

Germplasm Enhancement and Conservation



Breeding Pearl Millet with Improved Stability, Performance, and Resistance to Pests

Project ARS 101
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Introduction and Justification

Pearl millet is a staple food in the most difficult production environments of semi-arid Africa and Asia. It is used as a forage and cover crop in the U.S., Brazil, Canada, and Australia, but is also being developed for grain in these regions because of its superior water- and nutrient-use efficiency. Because of the dependability of harvests in harsh environments, and the potential for improvement, pearl millet will be a key component in the future prosperity of Africa, and will provide new economic opportunities for the U.S.

Advances can be made in production and use of pearl millet by targeting high-value and market-driven traits. In addition to increased yield, value for specific uses, such as fodder, grain for processed foods, poultry feed, or as ethanol feedstocks is needed for existing and developing markets is needed. The needs of growers must be met by facilitating crop production, and the needs of end-users must be met by providing a superior product. This project targets multiple traits including fertility restoration, staygreen, free-threshing grain, grain quality traits, and resistance to pests and diseases, including downy mildew, striga, nematodes, and grain molds.

The genetic diversity of open-pollinated varieties (OPVs) contributes to stable production in harsh environments. Early-maturing hybrids may have improved yield over the early OPVs, can increase grain availability during deficit periods, and will promote the development of a private-sector seed industry. Hybrid technology for Africa will require appropriate maintainer and restorer inbreds for the A1, A4, and A5 male sterile cytoplasm. Advancing hybrid technology for Africa will be facilitated through use of fertility restorer genetic stocks derived from African varieties.

Improving Yield and Stability through Resistance to Diseases and Pests

Genetically uniform hybrids can be more susceptible to biotic and abiotic constraints that cause low or unstable yield. The downy mildew pathogen (*Sclerospora graminicola*) has a high potential for epidemics. Multilocation screening is necessary to identify resistance that is broadly effective to diverse pathotypes. *Striga* (*Striga hermonthica*) is a serious parasite in regions where food security is lowest. Resistance provides a low-cost means of control.

Other pests contribute to chronic production problems. Nematodes are widespread in association with pearl millet. African varieties differ in resistance to root knot nematodes (*Meloidogyne* spp.), and in each variety tested, most plants were susceptible. In the U.S., susceptible pearl millets have lower grain yield, and can result in greater root damage and yield losses in subsequently grown peanut. Peanut and cowpea are grown in intercrop and rotation with pearl millet, and both legumes are severely affected by root knot nematodes in Africa. Resistant pearl millets will promote long-term sustainability of the production systems.

Grain molds are another chronic problem that can occur when crops mature before the rainy season ends. When poor rural farmers need to raise cash, highest quality grain is frequently sold into the market, and poorer quality grain is kept for on-farm consumption. Molded grain has poorer nutritional qualities, and may be contaminated by mycotoxins that are associated with cancers, and that compromise the health of individuals with HIV/AIDS or hepatitis C. Aflatoxins and fumonisins are considerably lower in pearl millet compared to corn, but other mycotoxins associated with *Fusarium* infection (such as trichothecenes and zearalenone) are common.

Improving Yield and Stability Through Tolerance to Drought and Low Soil Fertility

Drought and low soil fertility are significant abiotic constraints for pearl millet production in Africa. Drought stress during flowering through grain fill results in low and unstable yield. Staygreen is an expression of drought tolerance characterized by the retention of green leaf area at crop maturation and improved nitrogen utilization. The staygreen trait could further improve drought tolerance and nitrogen-use efficiency in pearl millet.

Improving Marketability through Value-Added and Grain Quality Traits

Manual threshing and winnowing are labor-intensive tasks primarily performed by women using a wooden mortar and pestle. Traditional threshing and winnowing techniques require 5 to 11 hours of women's labor to produce a 50 kg bag. Winnowing requires about 37% of the total time of these operations. Plant breeding may help to improve the efficiency of this post-harvest operation. A "clean threshing" inbred has recently been identified in the USDA-ARS pearl millet program. The seed does not shatter, but it is released from the glumes more easily, with a lower rate of abscission of the pedicle from the rachis. This trait may be useful in freeing up women's labor in post-harvest operations in the African setting.

Market demand is the most effective stimulus to increase pearl millet production. Quality traits that provide value to the end-user are needed. These market-driven quality traits include those valued for pearl millet-based foods, or traits for the recreational wildlife, poultry, or ethanol industries. Traits such as grain color, proximate composition, feed value, and fermentability are important criteria. The value of pearl millet in poultry rations is relevant to Africa. Pearl millet-based pre-starter rations increase chick body weights compared to a corn-based ration, and the performance and yield of broilers fed diets with up to 50% pearl millet are equal to or better than those fed typical corn-based diets. Demand for ethanol feedstocks is historically high, and pearl millet may be a useful supplemental feedstock. It ferments faster than corn, and the value of the distillers dried grains with solubles from pearl millet is greater than that from corn. Limited information exists on the differences in fermentability among pearl millet genotypes.

Objectives and Implementation Sites

Objectives

1. Improve the stability and performance of pearl millet by identifying and preserving germplasm with superior agronomic traits and resistance or tolerance to diseases, pests, and environmental stresses.

2. Enhance the production and marketability of pearl millet by improving pearl millet for yield, stability, consumer nutrition, and other market-driven quality traits.

3. Enhance the improvement of pearl millet genetic resources through the application of molecular genetic technologies.

4. Develop effective partnerships with national and international agencies, and other partners engaged in pearl millet improvement and the betterment of people who depend upon pearl millet for their livelihood.

Implementation Sites

The project will be coordinated through the USDA-ARS Crop Genetics and Breeding Research Unit at Tifton GA, and conducted with collaborators in the West and Southern Africa regions. Collaborative sites in West Africa include Maiduguri Nigeria and Kamboinse Burkina Faso. Collaborative sites in Southern Africa include Kaoma, Zambia.

Objective 1. Improve the stability and performance of pearl millet by identifying and preserving germplasm with superior agronomic traits and resistance or tolerance to diseases, pests, and environmental stresses.

Genetic Improvement of Nematode Resistant Pearl Millets

Root knot nematodes are important yield constraints in pearl millet and in peanut and cowpea, which are frequently grown in intercropping and rotations with pearl millet in Africa. Advanced inbred progeny derived from African varieties P3Kollo, Sosat-C88, Zongo, and Gwagwa were selected based upon resistance to *Meloidogyne incognita* in greenhouse evaluations. F5 progeny with greatest seed availability were distributed to collaborators in Mali, Nigeria, and Burkina Faso. Fifteen progeny from each of P3Kollo, SoSat-C-88 and Gwagwa, and ten progeny from Zongo were evaluated for downy mildew resistance and yield in two replications at each location. Downy mildew incidence and agronomic traits were recorded.

Research Results

Downy mildew incidence was significantly affected by location, variety, and entry within variety. Over all locations, downy mildew incidence was greatest at Mali (33.9%), intermediate at Burkina Faso (29.1%) and least at Nigeria (22.2%) (lsd0.05 = 6.8). Mean downy mildew incidence over all locations were Gwagwa (22.2%), P3Kollo (24.5 %), SoSat-C88 (32.8 %), and Zongo (36.9 %) (lsd0.05 = 7.9). There was a significant GxE interaction. The most striking example was that overall, the SoSat-C88 selections were most susceptible in Mali, the origin of SoSat-C88, whereas it was resistant in Nigeria. P3Kollo was more resistant in Burkina Faso and Mali than in Nigeria. Only a few inbreds were resistant across all locations, notably, entries 52 through 55, derived from Gwagwa. Two entries were susceptible across all locations; entries 31 and 32 derived from Zongo. The most common reactions appeared to be site specific reactions where resistance was effective at some locations, but not at others. Downy mildew incidence at Mali and Burkina Faso were correlated ($r=0.44$, $P<0.01$), suggesting that pathogen virulence was similar between the locations. Although not specifically designated as a variable for evaluation, some observations at several locations indicated that striga infestation was lower in the plots of pearl millet varieties with root knot nematode resistance.

Objective 3. Enhance the improvement of pearl millet genetic resources through the application of molecular genetic technologies.

A Modified Cost and Time Effective Procedure for Genotyping Pearl Millet in Resource-limited Laboratories

The need to genotype large mapping populations in pearl millet has increased for the improvement of various traits and for linkage mapping studies but the cost of production of per data unit remains a major impediment to fully harness the benefits of molecular genetic technology. We focused on reducing the cost and time for microsatellite genotyping from DNA extraction to PCR amplification to separation of PCR product using PAGE. A DNA extraction procedure was based on the premise that SSR markers require a relatively low concentration (5ng - 50ng) of average quality DNA. Post-PCR multiplexing of two or more SSR markers involving the simultaneous separation of PCR amplified products in a single gel lane requires prior information about base-pair sizes or differences between SSR primers used for PCR amplification of DNA samples. We experimented with techniques to save time and costs by eliminating some protocol steps and reducing volumes of various reagents used for DNA extraction, PCR amplification and multiplexing of PCR products during PAGE electrophoresis in resource limited laboratories.

Results

Through eliminating or changing several steps used in DNA extraction, PCR amplification and PAGE electrophoresis, we developed a modified procedure that reduced the cost of consumables and required less time without compromising data quality. In the revised procedure, DNA was extracted by incubating 0.5-0.7g ground young leaf tissue in 2% CTAB/ β -mercaptoethanol followed by refrigerated differential centrifugations with phenol:chloroform:isoamylalcohol. Steps such as additional phenol/chloroform treatments, DNA pellet drying followed by RNase treatments and incubation were eliminated, reducing use of costly and corrosive chemicals, and saving time. DNA produced from 174 genotypes exhibited an average concentration of 640ng/ μ L and average optical density ratio of 1.9. PCR amplification of SSR markers with this DNA produced clear and scorable bands following ethidium bromide stained agarose and silver stained polyacrylamide gel electrophoresis. Post PCR duplexing of two or more microsatellites based on different lengths of base pairs reduced the time and cost per unit data generation by up to half as compared to single marker per PAGE. The procedure is an intermediate between maxi- and mini-prep DNA extractions suited for resource limited laboratories engaged in molecular breeding requiring large volume of genotyping.

Objective 4. Develop effective partnerships with national and international agencies, and other partners engaged in pearl millet improvement and the betterment of people who depend upon pearl millet for their livelihood.

Development and Evaluation of a Pearl Millet Thresher

A barrier to increased commercialization of pearl millet in the African setting is the lack of improved technologies for post-harvest processing. Partnerships have been developed with Compatible Technology International, St. Paul, MN to develop effective technology for post harvest processing. Prototype devices developed by Compatible Technology International (CTI) for threshing, winnowing, and decorticating pearl millet were evaluated by the USDA-ARS at Tifton, GA. The CTI prototypes were developed from the following premises: Villages have 10 to 100 families with approximately 10 persons / family. The village would own the thresher, separator and a grinder for flour, which cost approximately \$800 (available through microloans). Families would use the village machines on an as-needed basis. Thresher mechanisms were to be developed for hand-operation. As higher capacity threshers are developed, opportunity exists to scale up capacity through the use of motors for commercial grain markets.

The processes for effective threshing consist of stripping the grain and florets from the rachis, dislodging the grain from the florets, and separating the grain from the chaff. Decortication requires removal of the seed coat from the grain. Identifying effective mechanisms to perform these operations were evaluated in stage 1 prototypes in 2008. Stage 2 prototypes that incorporated the most promising mechanisms were assessed in the current studies. After hand stripping panicles, the effectiveness of the Leary and Ewing threshers and the Wenkel separator were evaluated. Criteria considered were 1) ease of operation, 2) capacity, and 3) ability to produce clean, unbroken grains.

Results

Pearl millet florets can be hand stripped from the rachis reasonably quickly with sturdy devices. A box wrench was found to be highly effective for the process. Advantages of the Leary thresher were its comparative ease of operation and high capacity. Disadvantages included a higher frequency of cracked grain and a high level of chaff contamination in the final product. A winnowing step prior to the separation step improved the output quality by reducing residual chaff. Advantages of the Ewing thresher were its versatility, options for decortication, and the high quality of the resulting grain. Disadvantages included difficulty of feeding stripings into the device, difficulty with material discharge and cleaning after processing, and poorer performance when hand-cranked compared to the electric motor driven option. The Wenkel separator was modified by moving the hand crank to the discharge end of the shaft. Sieve screen sizes selected by CTI appear to be reasonably effective, but modifying the lengths of the sieve sections and providing an input hopper may improve the design. It will be necessary to develop shielding to prevent losses from scattering and flow controls to collect the output fractions more effectively. The Ewing device could be used as a decorticator, however, excessive grain breakage occurred with the pearl millet variety used in the evaluation. Additional evaluation is necessary to determine if breakage was due to the pearl millet variety used, or is an inherent property of the device. The Leary thresher showed some promise for processing sorghum. Modifications will be required to effectively thresh sorghum without breaking the grain. The Ewing device was ineffective for threshing sorghum. When coupled with

the other technologies in these trials, the Leary thresher could produce 50 kg of pearl millet grain in 10.9 hours, whereas the Ewing thresher with metal blades and electric motor would require 16.5 hours. It was recommended that the processing steps should be examined to determine if output can be improved to compare to the 5 to 11 woman-hours required to process 50 kg of grain by using traditional threshing and winnowing processes. If the capacity or quality achieved by the existing prototypes cannot meet this goal, additional prototype designs should be considered.

Results and recommendations from these evaluations were shared with the Battelle Institute. CTI and the Battelle Institute have developed a stage 3 prototype based on the recommendations from the ARS evaluation. This advanced prototype has eliminated the hand-stripping step, and instead has incorporated a stripping mechanism at the feeding stage. A fan winnower has been developed to eliminate the need for the separator. These two modifications should significantly increase throughput rates. The stage 3 prototype will be evaluated in Mali in December, 2009. The progress of the thresher development can be viewed at: <http://onelabinitiative.blogspot.com/>

Networking Activities

Workshops and Meetings

Presented “Post-Harvest Processing Technology for Pearl Millet and Developmental Needs” to Lemelson-MIT InvenTeam, Bridgewater NJ (via distance seminar technology) 9 Jan 09

Presented “Application of the Brazilian Pearl Millet Model to the U.S.” at the Pearl Millet Consortium meeting, Ft. Valley St. University, Ft. Valley. 27 February 09

Presented “Progress in Pearl Millet Improvement and the Importance of Genetic Resources. USDA-ARS Plant Genetic Resources Conservation Unit, Griffin, GA. 5 May 09

Presented “Progress and Priorities in Pearl Millet Improvement” at the UGA Institute of Plant Breeding, Genetics, and Genomics retreat. Griffin GA. 2 Jun 09

Presented “No-Till Production and Niche Marketing of Pearl Millet” Rillington Fields, Tifton GA. 4 Sep 09 (Invited SARE field day presentation)

Research Information Exchange.

Consulted by Compatible Technology International (CTI), St. Paul, MN and the Battelle Institute to assess pearl millet threshing and winnowing prototypes and deployment for evaluations in sub-Saharan Africa. Nov 08 – Jul 09

Provided pearl millet diseases images requested by the Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Taiwan, for use in quarantine data base for BAPHIC staff training and reference. 1 Dec 08.

Consulted by USDA-APHIS on disease implications of pearl millet illegally imported from Pakistan via Canada and being sold in Indian food markets in the U.S. 4 Dec 08

Consulted by Semetes Adriana (Brazil) 3 times to diagnose foliar and stalk rot diseases of pearl millet and for control recommendations Feb-Sep 09

Consulted by Invasives.org and the Bugwood Network to correct taxonomic problems with pearl millet and yellow foxtail. Nomenclature problems caused the crop to be classified in national databases as a noxious and invasive weed in subject to regulatory action. Mar 09.

Consulted by Plantation Seed Conditioners to diagnose pearl millet cropping problem and to recommend control measures in a 1000 acre planting. Newton, GA. 3 Jun 09

Consulted by Tommy Dollar of First United Ethanol LLC (Camilla GA) for information on ethanol production and DDGS quality from pearl millet feedstocks. 10 Jun 09.

Consulted by Bioversity International (Italy) to develop relevant key descriptors for researchers to access and utilize pearl millet genetic resources. 16 Jul 09

Met with representatives of the Mali Ministry of Livestock and Fishery - Mamadou Coulibaly (National Director of Production and Animal Industries), Fode Traore (Project Coordinator of Livestock Development in Liptako-Gourma Region) and Vincent Farley (Honorary Consul of Mali). Responded to request from the Consul of Mali to outline an action plan policy paper to address aflatoxins in Malian diets. Aug 09

Germplasm Conservation and Distribution

Prepared 11 MTAs and provided 212 pearl millet germplasms upon request to Alabama A&M Univ (AL), BioDimensions Inc (TN), Emory Univ (GA), Ft. Valley St. Univ (GA), Jefferson Institute (MO), Nu-Life Market (KS), Operation Double Harvest (VA/Haiti), Univ. GA (GA), Univ. Arizona (AZ), Universidad del Zulia (Venezuela), and Vibha Seeds (India)

Publications and Presentations

Journal Articles

Rajewski, J.A., Ni, X., Wilson, J.P., Dweikat, I., Buntin, G.D. 2009. Evaluation of resistance to chinch bug in pearl millet in temperate and subtropical environments. Online. Plant Health Progress doi:10.1094/PHP-2009-0112-01-RS.

Scully, B.T., Krakowsky, M.D., Ni, X., Wilson, J.P., Lee, R.D., Guo, B.Z. 2009. Preharvest aflatoxin contamination of corn and other grain crops grown on the U.S. Southeastern Coastal Plain. Toxins Reviews. 28(2-3):169-179

Miscellaneous Publications

Ni, X., Coy, A.E., Buntin, G., Wilson, J.P. 2008. Sorghum Midge Resistance in 16 Grain Sorghum Hybrids - 2008. GA Agric. Experiment Station Report. <http://www.swvt.uga.edu/2008/sysrsn08/RR718-SR-midge.pdf>

Abstracts

Gulia, S.K., Singh, B.P., Wilson, J.P., Ma, X. 2009. Successful application of new cost-effective procedures for genotyping pearl millets for genetic diversity and linkage mapping. 15th Biennial Agricultural Research Director's Symposium, Atlanta, Georgia. March 28 - April 1, 2009. p. 118.

Gulia, S.K., Whitehead, W., Singh, B.P., Wilson, J.P. 2009. Grain yield and component traits of pearl millet genotypes at different row spacing. 15th Biennial Agricultural Research Director's Symposium, Atlanta, GA. March 28-April 1, 2009. pp. 117-118.

Breeding Sorghum for Improved Resistance to *Striga* and Drought in Africa

**Project PRF 101
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Introduction and Justification

Sorghum is an important crop worldwide both in area of production and in total tonnage produced. It is a particularly important crop in Africa where it is the cereal of choice to cultivate because of its relative superiority in productivity under low input levels and where abiotic and biotic stresses prevail. In the United States, sorghum is the second most important feed crop for both poultry and livestock; it is also a major livestock feed in several countries around the world. The project has identified the two most important sorghum production constraints in Africa as its area of focus and concentration. Drought stress is the most important abiotic factor limiting crop productivity in Africa. It is most severe in marginal environments where sorghum is routinely grown, but a major constraint in most areas and every crop season. About one-third of the world's arable land experiences water deficits, and in these areas crop yields are significantly reduced by drought. The parasitic weed, *Striga*, is the most important biotic stress in semi-arid tropical Africa. *Striga* infestation is most severe in areas where moisture is the most limiting. Nearly 100 million hectares of field crops including sorghum, millets, maize are infested annu-

ally with *Striga* in sub-Saharan Africa. We focus on genetic improvement of sorghum for drought and *Striga* resistance through a collaborative interdisciplinary process involving colleagues in several national agricultural research services (NARS) in Africa. The project will have a research for development emphasis with a value chain approach. It will have as its major activities the breeding of drought and *Striga* resistant sorghum varieties and hybrids, deploying these superior cultivars with a package of well thought out crop management or agronomic practices, seeking market opportunities for those adopting the recommended packages of technologies, and resulting with increased income and well being of poor farmers.

Research Objectives

In this project period, the following research objectives were addressed:

1. Create genetic variation in epicuticular wax (EW) production in sorghum through chemical mutagenesis for future studies on the contribution of EW to drought resistance.
2. Develop an effective chemical mutagenesis protocol for

generating EW variants (bloomless and sparse bloom mutants) in sorghum.

3. Determine, through allelism tests, how many different loci affecting EW production are present in the mutagenized populations.

Research Methods

Create Genetic Variation in EW Production in Sorghum Through Chemical Mutagenesis

Seed from two drought-tolerant inbred *Sorghum bicolor* (L.) Moench cultivars designated P898012 and P954035 were treated with chemical mutagens and planted on the same day at the Purdue Agronomy Center for Research and Education in West Lafayette, IN and M1 plants grown to produce M2 seed heads. M2 plants were grown in a winter nursery in the Banderas Valley of Southwestern Mexico. Visual selection of the P898012- and P954035-derived mutant populations for plants with reduced visual deposition of EW (reduced glaucousness) on abaxial sheath surfaces was conducted during the pre-boot and boot stages from among the segregating M2 head rows. Nine mutants (initially designated bm1-bm4, bm4A-bm8) were isolated from the P898012 mutagenized population and 29 mutants (initially designated bm10-bm38) were isolated from the P954035 mutagenized population. The M2 mutant plants were self-pollinated to produce M3 seed. Up to 20 normal sibs from each segregating head row containing a mutant were self-pollinated in order to find heterozygotes in which single gene inheritance for the corresponding mutant allele could be established as well as a homozygous normal sib that could be propagated as an isogenic counterpart for each mutant.

Develop an Effective Chemical Mutagenesis Protocol for Generating EW Variants (Bloomless and Sparse Bloom Mutants) in Sorghum

P898012 and P954035 seed were soaked in diethyl sulfate (DES) and ethyl methanesulfonate (EMS) at concentrations of 5.7 mM DES, 7.6 mM DES, or 11.5 mM DES for 3 hrs at room temperature; and at concentrations of 4.7 mM EMS or 9.4 mM EMS for 18 hrs at room temperature. Seeds were then rinsed in distilled water for 1 hr and dried with a blow dryer. They were planted immediately after.

Since seed head tissues represent sectors derived from cell lineages originating directly from the seed meristem, the mean number of meristem cells in the original dormant M0 seed that were serving as target cells for mutagenesis can be calculated. Assuming 3:1 wildtype to mutant segregation for all mutant loci (i.e., nuclear recessive mutations), the average segregation ratio of wildtypes to mutants was calculated by simply predicting segregation of 3:1 in the M2 head row if one target meristem cell were present in the M0 seed. Assuming two equal sectors derived from two target cells (i.e., 3:1 for mutated sector and 4:0 for wild-type sector), the M2 segregation ratio would be 7:1. Three sectors (from three target cells) would generate an 11:1 segregation, four sectors a 15:1 segregation, five sectors a 19:1 segregation, and so forth.

Determine, Through Allelism Tests, How Many Different

Loci Affecting EW Production are Present in the Mutagenized Populations

Of the original 38 mutants isolated, seven lines were not advanced, because either they were difficult to classify under some environmental conditions (bm12, bm14, bm23, and bm29) or they were completely or partially male and/or female sterile and thus difficult to propagate (bm13, bm36, and bm37). Thirty one distinct mutants were selected for the allelism test.

Efforts were made to obtain genetic stocks containing the previously reported epicuticular wax loci *bm1*, *bm2*, *h1*, *h2*, *h3* and *h4* (Weibel, 1986a,b; Peterson *et al.*, 1982). Unfortunately representatives of only two of these loci (*bm2* and *h3*) were available. The *bm2* locus was represented by Txbm1 (first bloomless line received from Texas A&M) and CK60bm (Combine Kafir-60 bloomless) and the *h3* locus was represented by Neb31h. The epicuticular wax traits of these stock lines all had single gene recessive inheritance.

Regardless of whether mutants were the bloomless or sparse-bloom types, crosses were made for all possible genotype pairs of the 31 selected mutants and three available representatives of loci *bm2* and *h3*. Mutants were shown to have single gene nuclear inheritance based on 3:1 segregation within the F3 population derived from heterozygotes in the original M2 population. Hence only non-reciprocal crosses were made. Nevertheless, two or more test crosses were made for each combination of all independent mutants. Nonallelism was indicated when two or more wild-type plants arose in an F1 population and, the resulting F2 segregated for wild-type and mutant phenotypes. Allelism was indicated when no wildtype appeared in the F1.

Research Results

Sorghum EW Mutants

Segregating F3 head rows derived from heterozygotes in the original M2 population were tested for a 3:1 segregation ratio as would be expected from epicuticular wax mutations resulting from a single gene with nuclear inheritance. Chi-square tests for single gene recessive inheritance (Table 1) were calculated for individual head row families with 1 degree of freedom per family. Families were excluded if the expected value for either category was less than 5. In addition, outliers were excluded. Outliers were defined as families that gave nonhomogenous ratios compared to the majority of families for a mutant. Of the 31 original mutants tested for allelism, single gene inheritance was established by this method for 20. These were bloomless mutants bm2, bm3, bm4, bm4A, bm15, bm16, bm18, bm20, bm21, bm22, bm27, bm30, bm31, bm33, bm35, and bm38, plus sparse-bloom mutants bm11, bm19, bm24, and bm34. In addition, single gene inheritance was established by this method for one sparse-bloom mutant (bm23) that was not tested for allelism because of difficulty in classifying mutants.

In six other independent mutants that were tested for allelism (bloomless mutants bm6, bm7, bm8, and bm26, and sparse-bloom mutants bm1 and bm28) and one bloomless mutant that was not tested for allelism (bm14), heterozygotes were not among the

wild-type seed heads gathered from the original segregating head rows. Nevertheless, each of these showed segregation ratios in the F2 populations from the allelism studies consistent with single gene recessive mutations (data not shown). For these six original mutants, F2 segregation ratios were approximately the expected two loci ratios of 9:7 in bm-type crosses with other bm-types, and the expected 9:3:4 ratio in bm-type by h-type crosses. In addition, the test crosses allowed each of these mutants to be placed directly into allelic groups. Moreover, F2 populations generated from crosses of these six mutants with male sterile genotypes had observed segregation ratios consistent with the expected 3:1.

Five of the sparse-bloom mutants that were tested for allelism (bm5, bm10, bm17, bm25, and bm32) and two sparse-bloom mutants that were not tested for allelism (bm12 and bm29) had segregation ratios that did not fit the expected 3:1 ratio (Table 1). Nevertheless, it is still likely that these are in fact single gene recessive mutations. Based on our visual assessment, a lack of 3:1 segregation likely resulted from difficulties in distinguishing between wild-type and sparse-bloom phenotypes in our Indiana field plots. In these sparse-bloom lines, the visual wax phenotypes differed only slightly from the wild-type and these phenotypes were affected by precipitation that often removed visible waxes. Because of this difficulty, essentially all allelism studies between sparse-bloom mutants were conducted in a winter nursery in Banderas Mexico where there was no precipitation and plants were watered using sub-irrigation. Under conditions in Mexico, the sparse-bloom phenotypes could easily be distinguished from wild-type wax phenotypes. In Mexico, F2 segregation ratios from the allelism studies were consistent with single gene recessive mutations.

Finally, three bloomless mutants that were not included in allelism tests (bm13, bm36, and bm37) had segregation ratios that did not fit the expected 3:1 ratio (Table 1). In each of these cases, the number of mutants present in the population was very low. These mutants had extreme alterations in their phenotypes, being dwarfed, having wrinkled leaves, and with poor development of flowering heads. We interpreted this low proportion of mutants in segregating populations as being due to either low germination of mutant seeds, a failure in mutant fertilization, or inhibited mutant seed development. Further studies are needed to test these hypotheses. The bm13, bm36, and bm37 mutants were not included in allelism studies due to these questions about inheritance.

A few mutants had phenotypic differences from wildtype other than a reduction in visible epicuticular waxes alone. Of these, many were slightly reduced in height compared to wildtype. Besides a small height reduction, bm38 developed slightly chlorotic leaves, had no basal tillers, and the flower heads appeared later; bm24 had slightly wrinkled leaves and lacked basal tillers; bm16 and bm20 had more erect leaves and lacked basal tillers; and bm2, bm6, bm22, and bm33 had a rapid-water-loss phenotype. It is likely that these phenotypes were due to pleiotropy of a single gene mutation since the multiple phenotypes of these lines always cosegregated in segregating populations derived from backcrosses with the wildtype.

These mutants can be used in future studies investigating the contributions of EW load (amount of visible waxes) and chemistry

(specific wax constituents) to drought resistance. It is believed that EW contributes to drought resistance in sorghum by reflecting excess radiation during hot dry spells (reflective cooling) and reducing non-stomatal water loss (dehydration avoidance). Several loci affecting specific wax characteristics are represented in these mutagenized populations and therefore this germplasm will be valuable in testing the role of EW in overall drought resistance in sorghum.

Chemical Mutagenesis

Within the sorghum mutagenized populations, the epicuticular wax mutants segregated at a frequency of 0.88% of M2 head rows (Table 2). The highest mutation rate of 1.17% was found for the M2 population derived from seeds soaked in 4.7 mM EMS. The lowest rate of 0.39% was found for the M2 population derived from seed treated with 7.6 mM DES (Table 2). Overall, the EMS treatments produced higher average mutation rates of 1.13% than the DES treatments of 0.66%.

Table 3 shows the M2 segregation ratios for wildtypes to wax mutants in each of the mutants derived from the wild-type parent P954035. The overall average number of target meristem cells in the M0 seed was 5, with a range of predicted values of 2 to 13.

Allelism Tests

Our test of allelism with the 31 independent EW mutants selected for allelism studies allowed us to identify one existing EW locus and 18 new EW loci (Table 4). Of these, one locus represented the existing bm mutant locus bm2, nine represented new bm mutant loci, and nine others represented new h mutant loci (Table 4). The bm2 locus, previously described by Peterson et al. (1982), had the greatest number of alleles with six. In this study, four new bm2 alleles were identified. The bm3, bm4, bm6, and h7 loci had three alleles each, whereas the bm5 locus had two allelic members. Only six out of 19 loci with new mutants reported here had more than one allele. Therefore, thirteen new loci were represented by only one allele. In addition, all of the new EW loci were found to be independent of the existing sparse-bloom locus h3.

Training (Degree and Non-Degree)

Idris Amusan, a Ph.D. student from Nigeria working on Striga resistance in maize, completed his education and accepted a maize breeding position at Ag Reliant based in Aimes, Iowa.

Networking Activities

A group of US sorghum seed industry plant breeders visited Purdue sorghum research and walked our nurseries. We also had visitors from South America interested in sorghum as biofuel.

Gebisa Ejeta returned to Nairobi, Kenya and visited colleagues at the Alliance for Green Revolution in Africa.

Publications and Presentations

Peters, P.J., M.A. Jenks, P.J. Rich, J.D. Axtell and G. Ejeta. 2009. Mutagenesis, selection and allelic analysis of epicuticular wax mutants in sorghum. *Crop Science* 49: 1250-1259.

Rich, P.J. and G. Ejeta. 2008. Towards effective resistance to *Striga* in African maize. *Plant Signaling & Behavior* 3: 618-621.

Amusan, I.O., P.J. Rich, A. Menkir, T. Housley and G. Ejeta. 2008. Resistance to *Striga hermonthica* in a maize inbred line derived from *Zea diploperennis*. *New Phytologist* 178:157-166.

Table 1. Segregation ratios and sums of chi-square values from tests for single recessive gene inheritance predicted as a 3:1 ratio for segregating populations. Individual chi-squares were calculated for separate F3 families (head rows) derived from self-pollinated M2 heterozygotes. Individual chi-squares for separate families each had one degree of freedom. Sum of chi-square values were compared to sums of tabulated values for the individual family tests. Nonsignificant chi-square indicates acceptance of the hypothesized 3:1 ratio for single gene recessive inheritance. Mutants bm1-bm8 were derived from P898012, whereas bm10-bm38 were derived from P954035.

Mutant	Wildtype	Mutant	$\Sigma\chi^2$	df
bm2	450	142	19.03	10
bm3	272	112	16.14	8
bm4	680	213	13.64	12
bm4A	589	207	13.41	10
bm5	651	77	126.29 ** ¹	10
bm10	1227	152	190.21**	11
bm11	1004	335	11.02	11
bm12 ²	1292	124	220.89**	12
bm13 ²	924	162	80.38**	9
bm15	659	186	6.93	6
bm16	781	251	9.55	8
bm17	903	100	146.54 **	9
bm18	839	268	4.05	9
bm19	360	119	16.16	8
bm20	503	150	6.33	8
bm21	622	214	7.19	10
bm22	344	113	4.15	8
bm23 ²	318	83	5.2	6
bm24	434	107	20.58	9
bm25	786	83	134.91 **	9
bm27	420	156	4.75	8
bm29 ²	593	258	216.12 **	6
bm30	1149	349	13.57	11
bm31	447	144	12.27	5
bm32	274	551	1143.92**	12
bm33	786	250	6.29	12
bm34	796	233	24.57	11
bm35	581	156	15.81	12
bm36 ²	928	91	149.62**	8
bm37 ²	723	162	88.2**	9
bm38	684	192	17.17	9

¹*, ** Significantly different from 3:1 ratio at 0.05 and 0.01 levels, respectively.

² Mutant not tested for allelism.

Table 2. DES (diethyl sulfate) and EMS (ethyl methane sulfonate) mutagenesis treatments and mutation frequencies for epicuticular wax mutants of the Sorghum bicolor lines P898012 and P954035. Mutants bm1-bm8 arose from P898012, whereas bm10-bm38 arose from P954035.

Treatment	Mutation Rate ¹	Mutation Freq. (%)	Mutants Generated ²
5.7 mM DES	NA	NA	bm8
7.6 mM DES	3/780	0.39	bm22-bm24
11.5 mM DES	11/1340 0.82		bm1-bm4, bm4A, bm33-bm38
4.7 mM EMS	NA ³	NA	bm6-bm7
4.7 mM EMS	13/1110 1.17		bm5, bm10-bm21
9.4 mM EMS	8/744	1.08	bm25-bm32

¹ Actual number of mutants per M2 head rows.

² Designation for original mutant isolates [these do not represent loci].

³NA Data not available.

Table 3. Segregation ratios in the M2 head rows for the original P954035 derived epicuticular wax mutant isolates and estimates of the number of meristem cells in dormant M0 seed.

Mutant	M2 Segregation Ratios Wildtype:Mutant	Estimated Number of Meristem Cells in M0
bm10	30:1	8
bm11	24:5	2
bm12	36:3	4
bm13	36:3	4
bm15	34:1	9
bm16	52:3	5
bm17	37:4	3
bm18	37:3	4
bm19	26:2	4
bm20	46:3	5
bm21	27:2	4
bm22	28:1	8
bm23	21:2	3
bm24	46:1	13
bm25	26:3	3
bm26	25:3	3
bm27	30:2	4
bm28	32:2	5
bm29	29:1	8
bm30	25:6	2
bm31	34:4	3
bm32	26:1	7
bm33	33:1	9
bm34	26:2	4
bm35	33:5	2
bm36	37:2	5
bm37	30:6	2
bm38	36:1	10
Mean		5.1

Table 4. New bloomless (bm) and sparse-bloom (h) loci and their allelic members identified by allelism studies. The members of each group represent the isolates named in the original screens with the new designators presented. The original mutant isolates designated bm1-bm8 arose from P898012, whereas bm10-bm38 arose from P954035. Two or more test crosses were made for each combination of all independent mutants.

Locus	Number of Alleles	Original Isolates with New Loci and Allelic Designation in Parentheses
bm21	6	bm2 (bm2-1), bm6 (bm2-2), bm22 (bm2-3), bm33 (bm2-4), Txbm1 (bm2-5), CK60bm (bm2-6)
bm3	3	bm3 (bm3-1), bm4 (bm3-2), bm4A (bm3-3)
bm4	3	bm8 (bm4-1), bm15 (bm4-2), bm18 (bm4-3)
bm5	2	bm16 (bm5-1), bm20 (bm5-2)
bm6	3	bm21 (bm6-1), bm27 (bm6-2), bm30 (bm6-3)
bm7	1	bm26 (bm7-1)
bm8	1	bm7 (bm8-1)
bm9	1	bm31 (bm9-1)
bm10	1	bm35 (bm10-1)
bm11	1	bm38 (bm11-1)
h31	1	Neb31h (h3-1)
h5	1	bm1 (h5-1)
h6	1	bm5 (h6-1)
h7	3	bm11 (h7-1), bm19 (h7-2), bm32 (h7-3)
h8	1	bm10 (h8-1)
h9	1	bm25 (h9-1)
h10	1	bm28 (h10-1)
h11	1	bm17 (h11-1)
h12	1	bm24 (h12-1)
h13	1	bm34 (h13-1)

¹ Previously described loci (Peterson et al., 1982. Crop Sci 22: 63)

Developing Sorghum with Improved Grain Quality, Agronomic Performance, and Resistance to Biotic and Abiotic Stresses

Projects PRF 104
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Introduction and Justification

Sorghum is poised to play a key role in agricultural development and food security in developed and developing countries around the world. The role of sorghum in agricultural development is expanding as genetic, genomic, and agricultural technologies that have been developed for the crop are transferred to targeted regions throughout the world. The goal of this project focuses on research and training activities to deploy genetic technologies that will enhance the value and performance of sorghum into farmer-accepted varieties in developed and developing sorghum production regions. These efforts will be accomplished through collaborative programs with sorghum breeders and researchers in U.S. universities and national agriculture research systems throughout West Africa (WA) including Niger, Burkina Faso, Mali, and Nigeria and through interaction with private industry partners including DuPont Crop Protection and private seed industry partners. Other more basic research efforts focus on the development and use of emerging genetic and genomic technologies to develop new traits for sorghum and more efficiently use the natural genetic variation in sorghum to improve the crop.

Problem Statement

The West Africa (WA) region produces over 30% of the total acreage of sorghum in the world and the U.S. produces another 5% (FAO, 2005). Most of the grain produced in WA is used to prepare foods and beverages for human consumption including traditional stiff or thin porridges (e.g. tô and fura), granulated foods (e.g. couscous), and beer production (e.g. dolo) (Awika and Rooney, 2004). In the U.S., sorghum primarily is used in animal feed, but the food and biofuel markets are expanding rapidly. Opportunities in new and expanding markets, especially emerging food and feed markets, will require that more attention be given to combine

grain quality and end-use requirement traits with key defensive traits (e.g., *Striga* and weed management) needed to maximize production potential. These efforts will facilitate the growth of the rapidly expanding markets for sorghum and millet, improve food and nutritional quality to enhance marketability and consumer health, increase the stability and yield of the crop through use of genetic technologies, and contribute to effective partnerships with national and international agencies engaged in the improvement of sorghum.

Objectives and Listing of Implementation Sites

Recent research workshops of INTSORMIL scientists in West African and the United States highlighted the need to actively transfer technologies developed in previously funded research to improve sorghum crop production, performance, and value (West Africa Technology Transfer Working Group, 2007). These meetings and feedback from sorghum producers in developed and developing countries indicated the need to combine traits and strategies to more effectively manage problematic weeds including *Striga* in varieties with improved grain quality characteristics, especially cultivars with improved food and feed quality traits (e.g., tan-plant, white-grain, etc.).

The objectives, collaborators, and implementation sites to address these constraints include:

Develop sorghum varieties and hybrids having improved grain quality and production characteristics. This objective focuses on development of sorghum varieties and hybrids having improved food- and feed-quality characteristics for use in West Africa and the United States. Key collaborators and implementation sites include:

- Soumana Soumana, INRAN, NIGER
- Daniel Aba, INRA, NIGERIA

Deploy traits that enhance resistance to biotic stresses into locally adapted varieties and hybrids with excellent grain quality. This objective focuses on deployment of *Striga* resistance and herbicide tolerance traits into locally-adapted varieties and hybrids with excellent grain quality attributes. Key collaborators and implementation sites include:

- Soumana Soumana, INRAN, NIGER
- Mountaga Kayento, IER, MALI
- Hamidou Traore, INERA, Burkina Faso
- Daniel Aba, INRA, NIGERIA
- David Aupperle, Reginald Young, and John Beitler, DuPont Crop Protection, USA
- Gebisa Ejeta, Purdue University, USA

Identify and mine genes and alleles associated with improved sorghum performance from the natural sorghum gene pool. An Association Mapping (AM) panel of 300 sorghum lines and varieties selected to represent the genetic diversity of sorghum from around the world has been developed to identify genes and genetic diversity for important food, feed, industrial, and performance traits. Key collaborators and implementation sites include:

- Jianming Yu, Kansas State University
- Dr. Scott Bean, USDA-ARS, Manhattan, KS USA

This project and approach will directly contribute to the vision of the INTSORMIL CRSP for 2007-2011. The development of improved, locally-adapted, sorghum varieties and hybrids having enhanced food and feed quality traits will increase availability of high-quality grains. Improved access to these grains will facilitate market development for use in new food products with enhanced nutritional value. Efforts to incorporate *Striga* resistance and herbicide tolerance traits into locally-adapted sorghum cultivars will provide new tools that are desperately needed for management of *Striga* and grassy weeds, the most important biotic constraints to sorghum production in Africa and the U.S. These efforts will enhance the productivity and stability of sorghum production in those environments and contribute to integrated management of the most important biotic pests through use of genetic technologies. Finally, the use and conservation of sorghum genetic resources will be improved through use of new biotechnology strategies to study genes and identify alleles associated with important target traits. Each of these objectives will be accomplished through maintenance and expansion of established linkages with foreign collaborators which will afford opportunities to enhance national and international organizations in West Africa through short- and long-term training of students and research scientists.

Specific Research Strategy and Approach

Collaborative research efforts are focused in West Africa and are supported through short and long-term training programs, germplasm exchange and evaluation, and basic research. The overarching objective of this project is to develop and deploy genetic technologies that improve sorghum production, performance, and value through plant breeding. The germplasm sources needed

to create new breeding populations were identified or developed through evaluations of elite U.S and tropical germplasm in the target region. The populations are advanced and selected in summer and winter nurseries and then transferred to the target region for evaluation in conference with collaborating plant breeders.

The effort to develop and commercialize new herbicide tolerance traits in sorghum are focused on creating new tools for managing weed pests in the crop. Herbicides are an important component of most weed management programs in sorghum; however, preplant herbicides often fail or perform poorly in the dry conditions where sorghum is grown. New herbicides are needed to control broadleaf and grassy weeds. In 2005, a natural sorghum mutant with tolerance to ALS-inhibiting herbicides was identified. Genetic crossing and backcrossing are being used to transfer this trait into elite grain sorghum varieties. In 2006, a sorghum mutant with resistance to several ACCase inhibiting herbicides was identified. This trait also is being incorporated into elite sorghum parent lines through genetic crossing and backcrossing. In the United States, inbred lines used for hybrid seed production are being converted to ALS and ACCase herbicide tolerance to facilitate commercialization of this technology. In Africa, the ALS herbicide tolerance trait can be used with seed treatments to control parasitic witchweed infestations (Tuinstra et al. 2009). Seed treatments that combine herbicides, fungicides, and insecticides are being evaluated for efficacy in improving sorghum productivity through research collaborations with private industry collaborators in the United States and NARs scientists in West Africa.

Sorghum exhibits an incredible array of natural genetic diversity. Much of this diversity is not utilized for crop improvement because potentially useful alleles of genes are hidden in otherwise inferior genetic backgrounds. New association gene mapping strategies search for genes involved in complex traits at a population level using natural diversity rather than through individual bi-parental crosses. It tests for relationships between molecular polymorphisms at the gene level with phenotypic variation among diverse genotypes. An association mapping (AM) panel of 300 sorghum genotypes collected from around the world has been assembled that represents much of the natural genetic variation of sorghum. The PI is collaborating with Drs. Yu and Bean to characterize the AM panel for grain quality and plant performance traits to identify genes and sources of alleles that can be used to enhance the crop.

Research Results

Develop locally adapted sorghum varieties and hybrids having improved grain quality and feed value.

Sorghum has been grown as a food crop for many centuries in Africa and India. Food-grade sorghum is becoming an increasingly important crop in the developed world, especially as a cereal option for people with celiac disease. The highest quality sorghum flours and food products are produced using grain from food-grade sorghum varieties (Tuinstra, 2008). Food grade sorghum varieties and hybrids with white pericarp, tan plant color, straw color glumes, and medium- to hard-endosperm kernels have been developed to maximize food quality, but these types of sorghum tend to be more susceptible to mold than sorghum varieties with

a red pericarp. Grain molds and weathering in the field can have a major effect on sorghum grain quality and value. Seed quality is diminished not only for nutritive value, but the flour produced from molded grain generally has poor color quality and reduced aesthetic value.

In much of WA, the guinea sorghums have been found to possess superior head bug and grain mold resistance and are uniquely adapted to this region (Ratnadass et al., 2003). Continued improvement of the guinea varieties is needed since these types of sorghum varieties are nearly always preferred by farmers in the region from Burkina Faso to Senegal. Some progress has been made in use of these germplasms to produce locally adapted varieties with improved grain quality. The food-grade guinea sorghum variety Wassia is being used extensively to produce breeding populations for development of new varieties and inter-racial guinea hybrids.

Plant breeding efforts in Nigeria and Niger focus more on hybrid variety development using caudatum sorghums. Crop improvement efforts in these environments focus on development of very large-seeded and early-maturing hybrids for food production and use in the malting industries. Field trials in 2009 identified several hybrid combinations that appear to be highly productive and well-adapted to environments in Niger and Nigeria.

Develop and deploy technologies and strategies to manage weedy pests including Striga

Sorghum researchers and producers in the U.S. and WA indicated that weed infestations including parasitic witchweeds are among the most important production constraints for sorghum. *Striga* is recognized as a growing problem and it is estimated that more agricultural land in WA (3.5 million ha) is infested with *Striga* than in any other region. Efforts to breed for improved *Striga* resistance have been successful; however, no single technology has been shown to be completely effective in controlling *Striga* or containing its spread.

One new *Striga* management technology being developed in this project involves use of herbicide tolerance traits for managing

this weed. Low-dose imazapyr or metsulfuron seed coatings applied to herbicide tolerant varieties have been shown to be highly effective in controlling *Striga* infestation in field and greenhouse trials (Tuinstra et al., 2009). In 2008-9, replicated trials in Niger, Burkina Faso, and Mali indicated that seeds treated with metsulfuron-methyl (MET) had fewer *Striga* attachments and the greatest delay in attachment (Table 1).

A major focus of our crop improvement program is to develop ALS herbicide tolerant guinea and non-guinea sorghum hybrids that are adapted in the West Africa region. N223 is one of the important food-grade sorghum seed parents being used in West Africa. Two ALS herbicide resistant derivatives of N223 (PU-KS10 and PU-KS11) were jointly released by Purdue University and Kansas State University in 2009 (Table 2). These seed parents can be used to produce interracial guinea-type and caudatum-type hybrids. Preliminary observation trials of testcross hybrids produced using these seed parents suggest that these sorghums will provide an effective tool for deploying herbicide seed treatment technology for controlling *Striga* infestations in sorghum.

Weed control is an important problem for sorghum producers in the United States. The ALS herbicide tolerance trait and a second acetyl coenzyme A carboxylase (ACCCase) herbicide tolerance trait are being incorporated into U.S. sorghum germplasm to allow use of these herbicides for grassy weed control in sorghum. In 2009, Purdue University and Kansas State University jointly released 11 ALS herbicide resistant lines, 14 ACCCase herbicide resistant lines, and six ALS+ACCCase herbicide resistant lines (Table 2). The PI is collaborating with researchers from DuPont Crop Protection and Kansas State University to conduct field and laboratory research needed to commercialize ALS and ACCCase herbicides as part of an integrated weed management strategy that incorporates post-emergence herbicide applications to control broad-leaf and grassy weed problems in sorghum.

Identify and mine genes and alleles associated with improved sorghum performance in the natural gene pool

A project was initiated to systematically identify and exploit natural genetic variation in the sorghum genome using the genome

Table 1. Efficacy of metsulfuron methyl (MSM) seed treatments at controlling *Striga* infestation in field trials in Niger and Burkina Faso.

Seed Treatment	<i>Striga</i> Emergence	<i>Striga</i> at 60 d	<i>Striga</i> at 90 d	Sorghum Yield
	(days)	(<i>Striga</i> m ⁻²)	(<i>Striga</i> m ⁻²)	(kg ha ⁻¹)
0 herbicide	44.6	6.2	14.0	262.2
0.003 mg MSM	52.5	2.0	13.5	321.0
0.006 mg MSM	51.1	2.1	13.3	401.1
0.0125 mg MSM	56.7	0.9	10.9	371.3
0.025 mg MSM	58.7	0.6	8.8	498.3
LSD	5.0	1.9	4.8	161.3
P-value	0.0001	0.0001	0.1578	0.06

Table 2. The germplasms PU-KS1 to PU-K31 were jointly released by Purdue University and Kansas State University in 2009. These lines are tolerant to acetolactate synthase (ALS) and/or acetyl co-enzyme A carboxylase (ACCCase) herbicide inhibitors.

Number	Entry	Pedigree	Herbicide Tolerance
PU-KS1-R	09WL47	Tx2737/[Tx2737///Tx2737//90SN7/Tw]	ALS
PU-KS2-R	09WL53	Tx2737/[Tx2737///Tx2737//90SN7/Tw]	ALS
PU-KS3-R	09WL68	Tx430/[Tx430///Tx2737//90SN7/Tw]	ALS
PU-KS4-R	09WL69	Tx430/[Tx430///Tx2737//90SN7/Tw]	ALS
PU-KS5-R	09WL77	Tx430/[Tx430///Tx2737//90SN7/Tw]	ALS
PU-KS6-R	09WL80	Tx430/[Tx430///Tx2737//90SN7/Tw]	ALS
PU-KS7-R	09WL88	Tx430/[Tx430///Tx2737//90SN7/Tw]	ALS
PU-KS8-R	09WL90	Tx430/[Tx430///Tx2737//90SN7/Tw]	ALS
PU-KS9-R	09WL92	Tx430/[Tx430///Tx2737//90SN7/Tw]	ALS
PU-KS10-B	09WL101	N223//N223//N223/Tw	ALS
PU-KS10-A	09WL102	N223//N223//N223/Tw-A	ALS
PU-KS11-B	09WL119	N223//N223//N223/Tw	ALS
PU-KS11-A	09WL120	N223//N223//N223/Tw-A	ALS
PU-KS12-R	09WL1037	Tx2737//Tx2737/[Tx2737///Tx430//Tx430/Bol-71]	ACCCase
PU-KS13-R	09WL1045	Tx2737//Tx2737/[Tx2737///Tx430//Tx430/Bol-71]	ACCCase
PU-KS14-R	09WL1047	Tx2737//Tx2737/[Tx2737///Tx430//Tx430/Bol-71]	ACCCase
PU-KS15-R	09WL1068	00MN7645//00MN7645/[00MN7645///Tx430//Tx430/Bol-71]	ACCCase
PU-KS16-R	09WL1091	01MN7951//01MN7951/[Tx2737///Tx430//Tx430/Bol-71]	ACCCase
PU-KS17-B	09WL1093	OK11/[OK11///Tx623//Tx623/Bol-71]	ACCCase
PU-KS17-A	09WL1094	OK11/[OK11///Tx623//Tx623/Bol-71]-A	ACCCase
PU-KS18-B	09WL1145	Tx2752/[Tx3042///Tx623//Tx623/Bol-71]	ACCCase
PU-KS18-A	09WL1146	Tx2752/[Tx3042///Tx623//Tx623/Bol-71]-A	ACCCase
PU-KS19-B	09WL1179	Tx3042//Tx3042/[Tx3042///Tx623//Tx623/Bol-71]	ACCCase
PU-KS19-A	09WL1180	Tx3042//Tx3042/[Tx3042///Tx623//Tx623/Bol-71]-A	ACCCase
PU-KS20-B	09WL1255	Tx3042///Tx3042//Tx3042/[Tx3042///Tx623//Tx623/Bol-71]	ACCCase
PU-KS20-A	09WL1256	Tx3042///Tx3042//Tx3042/[Tx3042///Tx623//Tx623/Bol-71]-A	ACCCase
PU-KS21-B	09WL1259	Tx3042///Tx3042//Tx3042/[Tx3042///Tx623//Tx623/Bol-71]	ACCCase
PU-KS21-A	09WL1260	Tx3042///Tx3042//Tx3042/[Tx3042///Tx623//Tx623/Bol-71]-A	ACCCase
PU-KS22-B	09WL1273	Tx399//Tx399//Tx399/[Tx399///Tx623//Tx623/Bol-71]	ACCCase
PU-KS22-A	09WL1274	Tx399//Tx399//Tx399/[Tx399///Tx623//Tx623/Bol-71]-A	ACCCase
PU-KS23-B	09WL1281	Tx399//Tx399//Tx399/[Tx399///Tx623//Tx623/Bol-71]	ACCCase
PU-KS23-A	09WL1282	Tx399//Tx399//Tx399/[Tx399///Tx623//Tx623/Bol-71]-A	ACCCase
PU-KS24-B	09WL1231	Tx378//Tx378/[Tx3042///Tx623//Tx623/Bol-71]	ACCCase
PU-KS24-A	09WL1232	Tx378//Tx378/[Tx3042///Tx623//Tx623/Bol-71]-A	ACCCase
PU-KS25-B	09WL1283	Tx623//Tx623/[N223(ALS)//Tx623//Tx623/Bol-71]	ACCCase
PU-KS25-A	09WL1284	Tx623//Tx623/[N223(ALS)//Tx623//Tx623/Bol-71]-A	ACCCase
PU-KS26-R	09WL2002	[Tx2737///Tx2737//90SN7/Tw]/[Tx2737///Tx430//Tx430/Bol-71]	ALS+ACCCase
PU-KS27-R	09WL2050	[Tx430//Tx2737//90SN7/Tw]/[Tx2737///Tx430//Tx430/Bol-71]	ALS+ACCCase
PU-KS28-R	09WL2040	[Tx2737///Tx2737//90SN7/Tw]/Tx2737/[Tx2737///Tx430//Tx430/Bol-71]	ALS+ACCCase
PU-KS29-R	09WL2037	[Tx2737///Tx2737//90SN7/Tw]/Tx2737/[Tx2737///Tx430//Tx430/Bol-71]	ALS+ACCCase
PU-KS30-B	09WL2167	Tx623/[N223(ALS)//Tx623//Tx623/Bol-71]	ALS+ACCCase
PU-KS31-B	09WL2181	Tx623/[N223(ALS)//Tx623//Tx623/Bol-71]	ALS+ACCCase

DNA sequence as a tool to identify and relate variation in specific genes with phenotypic variation represented in the sorghum germplasm collection. We are collaborating with Dr. Jianming Yu, Kansas State University and Dr. Scott Bean, USDA-ARS, Manhattan, Kansas to collect phenotypic trait data in the association panel and relate that to gene function through a process called 'association mapping'. This information will allow us to target genes for selective modification to enhance sorghum performance. In preliminary studies, we are targeting genetic variation at the *dw3* locus. The *dw3* allele used in the commercial U.S. sorghum sector has been reported to be unstable resulting in increased seed production costs and height mutants resulting from instability at this locus. We are using the sorghum genome sequence to develop strategies whereby a stable *dw3* allele can be identified for commercial use.

Networking Activities

Workshops and meetings

Health, Research, and Entrepreneurship: Sorghum Food for Celiac Patients. Naples, Italy, October 19, 2009

Third Annual Plant Breeding Conference, Plant Breeding Coordinating Committee, Madison, Wisconsin, August 3-5, 2009

Sorghum Field Day, Purdue University, West Lafayette, IN, September 9, 2009

Research information exchange

Traveled with representatives from DuPont Crop Protection and visited research plots and collaborators at IER and ICRISAT in Mali, INERA in Burkina Faso, INRAN in Niger, and the Alliance for a Green Revolution in Accra, Ghana from Sept 25 to Oct 4, 2008

West Africa Research Coordination Meeting, DuPont Crop Protection, Wilmington, DE, Feb. 18-19, 2009

Sorghum Field Day, Purdue University, West Lafayette, IN, September 9, 2009

Meeting with representatives from DuPont Crop Protection to discuss herbicide trait development and Striga management, West Lafayette, IN September 9, 2009

Germplasm Conservation And Distribution

Released and distributed 31 ALS, ACCase, and ALS+ACCase sorghum inbred lines to the U.S. seed industry.

Distributed tissue of 300 sorghum lines representing the sorghum association panel to Dr. Clifford Weil to initiate an eco-tilling project to study natural genetic variation in sorghum.

Distributed seed of the sorghum association panel to Dr. Kartik Krothapalli to evaluate genetic variation in forage quality traits.

Distributed a replicated experiment to evaluate efficacy of herbicide seed treatments and host-plant resistance to Striga to NARs collaborators in Niger, Burkina Faso, Nigeria, and Mali.

Publications and Presentations

Journal Articles

Wang M, Zhu C; Barkley N, Chen Z, Erpelding J, Murray S, Tuinstra MR, Tesso T, Pederson G, Yu J. (in press). Genetic diversity and population structure analysis of accessions in the U.S. historic sweet sorghum collection. *Theoretical and Applied Genetics*.

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Ochanda N, Yu J, Bramel PJ, Menkir A, Tuinstra MR, Witt MD. 2009. Selection before backcross during exotic germplasm introgression. *Field Crops Research* 112:37-42.

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Breeding Sorghum for Improved Grain, Forage Quality and Yield for Central America

**Projects TAM 101
William Rooney
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Introduction and Justification

Background

Throughout Central America, (defined as the countries of Guatemala, Belize, El Salvador, Honduras, Nicaragua, Costa Rica and Panama), sorghum (*Sorghum bicolor* L. Moench) was grown and harvested for grain on approximately 250,000 hectares in 2005 (FAO, 2006). The majority of this production is located in the countries of El Salvador, Nicaragua, Honduras and Guatemala. The crop is typically grown in the dry season due to its enhanced drought tolerance and ability to produce a crop under limited water availability. Average yields in the region vary dramatically and are dependent on the production systems, environment and types of sorghums that are being produced. Depending on the situation, the crop is grown as a feed grain, animal forage and in many situations as a food grain when supplies of corn are limited.

Within the region, there are two distinct sorghum production systems. The first is a traditional hillside sorghum production system that uses landrace and/or improved sorghum cultivars known as Maicillos Criollos. These sorghums are a very distinct and unique group because they are very photoperiod sensitive, meaning that

they require short daylengths to induce reproductive growth. In fact, Maicillos require even shorter daylengths to initiate flowering than most photoperiod sensitive sorghum from other regions of the world (Rosenow, 1988). They are primarily grown in intercropping systems with maize on small, steeply sloping farms where the maize matures before the Maicillos begin to flower. Because they are drought tolerant, they are grown primarily as food security crop where the grain is used extensively primarily to produce tortillas. The forage and excess grain produced by these crops are valued as animal feed. Traditional landrace Maicillos Criollos varieties are typically low yielding with relatively low grain quality. Previous research has resulted in the release and distribution of several improved Maicillos Criollos cultivars with higher yield potential and better grain quality (Rosenow, 1988). In addition to Maicillos Criollos, hillside production systems also utilize earlier maturing sorghum (ie, photoperiod insensitive) for food and forage. Significant research has also been devoted to their improvement, resulting in the release of cultivars such as Sureno and Tortillero that are now commonly grown throughout the region (Meckenstock et al., 1993). These cultivars have been adopted and used in the region as a food grain on small farms as well as a dual purpose crop (grain, forage) in mid-size commercial farms.

In addition to small farm production, sorghum is also grown in significant quantities on commercial farms in the Central American region. While some of these producers utilize cultivars for this production, most have adopted hybrids and are growing the crop as a feed grain for use in poultry, livestock and dairy production. More recently, there is significant growth of the crop in the region for grazing, hay and silage. This interest in sorghum forage has been increasing due to the increased dairy and beef production in the region, combined with the inherent drought tolerance of the crop, especially in the second, drier cropping season. In both grain and forage, the hybrids that Central American producers use are usually sold by commercial seed companies. In most cases, research and development for sorghum improvement in the region is relatively minimal. Hybrids grown in this region usually rely on improved germplasm from national programs as well as U.S. based sorghum improvement programs.

Problem Statement

While the two production regions differ for types of germplasm, the constraints to productivity and profitability are similar. First, there is a continual need to enhance yield of both grain and biomass. The Maicillos Criollos cultivars have low but stable yield potential. Small farmers place a high value on stable yields as they grown to provide food security. Thus, they will adopt higher yield varieties only if they provide stability of yield as well. As feed grain demand continues to increase, yield increases are also needed in commercial hybrid production as well to make their production more economically profitable. Sufficient genetic variation is present in both germplasm pools to enhance yield potential, provided that effective evaluation, screening and selection can be completed in the region (Santos and Clara, 1988).

Improvement in grain and forage quality are also continually in demand. Most of the grain sorghum grown in the region is acceptable as a feed grain, but would not be acceptable as a food grain. The changes needed to make an acceptable food grain (plant color and grain color) are relatively simple and highly heritable traits that are easily manipulated. If adopted, these changes will facilitate to opportunity to partially substitute domestically produced sorghum flour for more expensive imported wheat flour (INTSORMIL report #6, 2006, www.intsormil.org). However, food quality sorghum must possess resistance to grain mold and weathering to protect the quality of the grain prior to harvest. For forage, there has been relatively little improvement in the forage quality of sorghum grown in Central America. The development and adoption of brown midrib forage sorghums in the U.S. indicate that high quality forage sorghums can be produced (Oliver et al., 2005). The challenge is to introduce these characteristics into forage sorghum adapted to the Central American region.

As improvements in yield and quality are made, these must be protected from both abiotic and biotic stresses that are commonly present in the region. The predominant abiotic stresses involve drought and fertility and both genetic and agronomic management approaches must be used to mitigate these problems. Biotic stresses also pose a significant threat to yield and quality in sorghum production. In Central America, the predominant SDM pathotype is P5 and this pathotype is known to cause significant yield reductions in areas of the region where environmental conditions are

conducive to disease development (Frederiksen, 1988). While chemical control is a possibility, the most logical and reliable control mechanism is the incorporation of genetic resistance. Another disease of importance is anthracnose (caused by *Colletotrichum graminicola*), a fungal pathogen that is capable of infecting all above ground tissues of the plant that is endemic throughout the region. Because it can infect all above ground parts of the plant, it can cause significant reductions in both forage and grain yield and quality. Again, genetic resistance provides the only effective mean of managing this disease. Finally, grain mold (caused by a complex of fungi) is a common problem throughout the region and it reduces the quality of the grain as both a feed and food grain. In all of these abiotic and biotic stresses, sorghum germplasm has sufficient diversity to enable breeding programs to identify and select for tolerance and/or resistance to the specific stress or pathogen.

Objectives and Implementation Sites

Given the goals of the Sorghum, Millet and Other Grains CRSP and the needs of the Central American region, the overall goal of this proposal is to enhance the genetic yield and quality potential of sorghum genotypes adapted to Central America for use as a feed grain, food grain and forage crop. To meet this goal, we will use previously established linkages with collaborators in the Central American region (i) to coordinate in-country research studies and breeding evaluations, (ii) to identify quality students for training through involvement in ongoing projects at Texas A&M University, and (iii) to enhance technology transfer for sorghum in the Central American region.

The objectives, the location of the research, and the collaborators include:

DEVELOP HIGH-YIELDING, LOCALLY-ADAPTED SORGHUM VARIETIES AND HYBRIDS WITH IMPROVED GRAIN AND/OR FORAGE QUALITY, DROUGHT TOLERANCE, AND DISEASE RESISTANCE USING BOTH CONVENTIONAL BREEDING TECHNIQUES AND MARKER-ASSISTED SELECTION TECHNOLOGY. The goal of this objective is to extend the breeding and molecular technology provided by the principal investigator to collaborators to enable the development of new varieties specifically adapted to the Central American region. When successful, this objective will be result in the release of improved, locally-adapted cultivars to be used for grain and/or forage production.

IDENTIFY AND MAP GENES RELATED TO FORAGE YIELD AND QUALITY. The purpose of this objective is to understand the genetic control of important components to forage yield and quality and generate genetic markers that can be used by sorghum improvement programs in the near future.

IDENTIFY AND CHARACTERIZE GENES RELATED TO DISEASE RESISTANCE IN SORGHUM WITH SPECIFIC EMPHASIS IN DOWNY MILDEW, ANTHRACNOSE AND GRAIN MOLD. UTILIZE THESE SOURCES OF RESISTANCE IN BREEDING IMPROVED CULTIVARS AND HYBRIDS FOR CENTRAL AMERICA. Over the past ten years our program has screened numerous accessions to identify specific sources of resistance to anthracnose, downy mildew and grain mold. These

lines and populations derived from them are being evaluated in domestic and Central American sites to determine which sources will provide the most stable resistance.

IDENTIFY AND MAP GENES RELATED TO GRAIN QUALITY SUCH PROTEIN DIGESTABILITY, NUTRACEUTICAL POTENTIAL AND GRAIN QUALITY PARAMETERS PER SE. Variants that possess unique grain traits such as increased protein digestibility and enhanced antioxidant characters have been identified and characterized in our program. The purpose of this project is to assess the feasibility of producing cultivars that possess these characteristics. In collaboration with the TAMU grain quality program (L. Rooney, D. Hays), we are assessing the feasibility of combining both grain mold resistance and enhanced digestibility.

PROVIDE TECHNOLOGY TRANSFER AND TECHNICAL ASSISTANCE IN PROMOTING THE USE OF IMPROVED SORGHUMS AS A FEED GRAIN, FOOD GRAIN AND A FORAGE CROP IN CENTRAL AMERICA. The purpose of this objective is to transfer the technology and knowledge needed to effectively produce and utilize the forage and/or grain produced from the improved sorghum cultivars (Maicillos Criollos, lines and hybrids). As appropriate, our program will coordinate these workshops with collaborating scientists in the specific area of expertise, such as animal feeding (J. Hancock) grain quality and utilization for human food (L. Rooney), and agronomy and forage quality (J. Blumenthal). The technical assistance efforts will focus on industry and academic leaders in El Salvador and Nicaragua.

These five objectives merge together to provide a project that will have both short-term and long-term results. Objective 1 is a long-term and continual goal that will utilize the technology developed in objectives 2 through 4 and proven conventional breeding approaches. Objectives 2 through 4 should provide results in the short-term that will be important to work proposed in objective 1. The expected results of objectives 2, 3, and 4 include the identification of DNA-based markers to serve as tags for more efficient breeding. Objective 4 is a medium-term goal that will make the breeding programs and nutritionists more efficient in producing new cultivars that have enhanced market value. Ultimately, the success of objective 1 will be measured by the productivity of cultivars and hybrids developed in this project and how effectively they are utilized throughout Central America. For objectives 1 through 4, training of students from cooperating countries will be an integral part of the projects and potential students will be identified based on recommendations from researchers in the region and the in-country interaction of the PI with potential candidates. Finally, objective 5 is crucial because if the first four objectives are successful, additional sorghum (both forage and grain) with improved quality will be produced. It is imperative that there be the infrastructure (both technological and scientific) to utilize this grain. It should also be realized that while the efforts of this project are primarily targeted to Central America, the technology, basic knowledge, and personnel developed in this project will also be useful to sorghum and millet improvement programs in the United States and around the world. Because of these factors and their interrelationships, this project will address directly or indirectly all seven major goals of the Sorghum, Millet and Other Grains CRSP.

Research Strategy and Approach

DEVELOP HIGH-YIELDING, LOCALLY-ADAPTED SORGHUM VARIETIES AND HYBRIDS WITH IMPROVED GRAIN AND/OR FORAGE QUALITY, DROUGHT TOLERANCE, AND DISEASE RESISTANCE USING BOTH CONVENTIONAL BREEDING TECHNIQUES AND MARKER-ASSISTED SELECTION TECHNOLOGY.

Maicillos Criollos Breeding

Because these genotypes are photoperiod sensitive and they are uniquely adapted to the Central America, the breeding must be completed in the region. Segregating populations of breeding material from INTSORMIL was grown and selected in El Salvador for desirability, yield and disease resistance (see Central America Regional Report). On a regular basis these selections are advanced and the most advanced material is evaluated in replicated yield trials. To facilitate future development, a set of advance breeding material was sent to College Station Texas; and breeding crosses were made in greenhouse and winter nursery sites. These F1's are being grown in winter nurseries and F2 populations will be sent to El Salvador for selection in the fall of 2009. Many of these crosses were made between photoperiod sensitive material and photoperiod insensitive types to introduce specific traits such as disease resistance or enhanced forage or grain quality. Emphasis in selection is placed on improved food-type and Macio tan-plant cultivars as well as hybrids (where feasible).

Photoperiod Insensitive Line and Cultivar Breeding

Breeding lines for use as cultivars and/or parents in hybrids will use traditional pedigree breeding approaches, with populations generated from the Texas A&M University/Texas Agricultural Experiment Station sorghum breeding program. Over 3000 segregating rows, ranging from the F2 to the F5 were grown in South Texas for selection. Advanced lines were evaluated for grain yield and adaptation in hybrid combination. The best performing material from these trials is provided to the Central American programs for evaluation and testing in Central America. Traits of emphasis in grain types include but are not limited to grain yield, grain quality, disease resistance and drought tolerance. Traits of emphasis in forage types include but are not limited to biomass yield, forage quality, regrowth potential, foliar disease resistance and drought tolerance.

Forage Sorghum Breeding

Forage sorghums have become increasingly important in the Central American region; development of new varieties and hybrids with improved forage quality are important. Specific improvement involves incorporation of the brown midrib trait into existing and improved cultivars. Segregating progenies have been grown and selections made from these populations in both Texas and El Salvador; these lines are currently in evaluation in both line per se and hybrid combinations. Most of these selections are brown midrib.

IDENTIFY AND MAP GENES RELATED TO FORAGE YIELD AND QUALITY. In both the U.S. and Central America, interest in sorghum as a forage crop (and even as a potential bioenergy crop) has never been greater. In Central America, both CENTA and INTA have released both varieties and hybrids for use as silage and forage crops (see Central America Regional Report). In addition to breeding for standard forage sorghums, our program has provided sudangrass pollinator lines with bmr genotype to the CENTA program; the goal is to develop bmr genotypes for Central America with greater digestibility and palatability (Oliver et al., 2005). Additional breeding and evaluation of both bmr lines and corresponding hybrids is ongoing in the Texas A&M program; we have identified numerous combination that have bmr and are agronomically desirable as well.

In addition to breeding efforts, additional information on the genetic basis of biomass yield and how it is partitioned in the plant in botanical terms (stalks, leaves, and panicle) and compositional terms (carbohydrate, protein oil, ash, etc.) is critical to optimize production for specific end uses (forage, grain, or bioenergy). Our program has, in collaboration with researchers at Cornell University, recently published on QTL analysis of biomass partitioning in botanical and compositional terms (Murray et al., 2008a and b). This project identified a total of 145 QTL for 28 biomass and composition related traits. The results indicated that altering genetic potential for non-structural carbohydrate (primarily starch and sugar) as grain and stem sugar yield had greater impact on harvestable energy than altering grain and stem sugar composition. In the leaf and stem structural carbohydrates (ie, lignocelluloses), a total of 158 QTL were detected among the 41 different biomass and composition traits that were measured. Many of these traits co-localized with loci for height, flowering time and density/tilering, indicating a strong albeit not surprising, pleiotrophic effect between these traits.

IDENTIFY AND CHARACTERIZE GENES RELATED TO DISEASE RESISTANCE TO ANTHRACNOSE, GRAIN MOLD AND QUALITY, AND SORGHUM DOWNY MILDEW, UTILIZE THESE SOURCES OF RESISTANCE IN BREEDING IMPROVED CULTIVARS AND HYBRIDS FOR CENTRAL AMERICA.

Anthracnose Resistance Mapping

In Central America as well as the southern U.S., anthracnose (caused by *Colletotrichum graminicola*) can be a significant disease of sorghum. The disease can infect all above-ground portions of the plant, although infection in the leaves and stalks is usually the most economically damaging. Due to this, the disease can be very destructive to forage production because even if it does not reduce yield it will reduce forage quality. Over the past ten years, our program has identified new and unique sources of anthracnose resistance and this was highlighted in by Mehta et al. (2005) who described four sources of resistance controlled by different genes and determined that each was highly heritable. Our program has collaborated with molecular geneticists to identify at least one anthracnose resistance locus from SC748-5 to the end of linkage group 5 (Perumal et al., 2008).

Our program is currently expanding efforts in mapping anthracnose resistance; focusing on more detailed mapping of re-

sistance in SC748-5 as well as two other sources. Two different populations were planted for anthracnose evaluation in 2009 in three US locations. Unfortunately, the environments in 2009 were not conducive to the development of the disease and scoring was not possible in the main growing season. Currently, there are plans to repeat this evaluation in 2010.

Sorghum Downy Mildew Resistance

Sorghum Downy Mildew (caused by *Peronosclera sorghii*) is a significant pathogen of sorghum in both Central America and South Texas (Frederiksen, 1988). In endemic areas, the disease can be so severe that genetic resistance is the only effective means of limiting the damage. Fortunately, there are numerous sources of resistance to the disease, but the exact pathotype present in a region determines the best sources of resistance for use in breeding. In Central America, pathotypes 1, 3, and 5 have been identified so sources of resistance to these are critical for the region (Frederiksen, 1988). Previous research (some INTSORMIL funded) has identified several sources of resistance have been identified and within our program. We are continually evaluating and selecting for resistance in this material.

In addition to breeding with existing sources of resistance, there is a need to identify and characterize new and different sources of resistance to the pathogen. Our program has actively conducted SDM screening in Texas for the past five years and has identified a set of material that shows good resistance to at least two different SDM pathotypes (Isakeit and Jaster, 2005). These lines were screened in multiple locations against pathotypes 1, 3 and 6 (Isakeit and Jaster, 2005) and a total of 12 different accessions were identified with resistance. To determine if these sources possess the same source of resistance, they were hybridized in a partial diallel and segregating populations were derived from each. Segregation analysis of these populations indicates that there are at least three different sources of resistance; another is possible but contingent on confirmation with addition crosses that are currently not available. At this time, the plan is to create segregating populations for each unique source to determine the inheritance of the resistance and to transfer it to more adapted and useful germplasm.

IDENTIFY AND MAP GENES RELATED TO GRAIN QUALITY SUCH PROTEIN DIGESTIBILITY, NUTRACEUTICAL POTENTIAL AND GRAIN QUALITY PARAMETERS PER SE. Our two main projects in grain quality are (1) combining improved protein digestibility with enhanced grain mold resistance and (2) the development and characterization of high antioxidant "healthy" sorghums. Our program, utilizing highly digestible lines from the Purdue University program, has introgressed the highly digestible trait into traditional grain sorghum parental lines in our program. We are currently evaluating these lines for grain mold resistance (summarized by Portillo, 2007). Initial efforts to determine if these two combinations are feasible in the same genotype indicate that they are, to a limited extent. These lines represent an intermediate step in the development of high digestibility sorghums with enhanced grain mold resistance. Because of the increased protein digestibility, it has been hypothesized that they may be more efficient for both malting and ethanol production. In 2008, bulk production of these lines was completed and testing

for their efficiency of malting and ethanol production are being investigated in collaboration with J Taylor (Univ. of Pretoria) and D. Wang (Kansas State Univ.).

Another group of specialty sorghum receiving interest is the health food sorghums. These are grain sorghums with high levels of tannin and/or unique colors (primarily black); they possess very high levels of unique phenolic compounds that show high levels of antioxidant activity. Our program has developed a set of parental lines for use developing a series of lines designed to combine these traits into a single sorghum hybrid that could be grown as a "health" grain. While this does not directly affect efforts within Central America, it does provide the potential opportunity to be used in food products in the area. This work is in cooperation with the TAMU cereal quality lab (L. Rooney) and labs in Central American in CENTA (El Salvador) and at the Escuela Agricola Panamerica (J. Bueso). In 2008 and 2009 our program produced 30 experimental hybrids that were planted in replicated yield trials in four locations (Weslaco, Corpus Christi, College Station, and Halfway, Texas) to evaluate their relative agronomic potential, their antioxidant content and the effect of environment and genotype x environment interaction on those traits. These trials have been harvested and analysis is currently underway. From these trials, it is apparent that both genotype and environment influence antioxidant compound production and degradation and that certain environments are more conducive to their production than others.

PROVIDE TECHNOLOGY TRANSFER AND TECHNICAL ASSISTANCE IN PROMOTING THE USE OF IMPROVED SORGHUMS AS A FEED GRAIN, FOOD GRAIN AND A FORAGE CROP IN CENTRAL AMERICA. Technology transfer in the project is primarily in the form of germplasm supplied to the Central American Program. Our program has sent over 100 different parental lines and germplasm of grain and forage sorghum for evaluation in Central America. Technology generated in this project will be accessible through improved germplasm, both parental lines and cultivars that can be used by small farmers and the seed industry to enhance productivity and quality. Cultivars directed at subsistence production will be distributed in cooperation with National research programs (CENTA in El Salvador and INIA in Nicaragua for example). Lines that have potential as parents in hybrids will be distributed to commercial seed companies (both domestically and internationally); use of these lines in commercial products will require some form of licensing that will be determined on a case by case basis in which the involved parties will write the agreements.

Impact

This program focuses on the genetic improvement of sorghum with strong collaborations established with expertise in cereal chemistry, molecular biology, plant pathology, and agronomy. This will provide the critical mass of expertise to address problems that may arise during the research in sorghum. Given the development of sorghum cultivars and hybrids with improved quality and yield potential, and protection from pathogens such as anthracnose and grain mold, these crops should be more competitive with other cereal grains for end-use application in products for human and animal consumption. This is particularly important in the dry season in Central America and the Central U.S. where sorghum are

an important cereal grain. Increases in quality will enhance marketing opportunities and the potential for more favorable pricing. This will result in more stable income for producers and processors requiring high-quality grains for product development.

The success of the proposed research will result in technology transfer that includes the development of nutritionally enhanced sorghum lines and hybrids that can be grown in Africa, Central America, and the U.S. as well as technical assistance to effectively utilize these grains in human food and animal feed products. In many developing countries, this research will provide new entrepreneurial opportunities for production of animal feeds and forage as well as other products including meat and eggs. In developed countries such as the U.S., tan-plant sorghum hybrids will have enhanced marketing opportunities to industries that do not currently utilize sorghum or millet grain, particularly the U.S. poultry and food industries.

The genetic analysis described in this proposal will result in a better understanding of the genetic basis and relationship of genes controlling disease resistance (anthracnose, grain mold and SDM), yield (biomass), and quality (forage and grain) and genetic marker associated with each set of genes. These maybe used as markers in MAB and/or useful in isolating the gene sequence provided additional funding and access to the soon to be complete sorghum genome sequence. While this may not have immediate impact on Central America sorghum production, it does impact long term sorghum breeding efforts and that will impact all sorghum production in the future. A key product of this research will be marked "genes" that can be easily transferred to well adapted local cultivars. The need to verify the efficacy of the transferred genes will encourage further collaboration among US and developing country participants.

In addition to providing new cultivars and the technology to utilize them effectively, this training program promotes the development of human capital for enrichment of participating countries. Graduate students and visiting scientists with interest in crop improvement, crop utilization, and molecular biology will complete much of the proposed research. For each objective, as specific research projects are identified, students from target areas will be recruited to conduct this research at Texas A&M University. As appropriate, the students will be expected to collaborate with other investigators within this project and at the other university. This approach should expose the student to interactive and interdisciplinary research that will enhance his/her productivity upon return to their homes.

Evaluation of Project Impact

Crop improvement is a long term, continual process and measuring short term impact is often a challenging, but necessary task. To that end, short-term measurements of impact for this program will include: (1) the number of Material Transfer Agreements written for germplasm produced from this program, (2) the number of publications generated from research in the project, and (3) participation in research workshops and production shortcourses. Over the long-term, progress is easier to quantify and assess the impact. Several of the methods that we will use include: (1) the number of germplasm releases (including parental lines and cultivars) which

have been released and may be utilized by subsistence producers and/or commercial seed industry, (2) the number of hectares of a released cultivar and/or hybrid that are being grown in the region (either domestically or internationally), and (3) the production levels of the new varieties and the relative value of that production, and finally (4) to survey potential or actual end-users to determine if the new material has enhanced value for their particular use, and if so, attempt to determine a monetary value to the enhanced value.

Training of U.S. and Host Country Personnel

The PI in this project supports the collaborators in both El Salvador and Nicaragua. The PI traveled to Central America to interact, evaluate and collaborate on active research projects in the region. Funds are budgeted for support of a graduate student; it has been extremely difficult to identify acceptable and interested potential students. Mr. Ostilio Portillo, a Honduran will join our program in January 2010 to pursue a Ph.D in plant breeding.

Contribution of Proposed Research to the Sorghum Millet and Other Grains CRSP

The objectives of this proposal are designed (1) to fit precisely within this CRSP's vision, mission and global strategy for research, and (2) to complement and extend the efforts and the expertise of the INTSORMIL research team. The team assembled for this proposal is interdisciplinary and international in nature with a focus on three regions of the world in which INTSORMIL activities are concentrated. The proposed research will result in new and more competitive grain markets for sorghum and pearl millet. Enhanced value of these crops will contribute to a shift of sorghum and pearl millet from subsistence to cash crops in developing countries. Improvements in nutritional as well as grain quality characteristics (i.e. food-grade sorghums) will make sorghum more competitive with other cereal grains for end-use applications in the U.S. and in host countries. In addition, the development of these value-enhanced grains and the transfer of animal feeding technologies will promote the development of new entrepreneurial opportunities for production of meat and other animal products in countries where these crops are grown. Finally, the development of more competitive sorghum and millet cultivars will allow producers to conserve water resources that would otherwise be used by less water-efficient crops.

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Breeding Sorghum for Improved Resistance to Biotic and Abiotic Stresses and Enhanced End-Use Characteristics for Southern Africa

Project TAM 102
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Introduction and Justification

Sorghum is a major food and feed grain in the semi-arid tropics. It is ideally suited to marginal semi-arid environments due to its efficient water use, tolerance to high temperatures, multitude of uses (grain, forage, biomass), ability to produce harvestable grain yield in diverse cropping systems, and performance in rotation systems. Sorghum production is constrained by less than desired yield, biotic (insect pests and disease pathogens) and abiotic (primarily pre- and post-flowering drought) stresses that reduce yield, lower value of grain and forage quality, and government policy. Primarily a feed-grain in the U.S. demand for sorghum for ethanol production and as a food grain or nutraceutical is increasing. Sorghum is an ideal crop to enhance the economic viability of U.S. Great Plains agriculture through improved utilization of limited water resources and increased yield, quality (grain and forage), and marketing opportunities. The overall objective for this project is to develop new genetic technology (germplasm, parental lines and cultivars) with enhanced adaptation, increased grain yield potential, and resistance to multiple abiotic and/or biotic stresses.

U.S. research is directed at developing germplasm and parental lines suitable for use as hybrid seed parents. Restorer lines (pollinators or males in A1 cytoplasm) have been selected for disease resistance, improved weathering resistance, and wide-adaptation. Analysis of data from on-station replicated grain yield trails led to the identification of several parental lines that will produce at least 5% more grain than a common check hybrid. The superior lines will undergo additional evaluations in subsequent years. If the re-

sults are confirmed the lines will be made available to private seed companies for further evaluation and possible commercialization.

In Mozambique several experimental breeding lines have been identified for possible release as varieties and replicated trials were conducted at several locations to evaluate for adaptation and grain yield potential. The lines represent nine different pedigrees and were selected from nurseries developed for resistance to sorghum midge, grain weathering, and drought tolerance. In South Africa and Botswana, potential new varieties express a high level of resistance to sugarcane aphid and grain yield potential at least equal to the standard checks.

Objectives and Implementation Sites

- Develop sorghum genetic technology (germplasm, inbred lines and cultivars) resistant to selected biotic stresses.
- Develop sorghum genetic technology resistant to pre- and post-flowering drought stress
- Develop sorghum genetic technology with improved grain quality and grain mold/weathering resistance
- Develop sorghum genetic technology with improved grain yield and adaptation for diverse cropping systems and environments
- Evaluate forage and sweet sorghums for biomass and potential use in cellulosic ethanol production
- Contribute to host-country institutional human capital development through short-term (non-degree) and long-term (M.S. and Ph.D.) educational opportunities

Segregating populations are developed in Texas and selected for adaptation and resistance to selected diseases and/or drought tolerance, and grain mold/weathering resistance. Appropriate germplasm provided to host country collaborators provides the opportunity to evaluate the populations in indigenous cropping systems for traits of interest and adaptation. The multi-disciplinary research team includes plant breeders, entomologists, plant pathologists, and food scientists with the expertise and programs to develop and deliver new technology. Texas nursery sites that provide geographic diversity for selection and evaluation include the Coastal Bend for tropical adaptation and resistance to grain weathering, sorghum midge and disease(s) and the Southern High Plains for a semi-arid temperate adaptation for yield potential and drought tolerance. A Puerto Rico winter nursery provides an extra growing season to reduce development time for new varieties, parental lines, or hybrids. Southern Africa locations provide additional evaluation environments - yield potential and adaptation nurseries in Zambia (Golden Valley Agricultural Trust at Chisamba), Mozambique (Nampula), Botswana (Sebele), and South Africa (Cedara), insect resistance screening in glasshouse and field facilities at the Botswana College of Agriculture (Sebele) and at the ARC-GRI (Potchefstroom) and Cedara, and disease resistance evaluation at Cedara (anthracnose, grain mold, and ergot). Cereal quality laboratories at the Univ. of Pretoria or the ARC-Grain Crops Institute Quality Laboratory (Potchefstroom) will provide the opportunity to analyze advanced germplasm for milling qualities in comparison with local checks. Collaboration with the ARC-Grain Crops Institute at Potchefstroom has been suspended due to lack of a signed memorandum.

Research Methodology and Strategy

Primary breeding methodology is the pedigree system. Segregating populations, advanced lines and hybrids undergo multi-location testing to identify plants with the genetic combinations for the best expression of the trait(s) of interest. Selection in diverse environments should identify widely adapted multiple stress resistant genotypes.

For Southern Africa primary biotic stress resistance traits are for adaptation to indigenous cropping systems, seedling and adult plant stage resistance to sugarcane aphid, sooty stripe, leaf blight, anthracnose, and grain mold with sorghum midge resistance incorporated as necessary. As needed, populations to combine drought tolerance with biotic stress resistance are developed. Grain from experimental entries with the highest grain yield will at the appropriate stage of development undergo standard grain quality analysis including diastasis (the chlorox bleach test, malting, germination, and distase), presence of polyphenols, abrasive milling, roller milling and meal color.

For the U.S. selection is practiced for resistance to head smut and foliar diseases including anthracnose, downy mildew, bacterial streak, bacterial stripe, rust, zonate leaf spot, grain weathering resistance, and drought resistance. Advanced lines are evaluated as hybrid parents for combining ability and adaptation. Seed of advanced lines and hybrids will be provided at the appropriate time to the TAMU Cereal Quality Lab for standard grain quality analysis. The entries will be screened for: density (g/mL), protein

and moisture and starch use NIR (near infra-red) non-destructive analysis, kernel hardness and weight, diameter (mm), and color.

Linkages with private industry facilitates identification and evaluation of new genetic technology. New genetic technology will be available to private industry through material transfer agreements.

Research Results

Research in the U.S. was hindered by extreme drought in the Texas Coastal Bend. Lack of rainfall resulted in insufficient soil moisture to establish research plots and selection nurseries. No plots planted in the region in 2009 reduced the scope of research activities.

Sugarcane aphid (*Melanaphis sacchari* (Zehntner)) trials were provided to collaborators at the University of the Free State and the Botswana College of Agriculture (BCA-Sebele). Research in South Africa was hindered by the seed shipment from the U.S. being held in South African customs and neither the sender or recipient receiving notification in time to plant the trials. The trials will be planted in the 2009-2019 growing season.

Trials planted at Cedara, South Africa in the 2008-09 growing season were from remnant from superior entries in previous years trials. A sugarcane aphid screening/yield trial of with 24 entries was planted at Cedara. The trial consisted of 16 entries from the 2008-09 sugarcane aphid trial, six entries from the 2008-09 sugarcane aphid yield trial and two local hybrid checks. Severity of infestation was evaluated when the majority of panicles reached the milk stage. Severity of infestation was evaluated on a 1 to 5 scale, where 1 = no aphids present on plants, 2 = light infestation with aphids present on a few leaves (no dead leaves), 3 = moderate infestation with aphids present on two to three leaves (one or two dead leaves may be present), 4 = high infestation with aphids on nearly all leaves (many dead leaves) and 5 = majority of plants in plot dying. Plants with a rating of 1 or 2 were considered resistant, while a rating of 3 indicated an intermediate level of resistance. Plants with a rating of 4 and 5 were considered susceptible.

Results indicated that 42% of the entries rated 1 on the scale, indicating none to very little damage (Table 1). Thirty-three percent of the entries were rated 2, 17% rated 3, and 8% were highly susceptible with a rating of 4. The high level of resistance expressed was not unexpected as the entries had been previously screened for resistance, and only those with a high level of resistance selected for subsequent evaluation. Yield of the experimental entries, all cultivars, varied between 0.94 and 4.5 tons per hectare. The standard hybrid checks produced grain yield of 2.13 t/ha (PAN 8420) and 2.87 t/ha (PAN 6848). The grain yield of five experimental cultivar entries was significantly better than the hybrid PAN 8420 and one of the entries also produced significantly more grain than the hybrid PAN 6848. Cultivars producing more grain yield than hybrids is unusual but encouraging and indicates the potential usefulness of the experimental germplasm. Hybrid should be made using the experimental cultivars for subsequent evaluation. While cultivars will initially work for small-holder farmers sustainable development progress will be greater with hybrids and the associated availability and access to inputs, primarily fertilizer. Culti-

Table 1. Evaluation of sorghum lines for sugarcane aphid resistance and grain yield at Cedara, South Africa.

Pedigree/Designation	Sugarcane aphid damage†	Grain Yield t/ha
(Segaolane*WM#322)-LG2-LG2-(03)BG1-LG1-LBK	1	4.46#
(Macia*TAM428)-LL2	1	3.59#
CE151	4	3.52#
(6BRON161/(7EO366*Tx2783)*CE151)-LG5-CG2-(03)BG1-BG2-LBK	1	3.17#
(Macia*TAM428)-LL9	1	3.15#
PAN 6848 (standard check)	1	2.87
Tegemeo	2	2.73
(9MLT176/(MR112B-92M2*Tx2880)*A964)-CA3-CABK-CCBK-CABK	3	2.36
(LG35*WM#322)-BE40-LG1-CA1-LGBK-CABK	2	2.14
PAN 8420 (standard check)	3	2.13
(9MLT176/(MR112B-92M2*Tx2880)*A964)-LG8-CABK-LGBK-LGBK	2	2.06
TAM428	2	1.90
(5BRON151/(7EO366*GR107B-90M16)*Tegemeo)-HG7-CC2-CABK	2	1.85
Segaolane	3	1.66
(Dorado*Tegemeo)-HW13-CA1-CC2-LGBK	2	1.54
(5BRON151/(7EO366*GR107B-90M16)*Tegemeo)-HG1-LGBK-CABK	2	1.43
(Kuyuma*5BRON155)-CA5-CC1-CABK	1	1.43
Kuyuma	1	1.42
(A964*P850029)-HW6-CA1-CC1-LGBK	2	1.42
(Dorado*Tegemeo)-HW14-CA1-CC2-CABK	3	1.38
SRN39	4	1.31
Ent62/SADC	1	1.22
(Dorado*Tegemeo)-HW15-CA1-CC2-LG1	2	0.94

† Rated on a scale of 1 = no aphids present on plants, 2 = light infestation with aphids present on a few leaves (no dead plants), 3 = moderate infestation with aphids present on 2 or 3 leaves (one or two dead leaves may be present), 4 = high infestation with aphids on nearly all leaves (many dead leaves) and 5 = majority of plants in plot dying.

Significantly higher yield than the standard hybrid check PAN 8420.

\$ Significantly higher yield than the standard hybrid check PAN 6848.

vars can be developed and released by the collaborative program but production and marketing of hybrids will require the participation of private seed companies.

Botswana College of Agriculture collaborators planted two trials provided by this project - a 22 entry advanced trial composed of 15 experimental lines previously evaluated for resistance to sugarcane aphid resistance and 7 local checks, and a 45 entry preliminary screening trial. In the advanced trial, average abundance of the sugarcane aphid infestation was significantly affected by genotype and plant age. Three experimental entries - (Macia*TAM428), (6BRON161*CE151), and (Segaolane*WM#322) - did not differ from the resistant check TAM428 for mean aphid numbers. In a grain yield trial at Cedara, South Africa the three entries produced the most grain yield in a yield trial and produced significantly more grain than the hybrid check PAN 6848. This indicates that a high level of sugarcane aphid resistance has been incorporated into lines with high grain yield.

Aphid infestation increased with plant age and the abundance of infested plants could be arranged in the order of 74 days old > 54 days old > 47 days old > 40 days old. The sugarcane aphid infestation rapidly increases with once initial infestation has occurred with an approximate 9.9x increase in proportion of infested plants. Thus maximum aphid infestation occurs later in the season and coincides with grain fill and maturation.

Entries in the preliminary screening trial exhibited no significant differences in the average abundance of sorghum plants attacked by the sugarcane aphid. However, damage ratings varied from 1 (0-20% damage) to 3 (41-60% damage) indicating that the entries express different levels of resistance. Lines rated a 1 or 2 at both 47 and 74 days after emergence would be classified as resistant. The trial will be repeated in the 2009-2010 cropping season and if the preliminary results are confirmed the entries will be advanced to a yield trial.

The purpose of the sugarcane aphid resistance breeding program is to develop improved cultivars suitable for use in small-holder production systems with resistance to sugarcane aphid. New cultivars should be tan plant and white grain with excellent resistance to aphid and foliar disease, grain yield at least equal to local checks, and good grain mold resistance. Results indicated that sugarcane aphid resistance has been incorporated into elite cultivars with grain yield potential equal to a standard local hybrid check and significantly better than common cultivar checks. Grain will be grown during the next growing season in on-farm trial to better identify performance in the local production system. The overall objective of the program is to release at least one improved variety. The research program is making excellent progress toward this objective.

The Mozambique national sorghum breeding program continued to evaluate the grain yield potential of germplasm from Texas A&M University sorghum trials provided to the National Agrarian Research Institute (IIAM). In 2008-09 lines were in replicated yield trials at several locations in Mozambique to evaluate for grain yield, adaptation and biotic (disease and insect) resistance. Designation/pedigree of the lines are:

- 03CM15067 (((((Tx2880*(Tx2880*(Tx2864*(Tx436*(Tx2864*PI550607)))))))-PR3-SM6-CM3-CM1-CM2-CABK-CABK-CGBK
- 03CM15012 (85OG4300-5*Tx2782)-SM5-CM2-SM2-SM1-CABK-CMBK-CMBK
- 02CM1104 (((((Tx2880*(Tx2880*(Tx2864*(Tx2864*PI550610)))))))-PR3-SM6-CM3-CM2-CG3-BGBK-CABK
- Sureño
- 01CS20538 (90LI9178 - (M84-7*VG153)-LBK-PR7-L4-L2
- 02CS30445 (99CA3019 - (VG153*(TAM428*SBIII))-23-B32-BE2-BE1)
- B409 (B1*(B7904*(SC748*SC630))-HF17B
- 02CS5067 (B1*BTx635)-HF8
- 01CS19225 (B35*B9501)-HD9

Preliminary data analysis indicated that several of the lines express grain yield better than the local check Macia (2.54 t/ha). Multi-location evaluation trials will continue and selections compared with the local checks Macia and Sima. Eventually, experimental entries that produce acceptable grain yield and end-use quality will be released varieties in Mozambique.

Due to drought the sorghum midge resistant breeding nursery was not planted at Corpus Christi. The drought was extensive and no other locations were available to plant the nursery in an environment to obtain a damaging pest population density at anthesis. The program will be resumed in 2010 if there is sufficient planting moisture at Corpus Christi.

To evaluate hybrid combining ability and grain yield potential of new germplasm releases and advanced experimental lines three replicated yield trials were conducted at the Texas AgriLife Research Center, Lubbock during 2008. All trials had three replications with a plant population of approximately 52,000 plants per acre. The experimental site received one pre-plant and two post-plant irrigations.

Yield trial 1 was developed to evaluate the grain yield of recently released pollinator lines on standard A-Lines and proprietary A-lines from a seed company. The purpose was to generate data that might be more relevant to private industry. The test mean was 5281 lbs/A. The standard checks ATx2752*Tx2783, ATx631*RTx430, ATx2752*RTx430, ATx399*RTx430 and ATx399*Tx2737 produced 7659, 7447, 7113, 6839, and 6286 lbs/A, respectively. Twenty-one hybrids produced more grain than the test mean with yield ranging from 5393 to 7340 lbs/A. The two best experimental hybrids both had the same pollinator, Tx2945, a tan plant and red grain line released in 2006. Test weight of the higher grain experimental hybrid was at least as good as the standard checks. All of the hybrids would be classified as medium maturing with days to anthesis of 54 to 58 days after planting. Grain yield of hybrids on proprietary A-lines did not differ from that of standard A-line checks. Thus while the proprietary A-lines may have different genetics the standard A-lines will produce useful data for identifying superior pollinators.

Yield trial 2 evaluated the combining ability of experimental tan plant and white or red grain pollinators on the standard A-line ATx631 and included 49 experimental hybrids and 5 standard

checks. Grain yield of the standard checks ATx2752*T_x2783, ATx2752*RT_x430, ATx399*RT_x430 and ATx399*T_x2737 was 7206,6585, 5984 and 4825 lbs/A, respectively. The standard tan plant white grain check ATx631*RT_x436 produced 5017 lbs/A (Table 4). Nineteen hybrids produced more grain than the test mean of 5246 lbs/A. One experimental hybrid, ATx631*6BRON277, produced more grain than four of the five standard checks. Test weight was not different between the checks and the experimental entries. Additional research will be conducted to evaluate the combining ability and grain yield potential of the experimental pollinators.

Yield trial 3 was to evaluate the grain yield potential of experimental tan plant and white or red grain pollinators on proprietary A-lines. The purpose was to generate data that might be more relevant to private industry. In the 72 entry test there were 5 standard checks (including traditional purple plant and red grain hybrids) and 67 experimental entries. Grain yield of the standard checks ranged from 7765 lbs/A for ATx631*T_x2737 to 7743 lbs/A for ATx2752*RT_x430 to 6040 lbs/A for ATx399*RT_x430 to 5801 lbs/A for ATx399*T_x2737. Twenty-eight hybrids produced grain yield better than the test mean (5213 lbs/A). The experimental pollinator 4BRON262 produced a hybrid with grain yield in excess of 7,400 lbs/A. Four of the top five hybrids are experimental entries with the two ATx631*6BRON277 and ATx631*03BRON172 produced 6680 and 6434 lbs/A respectively. Test weight was similar to the standard check.

Research with the TAMU Cereal Quality Laboratory continued to study the flavonones eriodictyol and naringenin in lemon yellow grain. The compounds have potential benefit as nutraceuticals in sorghum. Seed samples of 54 lemon-yellow grain color germplasms representing 7 different pedigrees were analyzed for presence of eriodictyol and naringenin. All of the samples are from tan plants, a prerequisite for high levels of the flavonones. Six samples had high concentrations of the compounds. Two samples with the pedigree of B.HF14*B8PR1011 are potential A-lines for hybrid production and will be entered into a sterilization nursery. Selections were made in new additional segregating populations to for lemon yellow grain color and better agronomic traits. Three experiments were initiated to study the accumulation of the flavonones. The first study involves the effect of sunlight on compound accumulation. Five panicles in different lines with known compound concentrations were bagged (a paper bag placed over the panicle prior to anthesis). Grain from bagged and unbagged panicles will be analyzed for compound concentration. The second study involves compound concentration accumulation during grain maturation. Panicles of Tx2953 were harvested at 7 days post-anthesis, and then every 7 days for 7 weeks. The third study is to evaluate growing of grain mold fungi and their potential use of the compounds as a food substrate. Seven lines with known concentrations were sprayed with a fungicide 1, 2 and 3 weeks after flowering. The fungicide should control growth of the grain molds. Samples from each study were collected but grain analysis has not been completed at the time of this report.

Interest in using sorghum for brewing and malting is increasing. The contribute to research in Southern Africa seed of the original sorghum malting cultivar ‘Barnard Red’ was obtain. The cultivar was crossed to elite adapted cultivars to initiate the

process of developing populations to select for enhanced levels of brewing and malting quality in elite adapted cultivars and to develop populations for potential graduate student research. Two additional cultivars, ICSV400 and ICSV111, were obtained and will be sent to the Puerto Rico winter nursery to develop populations for selection and research.

A Puerto Rico winter sorghum nursery contributed to research progress. The nursery was used produce samples for a lemon-yellow study, to produce seed of new sweet sorghum populations and grain populations with potentially unique grain yield genes, to incorporate the brown midrib (bmr) traits in grain populations, to grow F1 cross seed, make additional backcrosses for sterilization of potential new A-lines, and increase A-line seed to produced hybrids.

Achievement of Activities Proposed in Work Plan

All activities proposed in the Work Plan were accomplished. For the U.S. the proposed activities included: evaluation of germplasm and populations already in the breeding program to determine reaction to important biotic and abiotic stresses; increase lines and exotic cultivars useful in developing new populations; evaluate and select segregating germplasm for resistance to selected biotic (disease: headsmut, downy mildew, anthracnose, rust, zonate, grain weathering) and abiotic (pre- and post-flowering drought) stress; evaluate advanced lines as hybrid parents for grain yield, biotic and abiotic stress resistance, and adaptation; develop new segregating populations based upon results of trials; distribute to collaborators replicated trials of advanced germplasm potentially useful in southern Africa cropping systems; utilize a Puerto Rico winter nursery to develop new breeding segregating populations, identify F1 plants, increase exotic cultivars and adapted lines, continue sterilization of potential new A-lines; distribute seed of released lines; evaluate forage and sweet sorghum populations for adaptation to a semi-airod production system; provide seed of lemon yellow grain lines for analysis.

For Southern Africa the proposed activities included: travel to the region to consult with collaborators and develop specific work plans; collaborate with regional scientists to evaluate sorghum for the traits (adaptation, grain yield, disease resistance, insect resistance, drought tolerance, grain quality and grain weathering) necessary for developing improved sorghum cultivars for local production systems; distribute trials of germplasm potentially useful in the indigenous cropping system(s); develop new segregating populations based on research findings; select germplasm for use

Relationship and contribution to INTSORMIL Strategic Plan objectives, target, benchmarks and indicators

Objectives	Targets	Benchmarks and Indicators	Throughputs
Nutrition, health and grain quality	- Higher grain quality cultivars - Increased nutrition of food and feed products	Development of cultivars with improved grain properties	Release of cultivars with improved grain quality
IPM	- Increased grain quality - Reduced pesticide use	Tolerance to grain insects and/or pathogens	Release of insect and/or disease resistant cultivars
Genetic enhancement	- Stable yielding genotypes	- Genotypes with less variation in yield - Decrease in drought damage	Stable yielding and/or drought tolerant cultivars released
Genetic resource and biodiversity	Higher yielding genotypes	Selection of high yielding genotypes	Increase in yield of new genotypes

in local production systems; participate in graduate training for regional breeders as appropriate.

Progress can be measured in the eventual release of new germplasm or cultivars. A new released germplasm or cultivar may be classed into more than one objective.

Networking Activities

Participated in the Sorghum/Millet Germplasm Committee meeting, ATSA Corn and Sorghum Research Conference, December 11, 2008, Chicago, IL.

Participated in the Texas Seed Trade Association Production and Research Conference, February 2-3, 2009, Dallas, TX

Participated in the INTSORMIL Technical Advisory Committee meeting, July 16-17, 2009, Lincoln, NE.

Participated in the SICNA/Great Plains Sorghum Conference August 11-12, 2009, Amarillo, TX

Travel to Botswana and Zambia, October 31 - November 12, 2008. In Botswana participated in the Alternative Cereal Processing Technologies Workshop held at the National Food Technology Research Center, Kanye. The workshop was attended by approximately 65 participants. Met with Botswana College of Agriculture collaborator. In Zambia met with representatives of the National Institute for Scientific and Industrial Research to evaluate their research activities and possible collaboration with INTSORMIL. Met with representatives of the University of Zambia School of Agricultural Sciences to discuss on-going collaboration. Met with the SABMiller Lusaksa Technical Director to discuss the continued progress of Eagle clear lager beer.

Travel to Mozambique, South Africa and Zambia, February 24 to March 13, 2009. In Mozambique met with entomology and breeding collaborators to evaluate development of their respective research programs and evaluation of germplasm selected from Texas developed populations. In South Africa met with University of the Free State collaborator to discuss graduate training and evaluation of sugarcane aphid resistant germplasm for disease resistance. Met with ARC collaborator to discuss progress in evaluation of germplasm for resistance to sugarcane aphid. In Zambia met with collaborators from the Zambia Agricultural Research Institute and review status of the regional program.

Seed of the following nurseries/test was distributed: All Disease and Insect Nursery (ADIN), Uniform Head Smut Nursery (UHSN), Sugarcane Aphid Test (SCA), Sugarcane Aphid Yield Test (SCAY), Midge Line Test (MLT). Seed was provided to private companies as requested under terms of a Materials Transfer Agreement (MTA).

Publications and Presentations

Peterson, G.C., K. Schaefer and B.B. Pendleton. 2009. Registration of 16 sorghum germplasm lines. *J. of Plant Registrations* 3(2):203-205.

B. Jampala, D.B. Hays, B. Rooney, G. Peterson, D. Wang. 2009. Designer Sorghum: Combining the high digestible and waxy grain traits for improved nutrition, bioethanol, beer, food and feed products. SICNA/Great Plains Sorghum Conference, August 11-12, 2009, Amarillo, TX.

Peterson, G.C. 2009. Sorghum Breeding at Lubbock: 100 Years of Progress. Centennial celebration of the Texas AgriLife Research and Extension Center at Lubbock, September 17, 2009, Lubbock, TX.