)

RNA-Seq elucidates the molecular basis of charcoal rot resistance in grain sorghum

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Charcoal rot caused by *Macrophomina phaseolina* (MP) is one of the most destructive fungal diseases in sorghum. Although host resistance is the key management strategy, field screening for resistance is tedious. The objectives of this study were to: i) discover differentially expressed genes (DEG) between resistant and susceptible genotypes in response to MP inoculation and ii) understand their contribution to resistance, in order to select potential DEG to be deployed as molecular tags in resistance screening. SC599 (resistant) and TX7000 (susceptible) sorghum lines were grown in the greenhouse and inoculated with MP. Control plants were mock-inoculated with phosphate buffered saline. RNA was extracted from 3 biological replicates at 2, 7, and 30 d (3 stages) post-inoculation (d.p.i.) from stem tissues and subjected to RNA-Seq. Analysis for DEG was performed with DESeq2 and pathway analysis was performed to explore DEG. 8530 annotated genes were identified across 3 stages as significantly differentially expressed between 2 genotypes, out of which 2053 were components of 197 known pathways. 1722 DEGs were observed at 7 dpi, indicating the importance of *M. phaseolina*-mediated host expression profile changes as early as 7 dpi. Of those, 945 genes were constituents of 21 pathways involved in stress responses, nitrous oxide-mediate oxidative burst, basal immunity, hormonal regulation, and nutrition. Most metabolic pathways including nitrate reduction, ethylene, jasmonic acid, and trehalose biosynthesis and homogalacturonan, triacylglycerol and glycerol degradation were significantly upregulated in TX7000, while those of SC599 were non-significantly changed. Results revealed the involvement of non-upregulated susceptibility genes for manifestation of the resistant phenotype.

Paper#: SICNA2015-P37 (SOC2)

Model study on extraction of both fermentable sugars and no-structural carbohydrate from sweet sorghum using diffusion process

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Sweet sorghum is a specific type of sorghum that stores a high concentration of soluble sugars in the stalk and also produces grain in the panicle. This grain represents a significant amount of starch. Currently, the ethanol industry uses sugarcane processing methods for handling sweet sorghum. However, sweet sorghum is not identical to sugarcane in that sweet sorghum produces significant quantities of grain in the panicle, predominantly starch. The objective of this research is to enhance the economic attractiveness of ethanol production from sweet sorghum through the development of technologies to fully utilize fermentable sugars, starch in the panicle, and non-structural carbohydrates in the stalk for high efficiency and low-cost ethanol production. In this work, fermentable sugars and non-structural carbohydrates were extracted from chopped sweet sorghum biomass and grains by the diffusion process. Response surface methodology (RSM) was applied to explore the effect of diffusion time, diffusion temperature, the ratio of sweet sorghum biomass to grain on starch-to-sugar efficiency, total sugar recovery from sweet sorghum, as well as to optimize the diffusion conditions. RSM results showed that at an optimized time of 114.9 minutes, temperature of 95 °C and 22% grain loading, starch efficiency and sugar recovery efficiency of 96% and 98.5% were achieved, respectively.

Paper#: SICNA2015-SOC1

Nitrogen yield response and use efficiency in high biomass sorghums raised for ethanol production in Missouri

Maw, M.J.

Demands for high-yielding, alternative biofuel feedstocks elicits the need to fully evaluate high biomass sorghum (*Sorghum bicolor* (L.) Moench) yield response and nitrogen use efficiency (NUE) to varying N fertilization rates in the U.S. Midwest. The objectives of this study were to determine the optimum N fertilization application rates and nitrogen removal response for the production of two high biomass sorghum cultivars (ES5200 and ES5201) over two years in central Missouri. The effects of five rates of N fertilizer (0, 56, 112, 168, 224 kg ha⁻¹ N) were tested on dry matter (DM) yields, and theoretical lignocellulosic ethanol yield and stem-leaf dry matter ratios. Dry matter nitrogen content was used to determine NUE and nitrogen recovery efficiency (NRE) at each N rate. Nitrogen (N) rate was significant both years for dry matter yields and ethanol with no additional N benefit above 56 kg ha⁻¹. Total dry matter yields averaged 12.8 Mg ha⁻¹ and ethanol averaged 3,999 L ha⁻¹ across years, with stem DM more than double leaf DM. An average of 93.2 kg N ha⁻¹ was removed in the DM with the best NUE and NRE at the lower fertilizer rates. High biomass sorghum could potentially reach optimum yields in Missouri with minimal fertilizer inputs.

Paper#: SICNA2015-SOC6

Genetic diversity in *Fusarium andiyazi* populations from sorghum

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Fusarium andiyazi is an important pathogen of grain sorghum (*Sorghum bicolor*) causing grain mold and stalk rot. There are numerous reports of “*F. andiyazi*-like” strains from sorghum around the world. However, little is understood about the genetic variation in *F. andiyazi* and these *F. andiyazi*-like populations. The objective of this study was to evaluate genetic diversity

in *F. andiyazi* and *F. andiyazi*-like populations. Eighty strains from sorghum in Kansas (USA), Cameroon, and Uganda were analyzed. An unweighted pair group method with arithmetic mean (UPGMA) analysis of 56 amplified fragment polymorphism (AFLP) markers separated the strains into three clusters. The clusters were not based on geographic origin of the strains. Cluster 1 had 53 strains that grouped together with *F. andiyazi* tester strains. Cluster 2 and 3 contained 13 and 14 strains, respectively. Furthermore, STRUCTURE differentiated the 80 strains into two subpopulations. Subpopulation 1 consisted of all the 53 *F. andiyazi* strains, while subpopulation 2 was represented by the 27 strains in clusters 2 and 3. These results indicate the presence of at least one and possibly two groups that represent sister taxa of *F. andiyazi* that have not yet been adequately described and defined. More work is needed to further characterize these strains of *F. andiyazi*, their relationships to each other and other species in the genus, and to understand their role in pathogenicity of sorghum and other potential hosts.

Paper#: SICNA2015-SOC8

Grain sorghum response to water supply and environment

Broeckelman, J.

Irrigation water is becoming limited in many semi-arid crop production areas. With the limiting factor of available water to the crop, there arises a need to maximize the water use efficiency (WUE) from the yield and water use (ET) of the crop. Therefore, this project studies the response of four high yielding grain sorghum hybrids (Pioneer 85Y40, Pioneer 84G62, Pioneer 87P06, and Dekalb 53-67) under different irrigation levels at four different locations and environments across Kansas (Silver Lake, Scandia, Hutchinson, and Tribune). The previous crop yield response to water supply was in locations with a maximum crop ET of 530mm. Thus, there arises a need to see what the yield response curve and WUE look like beyond the well-recognized sorghum response curve by Loyd Stone. Across our different locations, we had a maximum ET of 760 mm of water use at Silver Lake fully irrigated down to 370 mm at Tribune dryland with varying ET values in between. The range of yields corresponding to the high and low ET levels were between 11.1 Mg/ha and 6.10 Mg/ha. From plotting the points of yield verses water use, it will be better understood at what point of available water from rainfall and available irrigation would it be better or equally good to plant grain sorghum rather than corn or soybeans.

Paper#: SICNA2015-SOC12

Mechanism of atrazine resistance in Palmer Amaranth population from Kansas

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Palmer amaranth is one of the aggressive, troublesome and economically damaging weeds in several cropping systems particularly, in grain sorghum across United States. Sorghum growers heavily depend on use of atrazine (PS-II-inhibitor) to control broad spectrum of weeds including Palmer amaranth. Extensive use of atrazine in corn and sorghum resulted in evolution of resistance to this herbicide in Palmer amaranth. However the underlying mechanism of resistance is unknown. The objective of this study was to investigate the mechanism of atrazine resistance in Palmer amaranth population from Kansas using a known sensitive Palmer amaranth population as reference. The dose response analysis revealed that resistant Palmer amaranth

population survived six times the field rate of atrazine whereas susceptible population was killed with very low rates (70 times less than field rate). Molecular basis of resistance was determined by sequencing and analyzing the *PsbA* gene from both resistant and sensitive plants. Interestingly, the most common mutation (Serine 264 Glycine) associated with atrazine resistance in weeds was not found in these Palmer amaranth populations. This result suggests that the atrazine resistance is possibly mediated by metabolism of this herbicide via glutathione S-transferases (GSTs) activity. Experiments are in progress to determine the role of GSTs in atrazine resistance in Palmer amaranth. Metabolism-based resistance to herbicides may confer resistances to several other classes of herbicides, thereby limiting herbicide options for weed control and sustainable agricultural production.

Paper#: SICNA2015-SOC14

Evaluation of Queensland and Texas grain sorghum germplasm in hybrid combination

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Texas A&M University

Plant breeders consistently evaluate germplasm for new allele combinations to improve traits of importance. In hybrid crops, hybrids produced using germplasm from two distinct breeding programs may provide a direct opportunity for development of such combinations. In hopes of finding superior inbred lines for hybrid combinations A-lines from the DPI/University of Queensland sorghum program in Warwick QLD, Australia were hybridized with R-lines from the Texas A&M University Sorghum breeding program. A set of 64 hybrids was evaluated in replicated trials in College Station and Monte Alto, Texas. Among these hybrids, at least eight were numerically higher in grain yield than existing checks and all were in the top yielding statistical grouping. These results indicate that combinations of Queensland/ Texas germplasm are unique and productive hybrids for Texas grain sorghum production environments.

Paper#: SICNA2015-SOC21

Genetic improvement of *Sorghum bicolor* (L. Moench) for resistance to sorghum anthracnose (*Colletotrichum sublineolum*) in Burkina Faso

Soutonnoma, N.Z.

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Sorghum bicolor (L.) Moench is a staple food and feed crop in Burkina Faso. Several high yielding varieties have been released by the breeding program in the country to boost sorghum yield. Unfortunately sorghum improvement was focus on high yield without taking into account resistance to biotic stresses as sorghum anthracnose disease. Indeed, sorghum anthracnose, due to *Colletotrichum sublineolum*, is an economically important fungal disease. It contributes to lower yield, up to 50% in susceptible varieties. The fungus affects the whole crop (stalk, leaves, inflorescence and grains) and its high number of biotypes makes its control very difficult through conventional breeding methods. It is well known that host resistance is the most effective control method to anthracnose diseases and resistant's hosts to anthracnose disease have proven their effectiveness. The assumptions of this study were (i) anthracnose biotypes have different level of virulence, (ii) at least one genotype will express a broad sense resistance, (iii) at least three quantitatively trait loci (QTLs) confer resistance to anthracnose. This study aims were to identify at least one broad sense resistance genotype in screen house and field conditions; to

develop promising lines resistant to anthracnose and to develop molecular markers linked to resistant QTLs. The results have shown difference on genotypes susceptibility, only two genotypes have shown high resistance and across all the three most virulent biotypes. Promising progeny were developed. From these progeny elite varieties could derive carrying both resistance and yield traits that will meet farmers' preferences.

Paper#: SICNA2015-SOC22

Biomechanics of bioenergy in sorghum [*Sorghum bicolor* (L.) Moench]

Gomez, F.

Stem lodging is a complex and a limiting factor on bioenergy sorghum yield worldwide. Stem lodging is defined as mechanical failure at the stem caused by external forces due to wind or rain. Current lodging ratings are frequently unreliable due to the various factors that cause lodging and there is uncertainty about which factors are responsible. Temporal and spatial unpredictability has also hindered progress on the systematic research on this issue. As a result, stem lodging resistance is considered as one of the highest priorities for a bioenergy sorghum breeding program. In this study, two experiments were designed to take a biomechanical approach by using a three-point bending test to quantify the biomechanical properties (i.e. Strength, E-Young's Modulus, and Flexural Stiffness) of several bioenergy sorghum genotypes and relate them to important geometric traits as well as their lodging ratings. In the first experiment the 3PBT was able to detect significant differences among six genotypes and their stems. In the second experiment the 3PBT was applied to 15 bioenergy sorghum genotypes with different lodging ratings and maturity levels at three environment in Texas. The repeatability for the traits measured ranged from (0.53-0.9), and significant genetic variation was identified for the 15 bioenergy sorghum genotypes that may allow to identify quantitative trait loci (QTL) related to these lodging related traits. Importantly, it was found that plan height, internode length, strength, volume, and flexural stiffness particular important traits that may aid in improving lodging resistant cultivars in a bioenergy sorghum breeding program. Interestingly, the genotype R. SOR2014 was found to be the strongest, stiffest, and most flexural genotype which may infer lodging resistance potential, and to its other sorghum derivatives.

Paper#: SICNA2015-38

Polymeric sorghum proteins and compositional relationships to protein digestibility

Ioerger, B.P., Bean, S.R., and Adrianos, S.L.

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The goal of this research was the application of a new extraction method designed to optimize recovery of intact polymeric proteins, thereby allowing determination of the specific polymers involved with and influencing *in-vitro* pepsin digestibility (IVPD). From a highly diverse 337 member non-tannin association mapping panel, a 27 sample subset was randomly selected to span a range of IVPD values. Subset IVPD values averaged 59.88%, and ranged from 44.32% to 79.27%. Several additional grain traits investigated for potential impact on IVPD included total protein, total phenolic content, phytic acid content, kernel hardness index, kernel weight, and kernel diameter, but none were found to significantly correlate to IVPD. An extraction method designed to obtain three fractions containing intact protein polymers was applied to the subset. Acquired fractions were analyzed by size exclusion chromatography (SEC) in reduced and unreduced form. Molecular weights (M_w) of SEC separated protein peaks were compared to M_w

standards, and ranged from <14.3kD to >669kD. Total SEC peak areas as well as the area for individual SEC peaks within each fraction were compared to IVPD values. Total peak areas for fraction 1 and 2 (F1, F2) did not correlate to IVPD. Protein polymers represented by individual SEC fraction peaks from F1 and F2, with one exception, were also not significantly correlated to IVPD. However, total peak areas of extracted fraction 3 (F3) were significantly correlated to IVPD ($r = -0.605$ at $p \leq 0.05$). Additionally, four of five observed individual SEC peaks from separations of F3 were significantly correlated to IVPD ($r = -0.465$ to -0.575 at $p \leq 0.05$). This work expands on previous research by focusing on polymeric proteins within sorghum grain found to significantly influence digestibility.

Paper#: SICNA2015-39

Improved method for extracting sorghum protein polymers for characterization by size exclusion chromatography

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A method for fractionating sorghum proteins using extraction solvents and techniques designed to obtain intact protein polymers was developed. Extraction and separation conditions were optimized in terms of completeness of protein extraction, sample stability, and analytical resolution. After removal of albumins and globulins, a 3-step sequential procedure involving no reducing agents was applied to ground whole sorghum flour. The three fractions represented proportionally different protein polymer contents as evidenced by comparative size exclusion chromatography. Extract stability was acquired for all fractions by the application of 80 °C heat for 2min. Both silica and polymer based columns were evaluated for separating polymeric sorghum proteins. The best resolution within all fractions was achieved with a silica based column with a nominal molecular weight range of 5kD – 700kD. Complete separation of all extracted proteins typically required 30min or less. Application of this extraction method to sorghum varieties varying in protein digestibility provides a useful tool for polymeric protein content comparison, and will help provide additional insight into how sorghum protein structure relates to digestibility.

Paper#: SICNA2015-40

Association mapping of early-season cold tolerance in sorghum [*Sorghum bicolor* (L.) Moench]

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Cold temperature is one of the most important abiotic stresses affecting sorghum production in temperate regions. It reduces seed germination, seedling emergence and vigor thus limiting the production of the crop both temporally and spatially. The objectives of this study were (1) to identify new sources of cold tolerance in low tannin or tannin free backgrounds for use in breeding programs, and (2) to identify molecular markers associated with early-season cold tolerance. A total of 136 sorghum accessions from cooler regions of the world were phenotyped for seedling emergence, vigor, height and dry weight through early planting in two environments. The accessions were genotyped using 67 simple sequence repeats (SSR) markers representing all ten linkage groups of sorghum, of which 50 highly polymorphic markers were used in the analysis. Association mapping was performed using general linear model (GLM) and mixed linear model (MLM). Several accessions distributed in all subpopulations showed either

better or comparable level of tolerance to the standard cold tolerance source, Shan qui red (SQR). STRUCTURE, neighbor-joining tree and principal component analyses identified four subpopulations in the entire population. Six marker-trait associations were identified, of which Xtxp34, Xtxp88 and Xtxp319 markers were associated with seedling emergence, Xtxp211 and Xtxp304 with seedling dry weight, and Xtxp20 with seedling height. These markers were detected on chromosomes previously found to harbor QTLs associated with cold tolerance in sorghum. Once validated they may serve as genomic tools in marker-assisted breeding and to screen larger pool of genotypes to identify additional sources of cold tolerance.

Paper#: SICNA2015-41

Population genomics of pearl millet (*Pennisetum glaucum* (L.) R. Br): comparative analysis of global accessions and Senegalese landraces

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Pearl millet is a staple food for people who live in arid and semi-arid climates regions in Africa and southern Asia due to its high drought tolerance and nutritional qualities. A better understanding of the genomic diversity and population structure of pearl millet germplasm is needed to support germplasm utilization and genetic improvement of this crop. We identified 83,875 single nucleotide polymorphisms (SNPs) in 500 pearl millet accessions, including 252 global accessions mainly from Africa, southern Asia, and the Americas and 248 Senegalese landraces from multiple agroecological zones in Senegal, using genotyping by sequencing (GBS) of *PstI-MspI* reduced representation libraries and those were used to characterize genomic diversity and population structure among the accessions. The Senegalese landraces had the highest levels of genetic diversity (π), while accessions from southern Africa and Asia show lower diversity levels. Principal component analyses and ancestry estimation indicated clear population structure among global regions, and between Senegalese landraces and the global accessions. In contrast, little population structure was observed across in the Senegalese landraces collections. Faster linkage disequilibrium decay was observed in Senegalese landraces than that in global accessions. Comparative genomic analysis of pearl millet with foxtail millet (*Setaria italica*) and sorghum (*Sorghum bicolor*) indicated extensive regions of synteny between of them, as well as some large-scale rearrangements in pearl millet genome. This study generated a good genomic resource for pearl millet genomic selection breeding and the result will facilitate improvement of these climate-resilient crops.

Paper#: SICNA2015-42

How legume cover crops and tillage affect sweet sorghum biofuel productivity

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Sweet sorghum (*Sorghum bicolor* (L.) Moench) is recognized throughout the world as a highly promising biomass energy crop due to its high yield of sugar enriched juice and lignocellulosic biomass. Optimization of production systems and management practices for sweet sorghum cultivation for biofuel have not been fully developed for the USA. Sporadic research efforts during recent decades have provided some insights into the production of sweet sorghum, primarily for fermentable sugar production. This study was conducted to evaluate nitrogen sources such as commercial nitrogen fertilizer, crimson clover (*Trifolium incarnatum*) or hairy vetch (*Vicia villosa*), and seedbed preparation on sweet sorghum production and soil characteristics. The experiment was conducted as a randomized complete block design with a factorial arrangement. Field plots were located at northern and southern regions in Louisiana for three growing seasons. Data of soil characteristics, biomass composition, juice composition, and sugar yield were collected. Statistical analysis was completed using SAS (ver. 9.2). Seedbed preparation (SP), different nitrogen sources (NS), and interaction between SP and NS were considered as fixed effects.

Section II: Presentations; list of sponsors

Keynote Presentation: Dr. Graeme Hammer

“Improving Sorghum Adaptation to Drought and Heat”

http://sicna.net/docs/2015SICNA_Hammer.pdf

Keynote Presentation: Dr. Tim Dalton

“The Global sorghum Economy”

http://sicna.net/docs/2015SICNA_Dalton.pdf

Session 1: Challenges from sorghum trait identification to technology transfer (Agronomy and Physiology)

Speakers: Drs. Igancio Ciampitti & Brian Arnall

“Challenges for Sorghum Technology Transfer: A View from the Public Sector”

http://sicna.net/docs/2015SICNA_Ciampitti_Arnall.pdf

Speaker: Dr. P. V. Vara Prasad

**“Physiological Responses of Grain Sorghum to High Temperature and Drought Stress:
Understanding, Opportunities and Challenges**

http://sicna.net/docs/2015SICNA_Prasad.pdf

Speaker: Dr. Mithila Jugulam

**“Investigating Opportunities and Addressing Challenges to Improve Options for Weed
control in Sorghum”**

http://sicna.net/docs/2015SICNA_Jugulam.pdf

Session 2: Novel phenotyping approaches to accelerate plant breeding and gene discovery (Breeding and Genetics)

Speaker: Dr. Wilfred Vermerris

**“A perspective on Novel Phenotyping Approaches to Accelerate Plant Breeding and Gene
Discovery”**

http://sicna.net/docs/2015SICNA_Vermerris.pdf

Speaker: Dr. Courtney Jahn

**“Identification of Physiological, Morphological and Metabolic Traits to Enhance Biomass
Accumulation and Drought Response in Sorghum”**

http://sicna.net/docs/2015SICNA_Jahn.pdf

Session 3: The challenge of sugarcane aphid and sooty mold in sorghum production (Entomology & Plant Pathology)

Speakers: Drs Deanna Funnell-Harris and Scott Armstrong

“Research Challenges in Sooty Mold and the Sugarcane Aphids”

http://sicna.net/docs/2015SICNA_Funnell_Harris.pdf

Speaker: Dr. Karen Harris-Schultz

“Aphid Resistance in Sorghum and a Host Range Study”

http://sicna.net/docs/2015SICNA_Harris_Schultz.pdf

Speaker: Dr. Raul Medina

“Genetic Characterization of an Emerging Aphid Pest in Sorghum”

http://sicna.net/docs/2015SICNA_Medina.pdf

Session 4: Grain sorghum: More than just an alternative to corn thanks to unique chemical components and unique applications (Chemistry and Utilization)

Speaker: Dr. Robert Moreau

“Grain Sorghum: More than just an Alternative to Corn”

http://sicna.net/docs/2015SICNA_Moreau.pdf

Speaker: Dr. Joseph Awika

“Sorghum Polyphenols as Bioactive and Functional Food Ingredients”

http://sicna.net/docs/2015SICNA_Awika.pdf

Speaker: Dr. Fadi Aramouni

“Challenges Facing Food Product Development with Sorghum”

http://sicna.net/docs/2015SICNA_Aramouni.pdf

Discussion Panel:

Speaker: Mr Earl Roemer

“Industry Perspective on Food Product Development”

http://sicna.net/docs/2015SICNA_Roemer_Zimmerman.pdf

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