Proceedings of the 2013 Sorghum Improvement Conference of North America (SICNA) Meeting

Theme: *"BRIDGING RESEARCH GAPS BETWEEN PUBLIC AND PRIVATE SECTORS"*

Venues: CSRL, USDA-ARS & International Cultural Center, Texas Tech University

Aug 28-30, 2013, Lubbock, TX

INTRODUCTION

The 2013 Sorghum Improvement Conference of North America (SICNA) meeting was held at the International Cultural Center, Texas Tech University, Lubbock, TX from Aug. 28-30, 2013. The meeting attracted a large group of participants from a wide spectrum of the sorghum research community, representing both public and private sectors. It was a huge success in bridging the gaps between public and private sectors by building networking capacities and in fostering strong interest in high quality research in sorghum among participants. A highlight of the meeting was the presentation given by the keynote speaker, Dr. Daphne Preuss, Chief Executive Officer, Chromatin Inc., on championing sorghum as a resilient and smart crop for an ever-changing landscape of US agriculture and on challenging participants to continue to advocate for sorghum and sorghum research.

You will find here the "Proceedings of the 2013 SICNA Meeting" which include the abstracts of oral and poster presentations plus access to select Power Point slides given by the invited speakers in each of the research disciplines covered in the meeting. You will 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 following the format from Crop Science (this will vary based on journal format/requirements) as follows:

Burke, J. J. 2013. Screening germplasm collections for water-deficit and low temperature stress tolerant sorghum using physiological analyses. Proceedings of the 2013 SICNA Meeting, Lubbock, TX, Aug. 28-30, 2013 (eds., G. B. Burow, J. J. Burke, C. Trostle), p7.

Thank you and we hope you will find this resource beneficial.

Editors

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Contents

Section I: Abstracts from Scientific Sessions, Student Competition and Posters

Agronomy & Physiology

Paper#: SICNA2013-AP1

Screening Germplasm Collections for Water-Deficit and Low Temperature Stress Tolerant Sorghum using Physiological Analyses

Dr. John J. Burke, Cropping Systems Research Laboratory, USDA-ARS, Lubbock, TX 79415; email: john.burke@ars.usda.gov

Sorghum [*Sorghum bicolor* (L.) Moench] is a versatile crop suitable for food, feed, and bioenergy. Although sorghum has vast collections of germplasm, most have not been tapped for sorghum improvement. We have begun development of sorghum lines that possess superior tolerance to abiotic stresses. The correlation between Dhurrin levels and the level of pre- or post-flowering drought tolerance has allowed us to rapidly evaluate germplasm collections. Novel sources of post-flowering drought tolerance have been selected, and photoperiod-sensitive lines have been converted into photoperiod-insensitive lines. New sources of cold-tolerance have been identified from the Ethiopian collection. Additionally, we discovered that roughly half of our Ethiopian lines selected for post-flowering drought tolerance, also exhibit early season cold tolerance.

http://sicna.net/docs/2013_SICNA_Burke.ppt

Paper#: SICNA2013-AP2

Canopy Temperature: A potential Trait in Selection for Drought Tolerance in Grain Sorghum

Drs. Raymond Mutava and V. V. Prasad, Dept. of Agronomy, Kansas State Univ., Manhattan, KS 66506; email: mutavar@missouri.edu

Sorghum (*Sorghum bicolor* (L.) Moench), a C4 grass and a close relative of corn, is the fifth most economically important cereal crop grown worldwide behind wheat, rice, maize, and barley. Sorghum can grow in rainy regions as well as semi-arid areas making it an important crop in areas too dry for corn production. Even though the world collections of sorghum contain over 37,000 accessions, the genetic base currently used in breeding programs is very small (about 13%). Thus, it is important to evaluate the existing germplasm for traits that can be used in breeding for drought tolerance. Selection for drought tolerance in sorghum has mainly been on the basis of the staygreen trait. But this does not necessarily mean that staygreen lines will perform well under increased VPD conditions and gives no indications of conservative water use. The slow wilting trait enables a plant to use water conservatively by imposing a

limitation in its transpiration rate. This is a drought tolerance mechanism that results in a warmer canopy due to reduced transpirational cooling and water availability for an extended period during the seed filling stages in environments with limited moisture. Other plants will escape drought/heat stress through increased transpirational cooling that will result in cooler canopies. The hypotheses for this research were that (i) canopy temperature can be used as a screening tool for drought tolerance in sorghum, (ii) grain sorghum has the slow wilting trait as a drought coping mechanisms and (iii) genotypes with the slow wilting trait will be high in transpiration efficiency (TE). The general objectives for this research were to (i) screen the sorghum diversity panel for physiological traits associated with drought tolerance, (ii) quantify variation in canopy temperature and canopy temperature depression using infrared (IR) sensors in sorghum genotypes under field conditions, (iii) evaluate slow wilting genotypes for TE under controlled environment. Studies were conducted (2006 to 2011) in the field and greenhouses and our results show that the slow wilting trait exists in sorghum, genotypes selected for the slow wilting trait were high in TE (> 5.7 g DM $kg^{-1}$of water used) and that IR sensors can be used to quantify variation in canopy temperature and canopy temperature depression under field conditions.

http://sicna.net/docs/2013_SICNA_Mutava.pptx

Paper#: SICNA2013-AP3

Energy Sorghums Research in Oklahoma

Dr.Vijaya Gopal Kakani, Dept. of Plant and Soil Sciences, Oklahoma State Univ., Stillwater, OK 74078; email: v.g.kakani@okstate.edu

Sorghum is a known high-energy, drought tolerant crop with the ability to adapt to various climates and soil conditions. Energy sorghums are amenable to both lignocellulosic and sugar platforms for biofuel production in Oklahoma. Research on energy sorghums in Oklahoma includes both high biomass sorghum (HBS) and sweet sorghum (SS). Current energy sorghum research program was initiated through the Oklahoma Bioenergy Center and is continued through the Sungrant and USDA-BRDi funded projects. The presentation will highlight research activities in OK including germplasm screening, development of best management practices, abiotic stress responses, biofuel production and life cycle analysis.

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Biotechnology

Paper#: SICNA2013-B1

Lignin Modification to Improve Sorghum Biomass for Bioenergy Uses

Dr. Scott Sattler, Grain, Forage and Bioenergy Research Unit, USDA-ARS, Keim Hall 137 , East Campus, Univ. of Nebraska-Lincoln, Lincoln, NE 68583-0937; email: scott.sattler@ars.usda.gov

Modifying lignin content and composition are major targets for bioenergy feedstock improvement. Sorghum is currently being developed as a dedicated bio-energy feedstock. Our goals are to improve sorghum biomass for both biochemical and thermal bioenergy conversion by developing experimental lines that have altered lignin content and composition beyond normal levels and to understand how these changes impact conversion technologies and plant fitness. To reduce lignin content and alter lignin composition, *brown midrib* (*bmr*) mutants, generated through chemical mutagenesis, are being utilized. *bmr6* and *12* sorghum lines have been shown to significantly increase ethanol conversion through saccharification and fermentation. To increase lignin content for thermal bioenergy conversion, a series of transgenic lines overexpressing phenylpropanoid biosynthetic genes and a pathway regulatory gene are being developed. We are also developing a range of techniques to understand how changes in lignin biosynthesis affect gene expression, protein accumulation, enzymatic activity and metabolism. In addition, lignin has long been implicated as playing a critical role in plant defense responses against pathogens and insect pests in several plant species. Thus, we are examining whether the lignin-modified lines being developed have altered responses to key sorghum insect pests and fungal pathogens. Our preliminary findings indicated *bmr6* and *bmr12* plants do not have increased susceptibility to the pests and pathogens tested, and in some instances show increased resistance. The overall goal is to modify lignin for bioenergy without sacrificing plant fitness.

Paper#: SICNA2013-B2

New Resources and Strategies for Genome-Wide Mapping in Sorghum

Dr. Geoff Morris, Dept. of Agronomy, Kansas State Univ., Manhattan, KS 66506; email: morrisgp@mailbox.sc.edu

The application of high-throughput genotyping-by-sequencing (GBS) to diverse germplasm and mapping populations in sorghum has vastly increased the opportunities for trait dissection and molecular breeding. Here we describe a genome-wide map of single-nucleotide polymorphisms (SNPs), currently covering >5,500 accessions and mapping lines genotyped at >700,000 SNPs, and provide examples of its use in genome-wide association studies (GWAS) and biparental linkage mapping. We summarize lessons learned from the first generation of sorghum GWAS and outline

experimental design strategies for high-powered mapping studies, which take into account the complex genetic history and population structure of this widely-adapted crop. Finally, we will provide an update on the development of nested association mapping (NAM) populations that combine some advantages of biparental linkage populations and diverse association populations.

http://sicna.net/docs/2013_SICNA_GMorris.pptx

Paper#: SICNA2013-B3

Strategies for Mitigation of Anthracnose Blight in Sorghum Natural Defense Through the Use of Phytoalexins

Dr. Surinder Chopra, Coordinator : Agronomy Program, Plant Science Dept., Pennsylvania State Univ., University Park, PA 16802; email: sic3@psu.edu

The 3-deoxyanthocyanidin phytoalexins are produced in sorghum leaves in response to Colletotrichum sublineolum. These flavonoid compounds have chemical structure similarities to the 3-deoxyflavonoids that are precursors of reddish brown phlobaphene pigments. Phlobaphenes are commonly observed in the pericarp of mature sorghum grains, while synthesis of 3-deoxyanthocyanidin phytoalexins is a site-specific response to infection with Colletotrichum sublineolum. We have taken a genetic approach to investigate the overlap between the two sub-branches of flavonoid biosynthesis in sorghum that lead to phlobaphenes and 3-deoxyanthocyanidin phytoalexins. We have used an endogenous transposon element of sorghum called Candystripe1 as a tagging system. Gene(s) responsible for resistance to anthracnose will be cloned using the Candystripe1 transposon tagging system. Genetic stocks carrying a Myb transcription factor along with different alleles of structural genes have been developed to dissect the flavonoid phytoalexin pathway that plays a role in anthracnose resistance in sorghum.

http://sicna.net/docs/2013_SICNA_Chopra.ppt

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Breeding & Genetics

Paper#: SICNA2013-BG1

Nitrogen Use Efficiency in Sorghum

Dr. Ismail Dweikat, Dept. of Agronomy & Horticulture, Univ. of Nebraska-Lincoln, Lincoln, NE 68583; email: idweikat2@unl.edu

Nitrogen (N) is one of the most important nutrients limiting crop growth and yield but crop plants utilize less than 30% of N applied. A vast amount of excess N is lost through leaching and contributes to environmental pollution. There is a critical need to develop genotypes with improved nitrogen use efficiency (NUE) for input efficient, environmentally friendly sustainable agriculture. Here, we attempted to map the QTLs and find differentially expressed candidate genes using Illumina RNA-seq by comparing root tissues of sorghum genotypes, Ck60 (low NUE), BTx623 (low NUE reference), San Chi San, China17 (high NUE china lines), KS78. Composite interval mapping (CIM) identified a total of 12 QTLs for eleven low N tolerance and one NUE traits for two years. The phenotypic variation explained by individual QTLs varied from 6.88 % to 19.1%. The high accuracy and quality of this map was evidenced by relative grain yield QTLs are consisted across environments, providing useful information for understanding the genetic mechanisms of the agronomically important traits responsible for the change of NUE. RNA seq data revealed large number of differentially expressed genes involving cellular, metabolic pathways related to stress.

http://sicna.net/docs/2013_SICNA_Dweikat.ppt

Paper#: SICNA2013-BG2

Genome-wide Association Study of Resistance to Stalk-rots in Sorghum

Dr. Adedayo Adeyanju, Dept. of Agronomy, Kansas State Univ., Manhattan, KS 66502; email: adenyanju@ksu.edu

Stalk-rot caused by numerous fungal organisms is an important disease of sorghum that often leads to severe quality and yield loss each year. Here we present a genome-wide association study of stalk-rot disease resistance based on sorghum association panel that contains about 300 diverse accessions. Out of 152,624 polymorphic SNPs included in the analysis, few were associated with stalk rot resistance with some of these linked to novel candidate genes worth pursuing. Similar to the phenotypic studies conducted earlier, the current results also suggest complicated molecular mechanism for resistance against stalk-rot diseases caused by both *Macrophomina phaseolina and Fusarium thapsinum*. However, given that the resistance trait is found in multiples of backgrounds, it was clear that genome wide association study (GWAS) is a useful

approach for identifying causal genetic factors for stalk-rot resistance in larger number of accessions simultaneously.----------00000----------

Chemistry & Utilization

Paper#: SICNA2013-CU1

Use of Sorghum Bran as an Antioxidant in Meat Products

Dr. Rhonda Miller, Dept. of Animal Science, Texas A&M Univ., College Station, TX 77843-2471; email: rmiller@tamu.edu

Pre-cooked pork and poultry products represent a $6 billion industry in the US. These products are traditionally manufactured and stored as frozen product. The development of off-flavors from lipid oxidation limits the shelf-life of these products to less than 12 to 6 months. Commercial antioxidants are added to increase shelf-life, but many consumers want “natural” ingredients. We have conducted 6 research projects using powdered Sumac and Black tannin sorghum bran as a “natural” antioxidant in refrigerated beef, pork, turkey and chicken nugget products. Results have shown that Sumac and Black Tannin sorghum bran at 0.5 to 0.75% additions were stronger or similar in antioxidant properties as BHA/BHT. Also, the addition of these ingredients did not affect pH, water holding capacity, color or flavor of the final product. These results indicate that Sumac and Black Tannin sorghum bran can be used as affective antioxidants while not negatively impacting quality or processing characteristics in the final product.

http://sicna.net/docs/2013_SICNA_Miller.ppt

Paper#: SICNA2013-CU2

Improving the Use of Sorghum Distillers Grains in Beef Cattle Diets

Dr. Jim MacDonald, Dept. of Animal Science, Univ. of Nebraska-Lincoln, Lincoln, NE 68583; email: jmacdonald2@unl.edu

The use of grain sorghum for ethanol production is beneficial in semi-arid regions because of the water use efficiency characteristics of sorghum. Grain sorghum is easily utilized in ethanol plants. However, the profitability of ethanol plants is increasingly dependent on capturing value from the distiller's grains used from livestock feed. Information on the feeding value of sorghum distillers grains is limited compared to the amount of information available for corn distillers grains. Animal research trials generally suggest that the feeding value of sorghum distillers grains is lower than corn distillers grains. This could be because sorghum fiber has a less hemicellulose compared to corn fiber, thereby reducing digestibility. The adoption and value of sorghum wet distiller's grains could be improved if the digestibility of the distiller's grains were improved. Enzymes have the potential to improve fiber digestion, but are typically most effective at a neutral pH whereas the pH of distillers grains is low. Therefore, the use of a buffer to neutralize pH prior to use of an enzyme may allow for greater improvements in fiber digestion due to enzyme activity. In vitro and in situ digestibility estimates suggest that buffered enzymes solubilize neutral detergent fiber in sorghum distillers grains, increase the digestibility of dry matter, and maintain or improve the digestibility of the remaining

fiber. However, in vivo digestibility estimates suggest only a small insignificant increase in the total tract digestibility of neutral detergent fiber in vivo, and no improvement in organic matter digestibility. Inclusion of 30% distillers grains in the diets of growing cattle suggests no difference in animal performance due to the use of buffered enzymes. New novel strategies are needed to improve the digestibility of distillers grains from sorghum to maximize its feeding value.

http://sicna.net/docs/2013_SICNA_MacDonald.ppt

Paper#: SICNA2013-CU3

Processing Sweet Sorghum for a Dual Bioenergy System

Dr. Danielle Bellmer, Dept.of Biosystems Engineering, OSU, Stillwater, OK 74078; email: Danielle.bellmer@okstate.edu

Sweet sorghum is a promising bioenergy crop due to its high productivity, low input requirements and versatility. Due to a relatively short harvest window and a lack of storability of the directly fermentable sugars, a processing plant dedicated to sweet sorghum in temperate climates may be operated only seasonally. In order to maintain a more continual feedstock supply and improve economic viability, a dual feedstock process utilizing sweet sorghum and sugar beets is being investigated. In the Southern Great Plains, winter beets and sweet sorghum would complement each other well in a dual processing scenario. Ideally, the same sugar extraction process could be used for both crops. The objective of this project was to evaluate a diffusion process for extraction of sugar from sweet sorghum. Diffusion experiments were conducted using a four stage countercurrent diffusion process. Sweet sorghum stalks were chopped to coarse and fine particle sizes using a Seydelman bowl chopper. Samples of 200g were used to test the effects of process temperature and solid-to-liquid ratio on sugar extraction efficiency. Three different temperatures (60, 70, 80 ^{O}C) and three different solid-to-liquid ratios (1:0.75, 1:1 and 1:1.5) were evaluated. Contact time for each sample was 10 minutes. Results showed that solid-to-liquid ratio is critically important in the sugar extraction efficiency, and temperature seems much less important. Sugar extraction efficiencies ranged from 50-90%, with the highest extraction occurring at the lowest solid-to-liquid ratio.

http://sicna.net/docs/2013_SICNA_Bellmer.pptx

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Communication and Extension

Paper#: SICNA2013-CE1

Agricultural Apps Available for Smart Phones and Tablets

Dr. Brian Arnall, Dept. of Plant & Soil Sciences, OSU, Stillwater, OK 74078; email: b.arnall@okstate.edu

This presentation will cover the majority of the Agricultural based applications currently available for IPhones, IPads, and Androids. Most of these apps are free downloads that could make everyday life on the farm just a little bit easier. Apps will cover topics from fertilizer rates, prices, nozzle selection, and plant identification. Throughout the presentation an editors' pick will be provided with insight on some of the more popular and useful apps.

http://sicna.net/docs/2013_SICNA_Arnall.pptx

Paper#: SICNA2013-CE2

The Sorghum Headworm Calculator: A Speedy Tool for Headworm Management.

Dr. Tom A Royer[a], G.F. Backoulou[b], N.C. Elliott[b], K.L. Giles[a], B.P. McCornack[c], B.B. Pendleton[d] and M.J. Brewer[e].

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The Sorghum Headworm Calculator is an interactive decision support system for sorghum headworm management. It was designed to be easily accessible and usable. It provides users with organized information on identification, sampling, and management using images, descriptions and research-based management information. Using grower input, it can help the user calculate an economic threshold for sorghum headworms in any sorghum field, and provide the appropriate sequential sampling data form so a user can easily scout a sorghum field. A step-by-step demonstration of its use will be presented. This web-based calculator was developed with partial support from the United Sorghum Checkoff Program.

http://sicna.net/docs/2013_SICNA_Arnall.pptx

Paper#: SICNA2013-CE3

Ag Producer and Social Media

Mr. Bob Brown, PO Box 519, Panhandle, TX 79068; email: brownfarms@amaonline.com

Listening to comments, concerns and misinformation about today's agriculture while attempting to answer, educate the consumers as to the ways of today's agriculture. Agriculture has active critics via social media that let emotion drive their thinking instead of scientific fact.

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Entomology

Paper#: SICNA2013-E1

Current Status of Greenbug Biotypes in Sorghum

Dr. Scott Armstrong, USDA-ARS Wheat, Peanut and Other Field Crops Research Unit, 1301 NW St. Stillwater, OK 74075; email: scott.armstrong@ars.usda.gov

The USDA-ARS Wheat, Peanut and Other Field Crops Laboratory in Stillwater, OK currently maintains economically important greenbug, *schizaphis graminum* (Rondani) biotypes and has done so for at least 25 years. These aphids are used for wheat, *Triticum aestivum* L., barley, *Hordeum vulgar* L., and grain sorghum, *Sorghum bicolor* L. (Moench), breeding programs mostly within the United States but also abroad. There are currently ≈20 identified biotypes that are maintained by keeping them parthenogentic on susceptible barley. Those utilized most frequently in breeding programs and other biological studies are those that pose an economic threat to small grains and grain sorghum, namely B, C, E, F, G, H, I, and K. Of these, biotype E, I and K are considered those encountered in the wheat and sorghum cropping systems of the Central Great Plains. Biotype E has a notorious reputation as a wheat pest that will transition to sorghum. Biotypes I and K are recognized more as sorghum pests that will survive and cause economic damage to wheat. One of our future research objectives is too look at the transition of these greenbug biotypes within the wheat-sorghum cropping system of the Central Great Plains. This will include intrinsic rates of increase studies on wheat and sorghum hosts, preconditioning of the biotypes when reared on one host and used to infest the other, and the temporal abundance and distribution of the differing biotypes in the wheat-sorghum cropping system of the Central Great Plains.

http://sicna.net/docs/2013_SICNA_Armstrong.pptx

Paper#: SICNA2013-E2

What's New for Insect Pest Management in Louisiana?

Dr. David Kerns, LSU Ag Center, Macon Ridge Research Station, Winnsboro, Louisiana 71295; email: dkerns@agcenter.lsu.edu

Grain sorghum in Louisiana and is susceptible to a number of arthropod pests throughout the plant's vegetative and reproductive stages. The most common seed and seedling pest complex is the red imported fire ant, *Solenopsis invicta* (Buren), and the chinch bug. The red imported fire ant feeds on the endosperm of the seed or on developing root and seedling tissue which results in seedling death and reduced stand densities. Severe infestations of fire ants are more likely to occur under dry conditions. Insecticide-treated seed is the most consistent control strategy for these pests and new insecticides are being developed that will increase residual efficacy. The sorghum midge, *Stenodiplosis sorghicola* Coquillett, is the one of the most destructive insect pests of grain sorghum across the U.S and Louisiana. This pest is capable of reducing yields >90% if not managed. Uniform, early-planting and resistant varieties can dramatically reduce the reliance on insecticides; however, insecticides are effective when applications are well-timed. However, the sequential of indeterminate flowering of sorghum and short residual activity form currently available insecticides makes it difficult to protect the entire panicle without having to rely on multiple short interval insecticide applications. Identification and registration of an insecticide that would offer protection with a single well-timed application would be ideal. There are several new insecticides that may fill this niche. The sorghum webworm, *Nola sorghiella* (Ril.); *corn earworm*, and *fall armyworm* constitute a panicle-feeding complex of Lepidopteran larvae. This complex of pests is another one of the most damaging pest of grain sorghum. These pests reduce grain yield by feeding on developing seed. Several insecticides are available for these pests; however, due to resistance in all these species to pyrethroids, research is needed to identify alternative products. In 2012, field data indicated that the pyrethroid insecticides did a poor job managing corn earworms in sorghum.

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Plant Pathology

Paper#: SICNA2013-PP1

Response of Near Isogenic Sorghum Lines, Differing at the P Locus for Plant Color, to Grain Mold and Head Smut Fungi

Dr. Deanna Funnell-Harris, Grain, Forage and Bioenergy Research Unit, USDA-ARS, Keim Hall room 137 , East Campus, Univ. of Nebraska-Lincoln,, Lincoln 68583-0937; email: deanna.funnel-harris@ars.usda.gov

Leaves and stalks of many sorghum plants accumulate dark red or purple pigments upon wounding, while “tan” plants do not. Unpigmented “white” grain grown on tan plants is more desirable for food. The hypothesis tested was that these plant pigments protect grain from the panicle diseases grain mold and head smut. Near isogenic tan and purple plant color genotypes with white grain were planted at Lincoln and Ithaca, NE, and Corpus Christi, TX. Field grown grain was plated onto semiselective media to detect the presence of sorghum grain-molding genera *Alternaria, Fusarium* and *Curvularia*. Results indicated that more *Fusarium* and *Curvularia* spp. were recovered from grain grown at Corpus Christi than at Nebraska; however there was no indication that grain from purple plants was more resistant to the three fungal genera than grain from tan plants. Most fungi identified morphologically were *Alternaria alternata.* Molecular identification of *Fusarium* species using translation elongation factor 1-α gene sequences, showed that *F. thapsinum* and *F. proliferatum* infected grain at all three locations. At Corpus Christi, head smut disease incidence was assessed, which is caused by the fungus *Sporisorium reilianum*. Surprisingly, purple plants had significantly greater disease incidence than tan plants. We propose that the tan plant color lines, which are promising for development of food grade sorghums, are not more susceptible than pigmented lines to either grain mold or head smut.

Paper#: SICNA2013-PP2

Screening Exotic Sorghum Germplasm to Identify New Sources of Stalk rot Resistance

Dr. Chris Little, Dept. of Plant Pathology, KSU, Manhattan, KS 66506; email: crlittle@ksu.edu

Sorghum grain yield is affected by abiotic and biotic stresses throughout development. Field and greenhouse studies were conducted with diverse exotic germplasm accessions and adapted lines to identify potential sources of *Fusarium* stalk rot and charcoal rot resistance and tolerance. Initial screening work has shown that three exotic genotypes, IS29233, PI565174, and IS2864 showed tolerance to both diseases. A newly formulated resistance-tolerance index (IndexRT) has been tested to rank lines for disease resistance. Also, the use of a "detached leaf" assay, "rolled towel" assay, and seed imbibement trials in the greenhouse are being tested as high-throughput disease screening tools for large-scale germplasm assessment. Now, we are embarking on better ways to understand the impact of stalk rot diseases on the development of yield components in sorghum as well as whole transcriptome analysis of sorghum responses to both stalk rot pathogens, *Fusarium*

thapsinum and *Macrophomina phaseolina*. Integration of drought and disease tolerance for use in breeding programs is an emergent priority and will be discussed as well.

http://sicna.net/docs/2013_SICNA_Little.ppt

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Student Competition and Posters

Paper#: SICNA2013-SC&P-1

Growing Dryland sorghum in Clumps as a Strategy for Improving Microclimate and Grain Yield

Sushil Thapa and Bob Stewart
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Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most drought-tolerant and water-use-efficient cereals grown in semi-arid environments. When sorghum is grown under dryland conditions, variety selection, planting geometry and planting population are the key factors that determine grain yield. The objective of this study was to compare the difference in microclimate and vapor pressure deficit (VPD) between growing sorghum plants in clumps and the same number of individually spaced plants in rows. The greenhouse experiment was conducted using a nested split-plot design with equal space plating (ESP) and clump geometry followed by high and low water levels in the lid, straw and bare surfaces in three replications. The field experiment was conducted using randomized complete-block design with ESP and clump geometry in straw and bare fields in four replications. EL-USB-2^{+} data loggers were used to measure the air temperature and relative humidity (RH) within the crop canopy. Data obtained at different growth stages showed that the temperature was consistently lower and RH higher in clumps than in ESP in both the greenhouse and field experiments. Consequently, clumps were observed less water stressed compared to ESP during the hot summer days. As aridity increases, the units of transpiration required to produce a unit of dry matter increases because of higher VPD. Growing sorghum plants in clumps helped to reduce the VPD and increased the likelihood for higher grain yield under dryland conditions.

Paper#:SICNA2013-SC&P-2

QTL for Leaf Wax is a Precursor to Effective Staygreen Related Responses in Grain Setting and Filling in Sorghum

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Staygreen as a drought tolerance phonotype has been severally reported in both C3 and C4 plants. Based on screening for rate of senescence and green leaf area at maturity, four major QTLs for staygreen (stg) in sorghum have been shown to individually exhibit tolerance to post flowering drought-induced senescence. However, physiological mechanisms for this drought response have not been fully elucidated. Here we show that severe heat treatment combined with partial terminal drought treatment, elicits physiological responses that clearly partition the four main staygreen loci (Stg 1, 2, 3, 4) into three categories of drought and heat tolerance phenotypes. This may point to a unique phenological, stress-dependent association of genomic regions for staygreen and leaf wax, from flag leaf emergence to physiological maturity of grain in sorghum. Five replications of BTx642-derived near isogenic lines in the post flowering senescent Tx7000 background were planted in four greenhouse conditions in summer (2012) and spring 2013). Several data points were generated at regular intervals for transpiration, stomatal conductance, fluorescence quantum yield, leaf temperature depression, leaf total cuticular wax, and leaf spectral reflectance, from flag leaf emergence to 25 days after pollination. The isolines that exhibited lower WL from flag leaf formation to five days after pollination had higher level of embryo abortion, poor grain filling onset, rolled leaves but elevated stomatal conductance under heat treatment. More results suggest that Stg 1, 2 3 and 4 strategies interact differently with leaf wax to repackage the way plant respond to drought, heat or a combination of both stresses.

Paper#: SICNA2013-SC&P-3

Overview of the sorghum interspecific hybridization program at Texas A&M University

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A new technology for the creation of interspecific and intergeneric crosses is utilization of the mutant sorghum [*Sorghum bicolor* (L) Moench] allele *Inhibition of Alien Pollen* (*iap*). The mutant allele, when in homozygous recessive form, overcomes the fertilization barrier imposed by the wild type form of the allele. Hybridization of *S. bicolor* with divergent sorghum species is desirable in order to introgress known resistance genes to biotic stresses such as midge [*Stenodiplosis* (*Contarinia*) *sorghicola* (Coquillett)] and sorghum downy mildew [*Peronosclerospora sorghi* (Weston and Uppal) Shaw]. The introgression of traits from both sorghum and sugarcane into a new crop

has the potential to create a crop that avoids cold weather by maturing in one season and is more drought tolerant than existing sugarcane clones. In addition, a seeded sugarcane-like crop could reduce the high cost and intensive labor currently associated with planting sugarcane. While previous efforts proved unsuccessful, utilization of the *iap* allele has produced hybrids between *S. bicolor* and *S. macrospermum* Garber, *S. angustum* Blake, and *S. nitidum* (Vahl) Pers. Recombination and introgression of the *S. macrospermum* genome with the *S. bicolor* genome has been demonstrated. Intergeneric hybrids have been recovered between *S. bicolor* and sugarcane (*Saccharum* spp.). Pollen tube growth to the sorghum ovary has been observed using corn (*Zea mays*), *Pennisetum* spp., and wheat (*Triticum aestivum*). Further research is currently being conducted to determine the utility of this mutant allele and the interspecific hybrids created, as well as cloning of the gene responsible.

Paper#: SICNA2013-SC&P-4

Effects of sorghum bran fortification on phenolic profile, proanthocyanidin content, and starch digestibility of wheat flour tortillas

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Phenolic compounds from sorghum bran have been shown to have beneficial health properties. However, their interaction and stability in food matrices remain largely unknown. Phenolic profile, proanthocyanidin content, and starch digestibility of wheat flour tortillas fortified with 25% (baker's) bran from wheat and white, brown, and black sorghum were investigated. Phenolic profile and proanthocyanidin content were determined by UV-Vis Spectroscopy and HPLC. Total (TS), rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS) fractions were evaluated in tortillas using *in vitro* digestion. Addition of brown and black bran significantly increased ($P<0.05$) the total phenolic content of tortillas to 43.3 and 52.4 (mg GAE/g), respectively, compared to 9.46 (wheat bran) and 12.09 (white sorghum bran). HPLC analysis did not show changes in 3-deoxyanthocyanin profiles between bran and tortillas. Extractable proanthocyanidin content of brown sorghum bran tortillas decreased from 2,122 µg/g in dry ingredient mix to 893 µg/g in dough and 407 µg/g (db) in 14 day old tortillas. As expected, addition of bran significantly decreased TS and RDS compared to control ($P<0.05$). Bran decreased the RDS from 61% (control) to 49-51% (db). There was no significant difference in RDS among the brans. Brown sorghum bran tortillas had significantly higher SDS (11.3%) than the other brans (6.38-8.15%); this may be due to starch interaction with proanthocyanidins in brown sorghum bran. RS was not significantly affected. Brown and black sorghum bran utilization in wheat flour tortillas may positively affect the starch digestibility while increasing polyphenol content.

Paper#: SICNA2013-SC&P-5

Microwave Accelerated Reaction System Increases Extractability of Sorghum 3-deoxyanthocyanin Pigments

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Demand for natural pigments as alternatives to synthetic colorants in food continues to increase. Pigmented sorghums have high levels of relatively stable 3-deoxyanthocyanins, pigments with good potential as natural food colorants. However, the grain pigments are bound to cell wall material making them difficult to extract thus limiting their economic potential. In this study, extraction of black (AO5028/RTx3362) sorghum 3-deoxyanthocyanin pigments, was carried out in a microwave accelerated reaction system (MARS) for varying time intervals ranging from 15 seconds to 40 minutes, at three different power levels (300W, 600W, 1200W), using 1% hydrochloric acid in methanol as the extraction solvent. Controls were extracted at 20 °C for 2 hours on a laboratory shaker. UV-Vis spectroscopy was used to determine pigment concentration, and HPLC was used to obtain a profile of the extract. MARS extraction resulted in a 2 to 6-fold increase in 3-deoxyanthycyanin pigments from black sorghum (2.7-1.2 mg/g sample) compared to control (0.5 mg/g). Longer microwave radiation exposure tended to increase 3-deoxyanthycyanin extraction. Three new flavone peaks were observed by HPLC. MARS caused minimal change in the overall pigment profile of sorghum which indicates that microwave radiation did not cause denaturation of 3-deoxyanthocyanins. MARS significantly increases extractability of 3-deoxyanthocyanins in sorghum opening new economic potential for 3-deoxyanthocyanins as food colorants.

Paper#: SICNA2013-SC&P-6

Evaluation of Exotic Sorghum Germplasm for Stalk rot and Drought Tolerance

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Stalk rots are major biotic stresses of sorghum [*Sorghum bicolor* (L) Moench], while drought is the most important abiotic stress. Identification of superior lines tolerant to both stressors and incorporating them into breeding programs is important to enhance productivity. The objective of this study was to screen 38 exotic lines and 8 checks to identify sources of resistance/tolerance for both stressors. Plants established in the field were inoculated with *Macrophomina phaseolina* (MP) and *Fusarium thapsinum* (FT) at 14 days after flowering. At harvest, plants were screened for disease severity using plant height (PH), stalk diameter (SD), lesion length (LL), relative lesion length (RLL), number of diseased nodes (NC), 100-seed weight (SW) and total seed

weight per panicle (TSW). Three physiological traits: chlorophyll fluorescence (Fv/Fm), chlorophyll content (SPAD) and leaf temperature (LT) were measured at 74, 84 and 94 days after planting (DAP). Results revealed a wide variation in terms of all disease severity related traits as well as the physiological traits used as drought tolerance indicators. The effect of pathogen treatment was significant for NC, LL RLL and SW. The line and census dates at which the physiological readings were taken had highly significant effects on all physiological traits. An integrated approach was used to rank lines based upon their relative performance in terms of disease and drought tolerance as well as yield potential. Certain lines revealed extreme performances in comparison to checks. The superior lines identified in this study will be deployed as parental materials for hybrids production.

Paper#: SICNA2013- P-7

Tx3364 – Tx3407 Grain Mold Resistant Sorghum Germplasm

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The sorghum [*Sorghum bicolor* (L.) Moench] germplasm lines Tx3364 through Tx3407 were developed and released by Texas A&M AgriLife Research, Lubbock, TX, in 2013. Breeding crosses for most of these lines were made in Lubbock, Texas and growout and selection of subsequent generations were completed in breeding nurseries throughout Texas over a period of years. Once selected these lines were tested for agronomic performance in replicated trials over a period of years. Tx3364 through Tx3407 germplasm lines are resistant to grain molds caused by diverse genera that include *Fusarium* spp., *Curvularia* spp. and *Alternaria* spp. They are of diverse pedigree and parentage and represent an array of combinations for grain color, plant color, and other agronomic traits. These lines provide the sorghum industry with sources of grain mold resistance in elite genetic backgrounds for both seed and pollinator parent sorghum germplasm.

Paper#: SICNA2013 P-8

Evaluation of Sorghum for Response to Salt Stress

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Sorghum [*Sorghum bicolor* (L.) Moench] cultivars were evaluated for response to salt stress in a greenhouse experiment. The cultivars were evaluated in a saline soil media. The objectives were to develop reliable, repeatable methodology to evaluate sorghum

cultivars for salt stress and to characterize selected cultivars for response to salt stress. The seeds were germinated in soil media with moisture from a reverse osmosis system. At 15 days after germination, a saline solution was prepared by mixing 91.2 grams of salt in seven gallons of RO water and 500 ml of the solution was added to each plant every 48 hours for approximately forty days. An ultrameter was used to determine the amount of salt ($Na^{+}Cl^{-}$) dissolved in the water (total dissolve solids) and to determine the pH of the solution. Plants grown in the saline soil were assessed for salt injury day-to-day. At maturity plants were evaluated for several characteristics including seed weight, plant height, peduncle dry biomass and root development. For seed weight 'SC56-14E' and 'Desert Maize' were least affected. Plant height of Desert Maize and 'FC8917 Chiltex' was least affected by salt. Root growth of FC8917 Chiltex was least affect by salt stress with treated roots longer than the control. FC8917 Chiltex was overall the cultivar most resistant to salt stress can is a good source of salt tolerance in sorghum.

Paper#: SICNA2013-SC&P-9

Genetic Diversity and Population Structure Analysis of Sorghum Accessions with Tolerance to Early-season Cold Stress

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Cold temperature is one of the most important abiotic stresses affecting sorghum production in temperate regions. In an effort to identify new sources of tolerance, we evaluated 136 sorghum germplasm accessions collected from cooler regions of the world for tolerance to early-season cold stress. The stress was imposed through early planting and genotypic responses were scored based on emergence percentage and seedling vigor ratings. Several accessions were shown to have better or comparable level of tolerance to the known standard source, Shan qui red. We further looked at the extent of genetic diversity and the pattern of genetic relationship among the accessions. The materials were genotyped using 67 simple sequence repeats (SSR) markers of which 50 highly polymorphic markers were selected and used in the analysis. The analyses were done using PowerMarker version 3.25 and STRUCTURE version 2.2.3, respectively. A total of 307 alleles were detected with an average of 6.1 alleles per locus. The average major allele frequency was 0.08 and mean allele diversity as depicted by polymorphism information content was 0.97. STRUCTURE and neighbor-joining tree analyses identified five subpopulations which correlated well with the geographic origins of the accessions and was further confirmed by Principal Component Analysis. Many of the accessions with improved tolerance to cold stress were grouped under various subpopulations indicating that tolerance to cold stress may have evolved at different times and places in different backgrounds. Thus, we suggest that future

efforts to identify sources of cold tolerance should consider broader range of accessions from diverse geographic regions.

Paper#: SICNA2013- P-10

Effect of Sorghum Tannin Bran on Resistant Starch and Dietary Fiber Contents of Toasted Bread

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There has been a high demand for healthy foods due to increase incidence of chronic diseases. Previous studies showed that sorghum tannins interact with starch decreasing its digestibility. However, there is little information about the role of tannins on the formation of resistant starch (RS) and total dietary fiber (TDF) in foods. This study determined the effect of toasting on RS and TDF contents of breads made with sorghum tannin bran. The main ingredients were wheat flour and 14.5% of tannin sorghum bran (% bakers). Total phenols, RS and TDF were determined. The amount of extractable phenols in the tannin breads decreased after toasting from 1104.3 to 970 µg/g. This amount was significantly lower (320 µg/g) when tannin bread was kept in a cooler (4^{o}C) for 10 days and then toasted. No changes were observed in the phenol levels of control bread. This indicated that tannins bound to starch/proteins, and thus became less extractable. RS increased from 7.5 to 10.5 mg/g after toasting tannin bread and reached 16 mg/g after cooling/toasting. The highest level observed in control bread was 7.8 mg/g. TDF in toasted tannin bread was 5.8%. It reached 7% and 4.7% in tannin and control breads, respectively, after cooling/toasting. Thus, interactions between sorghum tannins with other food compounds become stronger after cooling/toasting, increasing the levels of RS and TDF in breads. This study opens opportunities for potential applications of tannin sorghum bran to produce dark and healthier foods such as breads, cookies and cereals.

Paper#:SICNA2013 P-11

Thermal stability of sorghum 3-deoxyanthocyanin pigments

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There is increased interest in natural alternatives to synthetic dyes in food products. Pigmented sorghum grains and tissues are good sources of 3-deoxyanthocyanin pigments, which are promising natural food colorants. This work aimed to establish thermal stability of sorghum 3-deoxyanthocyanins under conditions typically used in food processing. Color and structural changes were determined at 95 °C/2 hr and 121 °C/30 min, in pH 1-7 in presence of different acidulants (HCl, formic and citric acids). Color stability of sorghum pigments was generally excellent at 95 °C for up to 2 hours (79-89% color retained). After 121 °C/30 min heat treatment, the color retention of sorghum pigments (65.0-84.4%) was much better than reported for anthocyanin pigments under similar conditions (15-25%). Generally, the sorghum pigments were more stable at lower pH conditions after heat treatments. Chalcones were identified as the major heat degradation products of sorghum pigments. The 3-deoxyanthocyanins with *O*-methyl substitution tended to increase the formation of chalcones, thus affected heat stability. Slow rate of chalcone formation and resistance to C-ring fission were identified as the major mechanisms for better thermal stability of 3-deoxyanthocyanins. The identified structure-stability relationship of 3-deoxyanthocyanins would help select sorghum varieties containing relatively more heat stable pigments. The thermal stability of 3-deoxyanthocyanins indicates good potential of utilizing sorghum pigments for food use.

Paper#: SICNA2013 P-12

Boosting Sorghum Yields with Novel Productivity Traits

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Increasing sorghum productivity can increase producer profitability and enhance the position of sorghum in agriculture. Yield of competitive sorghum hybrids is related to biomass productivity. Novel biomass productivity traits have been identified and may lead to yield advance. Increased radiation use efficiency: Productivity of forage-type sorghum can be similar to high-yielding corn, yet some breeding lines have 33% lower productivity. Upright leaves are expected to increase radiation use efficiency of commercial-height sorghum. We've identified over 80 sources of the upright leaf trait. Limited transpiration: Sorghum lines with limited transpiration don't use as much water under hot, dry conditions. Others have identified 7 breeding lines and 8 Sorghum Conversion lines with limited transpiration. Chilling tolerance for photosynthesis: Cool growing conditions challenges early-planted sorghum. Our previous studies have identified 9 advanced breeding lines with vigorous early growth. Heat tolerance for photosynthesis: Though sorghum is heat-adapted, temperatures above 98 F can damage leaves. Surveys of the Sorghum Diversity Panel indicated 16 lines with large grain yields and warm canopy temperatures, indicating these are potential sources of heat tolerance. The proven methods of classic crop breeding demonstrate that novel

traits can lead to yield advance. Sources of novel productivity traits can contribute to genetic gain in grain sorghum for years to come.

Paper#: SICNA2013-SC&P-13

Optimization of *Macrophomina phaseolina* Inoculum for Enhanced Disease Reactions in Sorghum.

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Charcoal rot is a devastating disease of sorghum [*Sorghum bicolor* (L) Moench] caused by *Macrophomina phaseolina* (MP). Resistance is the key for managing it in the field. Optimum inoculum potential is crucial for accurate disease reactions when screening germplasm and to avoid escapes. The objectives of this study were to assess the effect of differential blending time on the number of in vitro colony forming units (CFUs) and investigate the in vivo disease reaction of different MP inocula produced with different blending times. MP grown in PDB for five days was blended at 18000 rpm for 4 to16 minutes (treatments) in 500 ml volume. 100 µl of fragmented suspensions from each treatment were spread on PDA plates and incubated at 25°C. SC599 (resistant check) and BTX3042 (susceptible check) established in the greenhouse were inoculated with different MP treatments at 14 days after flowering. At harvest, plants were screened for disease severity reactions using lesion length (LL), relative lesion length (RLL), number of diseased nodes (NC) and 100-seed weight (SW). Results revealed a significant effect of blending time on CFUs and upon the degree of disease severity. After 4 days of incubation, significantly higher mean CFUs were recorded from the 8 min blending treatment. Inoculation with the 8 min treatment also revealed significantly increased mean LL, RLL and NC across the replicates of the two check lines. Hence, modifications of the inoculum preparation steps should be taken into account to optimize disease reactions when screening for charcoal rot.

Paper#: SICNA2013-SC&P-14

Biomass and Cellulosic Ethanol Production of Forage Sorghum under Limited Water Conditions

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Forage sorghum is one of the suggested crops to provide feedstock for biofuel production under water-limited conditions due to its stress tolerance and efficient water use; however, research is needed under these conditions to better understand its energy yield potential. This study presents results from a two year evaluation of biomass and potential cellulosic ethanol (EtOH) production potential of forage sorghum cultivars differing in brown midrib trait (*bmr*) under dryland (no irrigation) and limited irrigation (2.88 mm day^{-1}; subsurface drip) in the semiarid Southern High Plains of the U.S. Commercial cultivar Sorghum Partners 1990 (SP1990, conventional non-*bmr*) produced significantly more biomass (29-62%) than a *bmr*12 cultivar PaceSetter *bmr* (PS *bmr*) under irrigated and dryland conditions during both years of this study. However, PS *bmr* biomass had higher cellulosic EtOH conversion efficiency than SP 1990 in both years according to simultaneous saccharification and fermentation analysis. Irrigation resulted in 26-49% more biomass and 28-72% more cellulosic EtOH production during both growing seasons, indicating that limited irrigation had favorable effects on both biomass and biofuel production. In the first year, when precipitation was below average, both cultivars produced similar amounts of cellulosic EtOH. During the second year, when precipitation was above average, higher biomass production of SP 1990 resulted in 28% higher cellulosic EtOH production than PS *bmr* when averaged across both irrigated and dryland. The large range of cellulosic EtOH production (1,600 to 3,380 L ha^{-1}) during the two years of this study was primarily driven by differences in water availability that resulted from precipitation and irrigation. This study indicates that chemical composition and biomass yield potential of sorghum cultivars are critical factors that affect biomass and biofuel production under limited water conditions.

Paper#:SICNA2013-P-15

Early changes due to sorghum biofuel cropping systems in soil microbial communities and metabolic functioning

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Evaluation of biofuel production cropping systems should address not only energy yields but also the impacts on soil attributes are important for long-term sustainability. In this study, forage sorghum (*Sorghum bicolor* L. Moench) cropping

systems were initiated on a low organic matter soil (< 0.9%) with a history of intensively-tilled low-input cotton production in the semiarid Southern High Plains of the U.S. Sorghum cropping systems were evaluated in a split-plot design with sorghum cultivar as the main plot and the combination of irrigation level (non-irrigated and deficit irrigated) and aboveground biomass removal rate (50% and 100%) as the split plot. The sorghum cultivars used varied in yield potential and lignin content, which are important features for feedstock-producing crops. Within one year, the transition from long-term cotton cropping systems to sorghum biofuel cropping systems resulted in increased soil microbial biomass C (16%) and N (17%) and shifts in the microbial community composition as indicated by differences in fatty acid methyl ester (FAME) profiles. Additionally, enzyme activities targeting C, N, P and S cycles increased 15-75% (depending on enzyme) after two growing seasons. Increased enzyme activities (16-19%) and differences in FAME profiles were seen due to irrigation regardless of aboveground biomass removal rate, which may be due to an increase in belowground biomass production even with limited irrigation. Biomass removal rate and the cultivar type had little effect on the soil microbial properties during the time frame of this study. Early results from this study suggest improvements in soil quality and the sustainability of sorghum biofuel cropping for low organic matter agricultural soils.

Paper#: SICNA2013- P-16

Enhancement of Cold Tolerance in Sorghums

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Sorghum is a warm season crop that originated from the hot and dry regions of Africa and generally lacks cold tolerance during germination and reproductive stages. Thus sorghum is conventionally planted later in the season preventing farmers from utilizing spring moisture, disposing the crop to competitive disadvantages with other cereal/field grown agronomic crops and compressing growing season for realizing higher yield potential of cultivars. This project focuses on screening and characterization of resilient untapped genetic diversity for cold tolerance in sorghum, conducting pre-breeding and genetic studies to develop cold tolerant germplasm that will support the requirements of the sorghum industry/ producers for excellent stand establishment at an earlier period of sowing and for longer growth duration with reproductive stage tolerance

to cool conditions. A key accomplishment of this project include the development of 3 recombinant inbred (RI) population resources and assemblage of top performing lines concurrent with distribution of these new germplasm to sorghum research community. Additionally, the completion of genetic and phenotypic characterization of the BTx623xPI567946, one of the 3 strategic populations developed for combining germinability and seedling vigor under cool (54-56 F) soil condition]) was achieved.

Section I I: Agenda & Sponsors

http://sicna.net/docs/2013_SICNA_agenda_sponsors.pptx