# Germplasm Enhancement and Conservation

# Breeding Pearl Millet for Improved Stability, Performance, and Pest Resistance

## Project ARS 206
## Jeffrey P. Wilson
## USDA-ARS


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### Summary


The goals of project ARS 206 have been to increase diversity of pearl millet germplasm available to breeders and researchers, identify sources of disease and pest resistance for pearl millet improvement, develop pearl millet with high yield, disease resistance, and superior grain quality for diverse uses, and expand markets for pearl millet.

Greater collaboration among researchers was fostered. Linkages and germplasm exchanges were established among relatively isolated pearl millet breeding programs within and across the West and Southern African regions. Simon Awala (Namibia) participated in short-term training with USDA-ARS, and Ferdinand Muuka (Zambia), Issaka Ahmadou (Niger), and Isaac Mbaiwa (Botswana) participated in the "International Training Course on Pearl Millet Improvement and Seed Production" at Patancheru, India.

Pearl millets Gwagwa and ICMV IS 89305 were high yielding and resistant to downy mildew across multilocation trials in West and Southern Africa. These and other distributed germplasm have been used as parents to introduce new traits into locally-adapted varieties. In similar evaluations for combining ability in hybrid combination, SoSank and SoSat C-88 tended to produce the highest-yielding hybrids.

Advances in breeding for resistances to other pests were made. Among diverse pearl millets from West Africa, SoSat C-88 was among the most resistant to the root-knot nematodes. Resistant progeny lines were produced from SoSat, Zongo, Gwagwa, and P3Kollo to develop nematode-resistant versions of these popular varieties. Wild pearl millets with resistance to *Striga hermonthica* were genetically diverse and may represent distinct sources for resistance breeding.

Tolerance to drought and improved nitrogen utilization is associated with stay-green. A non-destructive method for quantifying staygreen in pearl millet was developed. Staygreen was expressed as a dominant or overdominant trait, which will simplify its use in hybrid production.

Promising new hybrids for the U.S. were identified through multocation trials in Georgia. Hybrids (506 x 2304) and (606 x 2304) had yields ranging from 17 to 38% greater yield than the commercial standard Tifgrain 102. Increased yield will improve grower profit and expand cultivation.

Expanding markets will stabilize prices for grain. Pearl millet was shown to be a viable supplemental feedstock for ethanol production using standard maize-to-ethanol processes. Pearl millet ferments 30% faster than maize, and the net value of the products has the potential to result in greater economic value than maize feedstocks.

Significant advances have been made in developing collaborative research networks, identifying pearl millets with stable yield and downy mildew resistance across diverse locations, advancing the development of resistance or tolerance to biotic and abiotic

stresses, and improving profitability and developing new uses for U.S. growers.

## Objectives, Production and Utilization Constraints

### *Research Objectives*

**Increase yield and economic growth through:**

- Increasing diversity of pearl millet germplasm available to breeders and researchers
- Identifying sources of disease and pest resistance for pearl millet improvement
- Developing pearl millet with resistance to multiple diseases, high yield, and superior grain quality for diverse uses
- Expanding market opportunities for pearl millet by identifying new uses

### *Production and Utilization Constraints*

New pearl millet genotypes must be an acceptable type for cultural practices in their target production areas. The concept of desirable plant type differs dramatically between African and U.S. production systems. Yet, in both systems the production of high-quality pearl millet grain and forage is a result of the interaction between plant variety, diseases and pests, and environmental production constraints. The effects of these factors must be assessed when developing new germplasm and varieties. Developing the commercial potential of pearl millet requires an understanding of the needs of and constraints faced by producers, providing a consistent product that meets market standards, and identifying uses in which pearl millet has a competitive advantage compared to alternatives.

## Research Findings and Project Output

### *Improve Institutional Capacity*

*Linking and Building Capacity in African Pearl Millet Breeding Programs*

Linkages were developed for more effective research, dissemination of advances from individual research programs, and institutional capacity building. Samples of 86 pearl millets were acquired from breeding programs in West and Southern Africa. These represent new cultivars and advanced breeding lines with high grain quality, fertility restoration for specific cytoplasms, resistance to diseases or pests, or superior agronomic traits. Forty of these germplasms were distributed to collaborators in West Africa (Senegal, Mali, Burkina Faso, Ghana, Niger, and Nigeria) and Southern Africa (Zambia, Namibia, South Africa, and Botswana).

Through this network new germplasm was widely disseminated across essentially isolated pearl millet breeding programs throughout Africa. Linkages between the West and Southern Regions were fostered. These germplasms have been used by the breeders to introduce new traits into their programs, and will lead to varieties with improved yield and adaptation.

Simon Awala (Namibia) participated in a short-term training opportunity with USDA-ARS, and Ferdinand Muuka (Zambia), Issaka Ahmadou (Niger), and Isaac Mbaiwa (Botswana) participated in the “International Training Course on Pearl Millet Improvement and Seed Production” at Patancheru, India.

### *Increase Yield*

*Pearl Millet Germplasms with High and Stable Yield and Disease Resistance*

Multi-location evaluations can quickly identify genotypes with superior grain yield and quality, and resistance to diseases and pests. Collaborative, trails were established throughout West and Southern Africa to characterize selected pearl millets for high and stable yield over a range of production environments. Trials were conducted in 2003 and 2004 in Burkina Faso, Ghana, Mali, Niger, Nigeria, Senegal, Zambia, and South Africa.

Grain yield across locations was greatest for varieties CIVT, ICMV IS 89305, SoSat C-88, Gwagwa, SoSank, NKK, and GB 8735. Across locations, the most downy mildew resistant accessions were Gwagwa, ICMV IS 89305, ICMV IS 90311, Synthetic 1-2000, Zatib, ¾ ExBornu, and ¾ Souna. Grain yield was correlated with days to flowering, plant height, and panicle length. Grain yield was negatively correlated with downy mildew incidence. High-yielding varieties within locations varied, and germplasm introduced from other programs was frequently ranked among the top-yielding varieties.

*Combining Ability for Grain Yield and Pest Resistance in Pearl Millet*

Determining combining ability is valuable to identify prospective parents for hybrid production. Pearl millets were assessed for general and specific combining ability for yield and resistance to diseases in multilocation evaluations. Varieties that had superior performance across multilocation trials in 2003, or superior performance within each test location were crossed in a half-diallel design and grown in Burkina Faso, Ghana, Mali, Niger, Nigeria, Senegal, and Zambia for evaluation in 2005. This network of researchers characterized the germplasm to assess yield, agronomic characteristics and superior resistance to pests and diseases.

Across all locations, (SoSank x SoSat C-88) was the top-yielding hybrid combination, followed by (SoSat C-88 x Indiana 05), (SoSank x ICMV IS 89305), (SoSank x Gwagawa), and (SoSat C-88 x GB 8735). Yields were lowest for (Manga Nara x SoSat C-88), (GB 8735 x CIVT), (Gwagawa x CIVT), (Manga Nara x GB 8735), and (CIVT x ICMV IS 89305). Yield was correlated with days to flowering, plant height, and panicle length. Grain yield was negatively correlated with downy mildew incidence.

Downy mildew incidence was recorded in all locations except Burkina Faso and Zambia. Downy mildew was greatest in Mali, and least in Nigeria. Over all locations, the most resistant hybrids were (Indiana 05 x ICMV IS 89305), (Gwagawa x SoSat C-88), (SoSank x Indiana 05), (SoSank x ICMV IS 89305), and (SoSank x Gwagawa). The most susceptible hybrids were (Manga Nara x SoSat C-88), (GB 8735 x ICMV IS 89305), (GB 8735 x CIVT), (Manga Nara x GB 8735), and (HKP (GMS) x GB 8735).

*Striga* was observed only in Niger and Nigeria, and infestation was greater in Niger. In these environments, SoSat C-88 and (SoSank x SoSat C-88) were more susceptible, and HKP (GMS) and Indiana 05 were the most resistant entries. Smut was recorded only in Ghana. All entries had some level of smut, but (GB 8735 x CIVT) was most susceptible. Grain mold data were recorded only in Zambia. All genotypes had some level of grain molds. GB 8735, (SoSat C-88 x Indiana 05), (Gwagawa x GB 8735), (GB 8735 x Indiana 05), and (CIVT x Indiana 05) were more susceptible to grain mold.

*A1 and A4 Restorers for African Pearl Millet Hybrids*

Suitable fertility restorer inbreds are required for the development of pearl millet hybrids for African production systems. Crosses were made between diverse African pearl millet cultivars and Tift 454 (cms A1 restorer) or 99-70 (cms A4 restorer). Head-to-row selections were made in Tifton GA from 2003 to 2005. From these selections, 82 promising F4 lines were grown at Nigeria and Ghana in 2006 to assess days to flowering, downy mildew, panicle length, and grain yield.

Of the progeny with Tift 454 parentage, derivatives of (SoSat C-88 x Tift 454) had greater downy mildew resistance coupled with higher yield. Derivatives from Zongo tended to be most susceptible to downy mildew. Of the progeny with 99-70 parentage, derivatives from SoSat and Ankoutess had greater downy mildew resistance and higher yield. Downy mildew resistance in (SoSat x 99-70) progeny were greater than the counterparts derived from Tift 454. Progeny derived from 99-70 probably acquired some resistance from this ICRISAT-developed inbred. Selections (SoSat x Tift 454) F4-9, (SoSat x 99-70) F4-2 and F4-7, and (Ankoutess x 99-70) F4-5 and F4-4 are good candidates to use as parents for further development of downy mildew resistant A1 and A4 restorer inbreds for African hybrids.

*Root Knot Nematode Resistance in African Pearl Millets*

Root knot nematodes can reduce yield of pearl millet and of peanut and cowpea, which are often intercropped with millet. Seventeen diverse pearl millet varieties from Africa were evaluated for resistance to Meloidogyne incognita in greenhouse experiments. Plants were inoculated with eggs of *M. incognita*, and reproduction of the nematode was determined

Reactions within each African variety varied from resistance to extreme susceptibility. SoSat C-88 was among the most resistant varieties, Zongo and Gwagwa were intermediate, and P3Kollo was among the most susceptible. Patterns of apparent segregation of resistance varied among S1 progeny. Discreet resistant and susceptible phenotypes were identified in Zongo, and it was estimated that two dominant genes for resistance segregated in this variety. Reproduction of *M. incognita* on S2 progeny tended to confirm results from S1 progeny. Heritability of resistance determined by parent-offspring regression was 0.54. Realized heritability determined by divergent selection was 0.87. Selected progeny lines from these experiments will allow reconstitution of nematode-resistant versions of these popular varieties.

*Genetic Variability of Wild Pearl Millets with Striga Resistance*

Wild pearl millets (*P. glaucum* subsp. *monodii*) have been identified as potential sources of resistance to the hemi-parasitic weed, *Striga hermonthica*. Eighty wild pearl millet accessions, 9 U.S. inbreds and 7 African open-pollinated varieties were evaluated with 35 SSR primers and 60 EST primers to identify genetic diversity and identify polymorphic markers that would be useful for facilitating transfer of resistance. Genomic DNA was evaluated with EST and SSR primers. PCR fragments were scored for presence or absence of DNA fragments in each genotype. Dendograms were constructed the pearl millets grouped in distinct clusters. Cluster analysis was conducted and level of genetic diversity within and between populations was calculated.

Out of sixty EST primers tested, 30 produced scorable and reproducible fragments. Out of 35 SSR primers, 33 primer pairs gave amplification products in most of the accessions. Twenty eight marker loci were polymorphic out of 33 amplified primers. In total, 96 putative alleles were observed. A dendrogram constructed using the combined data of SSR and EST data resulted in 23 clusters at 85% similarity coefficient. The 7 West African varieties were grouped in one cluster, and the U.S. accessions were clustered in two groups. The wild accessions were grouped independently from the U.S. and African cultivated varieties. *Striga*-resistant accessions PS 202, PS 637, PS 639 and PS 727 tended to be located in different clusters, suggesting they are unrelated and may possess different resistances.

*Expression and Segregation of Stay-green in Pearl Millet*

Delayed senescence, or “stay-green” is a mechanism of drought tolerance characterized by the retention of green leaf area at crop maturity. Based upon information from the sorghum model, stay-green should have multiple benefits in pearl millet improvement. The chlorophyll content of a stay-green and normal senescent pearl millet were quantitatively compared over time, and preliminary information on the inheritance of stay-green was obtained through segregation in an F2 population.

Stay-green pearl millet 02F266-4 was crossed with a normal senescent line Tift 454. Relative chlorophyll content of 02F266-4, Tift 454 and their F1was measured on the top three leaves of the main tiller with a SPAD 502 Chlorophyll Meter. Data were collected at 7 d intervals for a total of 5 ratings. Segregation of stay-green was assessed in 02F266-4, Tift 454, and their F1 and F2. Using the SPAD meter, relative chlorophyll measurements were taken on the second uppermost leaf of the main tiller. A stay-green value was calculated which reflected the magnitude and duration of the relative chlorophyll content.

Minor differences in SPAD ratings among genotypes were observed at stigma emergence, but over time the upper leaves of 02F266-4 and the F1 maintained greater levels of chlorophyll than Tift 454. SPAD ratings of 02F266-4 were similar to the F1, but greater than Tift 454 in weeks 1 and 2. In weeks 3 and 4, SPAD rating of the F1 was greater than 02F266-4, and ratings of both genotypes were greater than Tift 454. The expression of relative chlorophyll content in the F1 indicates stay-green is a dominant or over-dominant trait with degree of dominance = 1.46. Stay-green in the F2 was skewed toward normal senescent types. Use of the SPAD meter to measure relative chlorophyll content provided a quantitative assessment of the stay-green trait. The data confirmed that 02F266-4 expressed stay-green characteristics. Trait expression was greatest in leaf 2. Using SPAD ratings, a stay-green value could be calculated as a measure of the magnitude and retention of chlorophyll content over time to assess segregation within populations.

*Promising New Pearl Millet Hybrids for the U.S.*

Pearl millet for grain is making inroads as a new-use crop for the U.S. but higher yield will improve returns to growers. Seven experimental hybrids and a pollinator were compared to the commercial standard Tifgrain 102 (TG102) in four locations in Georgia (Moultrie, Tifton, Watkinsville, and Newton) to identify superior yielding varieties under minimal inputs. No-till production practices were compared to conventional tillage in Watkinsville, GA with the goal of lowering production costs. Grain yield, 100 grain weight, protein, fat, starch, and fermentation efficiency were assessed.

Experimental hybrid (606 x 2304) was among the top yielding hybrids at all locations and had 17% higher yield than TG102. (606 x 2304) had 40% higher 100 grain weight, 7% lower protein content, similar fat, 50% greater starch, and 14% greater fermentation efficiency compared to TG102. In the no-till experiments in Watkinsville, experimental hybrid (506 x 2304) was among the top yielding hybrids and had a 38% greater yield than TG102. Over all treatments, (506 x 2304) had 10% higher 100 grain weight, similar protein and fat, 1% greater starch, and 1% lower fermentation efficiency compared to TG102. Hybrid (606 x 2304) had 30% greater yield than TG102. Over all treatments, (606 x 2304) had 17% higher 100 grain weight, 6% higher protein content, similar fat, 1% greater starch, and 1% lower fermentation efficiency compared to TG102. Yields were greatest in conventional tillage plots, which had better stand establishment than no-till plots. Grain from no-till plots had greater 100 grain weights, greater protein content, similar fat, lower starch, and greater fermentation efficiency. Hybrids (506 x 2304) and (606 x 2304) appear promising for additional testing. No-till has the potential to reduce production costs, but techniques to improve stand establishment must be identified.

*Pearl Millet as a Feedstock for Ethanol Production*

Grower profit can be improved by identifying applications in which pearl millet has advantages compared to alternatives. It is a superior grain in game bird rations and for broiler production, but diversified market options will stabilize grain prices. The development of the ethanol industry in the southeastern U.S. is limited by the amount of feedstocks produced in the region. Biological and economic information is needed on the value of pearl millet as a potential feedstock for this market.

Two fermentation evaluations were conducted. The first at KSU used four pearl millet varieties. The second at ICIA compared experimental variety “2304” to maize. Samples were evaluated using standard corn-to-ethanol protocols. The economic feasibility of using pearl millet as a feedstock was compared to maize by using the Superpro Designer Dry Grind model. This analysis simulates costs associated with the production of ethanol, as affected by the composition of raw materials entering the process. Other variables used in the model include the sizing of equipment, utility consumption, operating costs, capital costs required for a facility with a 40 million gallon per year capacity.

Ethanol yield from pearl millet was 8% less than from maize. Pearl millet fermented more quickly and reached 85% fermentation 12 hr earlier than maize. Fermentation efficiencies of pearl millet, on a starch basis, were comparable to those of maize. DDGS from pearl millet had 6% lower moisture compared to DDGS from maize. On a dry basis, the DDGS from pearl millet had 16% greater protein, 53% greater fat, 45% higher ash, 19% lower crude fiber, and 20% lower nitrogen-free extract content compared to DDGS from maize.

The protein content of pearl millet would result in a 13% greater DDGS value compared to maize DDGS. The analysis suggests that even with a 10% premium on the cost of pearl millet, the net cost of ethanol production is $0.06 per gallon less than production using maize. Total net profit from a facility using pearl millet as the sole feedstock was estimated as $25,175,000 per year compared to $23,758,000 for maize feedstocks, a $1.4 million advantage. Two additional sources of savings not captured in the analysis are the lower energy requirements to grind pearl millet, and a potentially faster batch processing allowed by the faster fermentation rate. Grinding rate of pearl millet is 53% faster and requires 40% less energy to grind than maize. For grain quantities indicated by the process and cost analysis, electricity costs can be reduced an additional $20,200 because of the lower energy requirements for grinding. The economic benefit of the faster fermentation rate could be significant, but may be difficult to capture if pearl millet is used as a supplement, rather than a principal feedstock. If used as a sole feedstock, faster fermentation could increase gross returns by 25%. Biologically and economically, pearl millet is a feasible supplemental feedstock for dry-grind maize-to-ethanol facilities. Pearl millet should benefit the economies in the southeast that must import feedstock for ethanol production.

## Networking Activities

Seed of superior Ghanaian pearl millet varieties were provided back to the Savannah Agricultural Research Institute after problems with storage facilities resulted in the loss of SARI’s elite pearl millet germplasm repository.

Seed of forage pearl millet populations with brown midrib and staygreen traits was sent to René Clará Valencia, CENTA, El Salvador for evaluation and selection.

Experimental pearl millet hybrids were provided to Pronaca, Ecuador for evaluation in agronomic production and poultry feeding trials.

Collaborated with Compatible Technology International (MN), Hampshire College (MA), and Bicycling Empowerment Network Namibia, in developing a prototype for a manually operated pearl millet thresher suitable for African village settings. F. Muuka (Zambia), I. Mbaiwa (Botswana), and A. Issaka (Niger) contributed to design specifications, and a prototype has been evaluated with pearl millet panicles sent to CTI and Hampshire College.

Collaborated with USDA-NRCS and the Smithsonian Institute to rectify incorrect information for pearl millet in the USDA-NRCS Plants Database. Pearl millet had been previously listed with incorrect taxonomic information and was classified as a noxious weed in this internet database used by NRCS field offices nationwide for making recommendations to growers.

Organized and discussed pearl millet field demonstrations at the Sunbelt Ag Expo in Moultrie, GA. (July 11, 2006), and Pearl Millet Field Day at Newton, GA (October 10, 2006).

Discussed pearl millet production and markets at demonstration plots at the Alternative Crops Workshop, Western Illinois University, Macomb, IL (July 20, 2006).

Presented "Biological and economic feasibility of pearl millet as a feedstock for ethanol production" at the 6th New Crops Symposium, Association for the Advancement of Industrial Crops. San Diego, CA October 14-18, 2006.

Presented "Pearl Millet: New Opportunities for Georgia Agriculture and Agri-Tourism" at Emanuel County Ethanol Producers, East Dublin GA (August 18, 2006), Sigma Xi Seminar, Tifton, GA (October 12, 2006), Johnson County Georgia Young Farmers Association, Wrightsville, GA (November 28, 2006), and Workshop for Small, Beginning, and Limited Resource Farmers, Heart of Georgia Technical College, Dublin, GA (December 14, 2006).

Hosted visit by Tom Hash, Principal Scientist (Molecular Breeding, Pearl Millet), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India; December 15, 2006.

Hosted visit by Stephen Nutsugah, Savannah Agricultural Research Institute, Ghana, in complementary project, "Identifying sources of resistance to grain molds and mycotoxins in pearl millet", funded by USDA-FAS.

Collaborated with Bettina Haussmann, ICRISAT Niger, in development of complementing proposal, "Introgressing resistance to *Striga* hermonthica from wild to cultivated pearl millet", funded by USAID Initiative on Development of Linkages with the International Agricultural Research Centers.

## Publications and Presentations

### *Journal Articles*

Bean, S.R., Tilley, M., and Wilson, J.P. 2006. Separation of pearl millet proteins by high-performance capillary electrophoresis. International Sorghum and Millets Newsletter 47:167-169.

Buntin, G. D., Hanna, W. A., Wilson, J. P., and Ni, X. 2007. Efficacy of insecticides for control of insect pests of pearl millet for grain production. Online. Plant Health Progress doi:10.1094/PHP-2007-0219-01-RS.

Chintapalli, R., Wilson, J.P., and Little, C.R. 2006. Using fungal isolation rates from pearl millet caryopses to estimate grain mold resistance. International Sorghum and Millets Newsletter 47:146-148.

Maw, B.W., Wilson, J.P., Sumner, P.E., and Hanna, W.W. 2006. Drying properties of pearl millet grain for long-term storage. International Sorghum and Millets Newsletter 47:165-166.

Timper, P., Brenneman, T. B., Hanna, W. W., and Wilson, J. P. 2007. Pearl millet as a rotation crop for peanut. Online. Plant Health Progress doi:10.1094/PHP-2007-0202-02-RS.

Wilson, J.P., Wilson, D.M., and Jurjevic, Z. 2006. Equilibrium moisture content of pearl millet. International Sorghum and Millets Newsletter 47:120-122.

Wilson, J.P., Tapsoba, H., Rajarajan, J. 2006. Mixture design analysis of rust infection in three-component pearl millet mixtures. International Sorghum and Millets Newsletter 47:143-145.

### *Books, Book Chapters, and Proceedings*

Wilson, J.P., Timper, P., Truman, C.C., Dale, N.M., Batal, A.B., Ni, X., Gitaitis, R., McAloon, A.J., Shumaker, G., Dowling, G. Brown, J. Webster, T., and Maas, A. 2006. Economics-driven research and incentives for pearl millet production in the United States. Proceedings of International Pearl Millet Breeding and Seed Production Workshop. ICRISAT, Hyderabad, India. (CD-ROM)

### *Abstracts*

Wilson, J.P., McAloon, A.J., Yee, W., McKinney, J., Wang, D., and Bean, S.R. 2006. Biological and economic feasibility of pearl millet as a feedstock for ethanol production. Abstracts of the 6th New Crops Symposium, p. 47, Association for the Advancement of Industrial Crops.

Gulia, S.K., Wilson, J.P., Singh, B.P., and Carter, J. 2006. Progress in grain pearl millet research and market development. Abstracts of the 6th New Crops Symposium, p. 48, Association for the Advancement of Industrial Crops.

# Breeding Grain Mold Resistance in High Digestibility Sorghum Varieties

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### Summary


The goal of this proposal is to combine the improved nutritional grain quality sorghum (i.e., high protein digestibility HD) with high levels of grain mold resistance. Sorghum grain mold, which is vectored by over 40 fungal species, is a primary constraint to sorghum production worldwide. The HD trait derives from a modified grain endosperm mutant possessing irregular and invaginated kafirin protein bodies. The HD grain confers a gain in function in human protein bioavailability and increased lysine amino acid content. Our project goal is to commercialize designer sorghums that combine disease and environmental stress resistance with grain possessing enhanced malting, fuel, and human and animal nutrition quality via the HD and waxy grain endosperm traits. To accomplish this goal we teamed with Southern African and Central American scientists and students and U.S. University and USDA, ARS scientists. The PI leveraged the $178,500 in funds from INTSORMIL with $120,000 in research and $212,700 in graduate fellowship funds obtained from competitive Federal and State programs during the 3-year funding period to accomplish the goals of this project. The fellowship funds supported two graduate students from Central America and two from the U.S.

In the past 2+ years we have developed molecular markers linked to single genetic loci for grain mold resistance to the two predominant vectors of the grain mold disease, Fusarium thapsinum and Curvularia lunata. The individual loci for resistance to the two pathogens are unlinked to each other and are independent of plant height, grain color, and grain hardness (Figure 1). These are critical and important new result the PI and graduate students obtained in terms of changing grain mold resistance breeding strategies and increasing grain yield. We are currently using marker-assisted-selection (MAS) in conjunction with Texas A&M sorghum breeders to combine resistance to these two pathogens into new inbred and hybrid lines. The mapping of genetic loci for the HD trait will be complete by summer 2007 and will be used to combine grain mold resistance with the HD trait.

The PI and graduate students also completed a genotype x environment (G x E) study to determine the other predominant pathogens vectoring sorghum grain mold, and the viability of HD germplasm in humid grain mold prone environments. Several HD lines were identified that were as resistant as normal endosperm lines to grain mold. No statistical correlations between assays for the HD trait and grain mold susceptibility were found. This means that grain mold resistant high nutritive HD sorghum hybrids and inbred cultivars can be developed for humid environments in Africa and Central America. Other predominant grain mold pathogens species that impact grain germination and grain quality were also identified. New resistance loci and linked molecular markers for these pathogens will be developed by the PI if refunded and combined with the resistance to *F. thapsinum* and *C. lunata* using MAS.

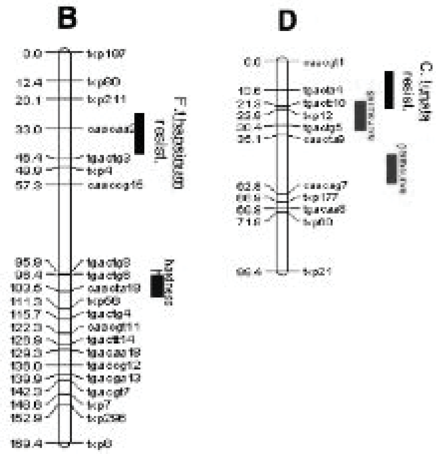


**Figure 1. Framework genetic map of sorghum linkage group B and D of the inbred RIL population derived from *Sorghum bicolor* RTx430 and Sureño highlighting the locations of genetic loci for resistance to *C. lunata* and *F. thapsinum.***

The PI, graduate students and collaborators in the Texas A&M Department of Biological and Agricultural Engineering have also demonstrated the utility of the HD sorghum trait for the bio-ethanol market. We have shown that the HD trait yields 15 to 20% more ethanol with lower energy input than normal endosperm sorghums and corn. We are now using MAS to combine the HD and high waxy endosperm traits to improve on this result with a target for the bio-ethanol and brewery industry in Africa and the U.S.

## Objectives, Production and Utilization Constraints

### *Objectives*

**The objectives for the 3-year funding period were as follows:**

- Determine the combinability of the high protein digestible trait with grain mold resistance
- Identify QTLs regulating mold resistance and high grain protein digestibility.
- Link QTLs controlling mold resistance to changes in the expression of genes that contribute to the mold resistance for future utilization in genetically engineering improved resistance to grain mold.
- Test the physical and functional properties of highly digestibility sorghums in fuel, brewery, food and feed products.

### *Sorghum and Millet Production or Utilization Constraints Addressed*

The development of new high yielding sorghum varieties with improved nutritional quality is a key attribute needed to increase the commercial utilization of sorghum. The high grain protein digestibility (HD) trait that is also associated with high lysine content is one such attribute that may spur increased utilization of sorghum. However, for the HD trait to be widely adopted lines must be developed with hard endosperms for improved milling capacity and better food application potential, as well the trait must be incorporated into lines possessing grain mold resistance. The overall goal of this project is to use molecular techniques to facilitate the development of HD varieties with optimal endosperm characteristics and viable levels of grain mold resistance. The HD lines have also been analyzed for their improved performance in the growing African brewery and biofuels industry

## Research Findings and Project Output

Grain mold and other disease resistance breeding: The PI (Hays,TAM230) in conjunction with other INTSORMIL CRSP projects (William Rooney, TAM 220c, and Gary Peterson, TAM 223) have developed molecular markers and mapped loci linked to resistance to two prominent grain mold pathogens, namely *Fusarium thapsinum* and *Curvularia lunata* (Figure 1). The genetic loci for resistance to these two pathogens were identified using control inoculations at anthesis of the individual pathogens in a set of ‘Tx430’ x ‘Sureño’ white grain recombinant inbred lines. Resistance was based on the presence or absence of ¼ PDA mycoflora colonies of the inoculated pathogen from surface sterilized grains. The loci for resistance to the individual pathogens are unlinked to each other (Figure 1). As well, they are unlinked to previously suspected phenotypic modes of resistance such as grain hardness, plant height, or plant color (not shown). It is also important to note that mycroflora resistance was poorly correlated with visual resistance scoring, but was highly correlated to controlled grain weathering by the same pathogen (i.e., dry mature grain maintained in a wetted condition). This indicates that while appearing resistant, grains can still harbor fungal mycelium that can, under the right condition, contribute to grain weathering or storage damage to grain quality. The identified markers for resistant loci are suitable for MAS. MAS and other fast breeding techniques discussed below are being used to pyramid grain mold resistant loci to *F. thapsinum* and *C. lunata* with the high value HD and waxy endosperm traits.

We have also conducted a genotype x environment (G x E) study in Texas to determine other key vectors of grain molds, and the viability of HD germplasm in a humid grain mold prone environment. Several HD lines were identified that were as resistant as normal endosperm lines based on visual and mycoflora scores. No statistical correlations between assays for the HD trait and grain mold susceptibility were found. This means that grain mold resistant high nutritive HD sorghum hybrids and inbred cultivars can be developed for humid environments in Africa and Central America. The prominent vectors of grain mold found in this study were *C. lunata*, *F. thapsinum*, *F. semitectum*, *Rhizopus mucor*, *Bipolaris*, *Alternaria*, *Phoma*, *Penicillium*, and *Aspergillus*. Some pathogens have serious correlative detrimental impacts on grain quality and germination (i.e., *Fusariums*, *Curvularia*, and *Alternaria*), while others appear to be passive pathogens with little correlative impact on grain quality and germination (i.e., *Penicillium*). Resistance loci and linked molecular markers to those individual pathogens that appear to have negative impacts on grain quality and germination are being developed in the same RIL population described above and in new RIL populations derived from novel sources of grain mold resistance. These will be used for MAS and mapped based cloning. Use of these tools, MAS and other fast breeding techniques will be trained and disseminated to collaborators in Africa and Central America. Efforts are being made to develop and provide hemizygous PCR marker pairs for simple scoring resistant and susceptible individuals in breeding populations.

The G x E study also revealed interesting antagonistic relationships between groups of grain mold pathogens. For instance *Alternaria* and *Penicillium* conferred no detrimental impacts on grain quality, had a positive impact on grain germination and were negatively associated with more pathogenic fungal pathogens such as *F. thapsinum* and *C. lunata* (not shown). This result opens some tantalizing possibilities for domesticating casual grain mold agents such as *Penicillium* as biological controls against more pathogenic grain mold agents.

High value high digestibility (HD) and waxy sorghum breeding. The PIs in conjunction with other INTSORMIL CRSP projects (William Rooney, TAM 220c, and Gary Peterson, TAM 223, Lloyd Rooney, TAM 226) have breed, and developed fast breeding technologies for the HD and high amylopectin waxy endosperm traits. These include MAS and near-infrared spectroscopy (NIR) early and late generation grain selection and sorting technologies for separating HD, waxy and HD-waxy grains with a hard flinty endosperm texture in segregating populations (being developed collaboratively with Drs. Floyd Dowel, Scott Bean, Michael Tilley, Cereal Chemist USDA,ARS,GMPRC). We have identified hard flinty, HD sorghum lines with no protein highways surrounding the starch granules. Similar lines have been identified by Bruce Hamaker (Tesso et al., 2006). The PIs have also worked collaboratively with cereal chemists and agricultural engineers from South Africa (John Taylor) and (Sergio Capareda, Texas A&M) to demonstrate the positive gain in function obtained from HD sorghums for malting and bio-ethanol systems (Figure 2). While only one HD sorghum line has been tested for bioethanol conversion to date, this one line conferred a 15-20% greater ethanol conversion rate versus corn or normal endosperm sorghums (Figure 2). Based on starch gelatinization data, we have identified additional HD lines which will confer an even higher increase in ethanol conversion rate versus normal sorghum or corn. These lines are currently being tested. Similar results have been reported for waxy sorghums (Moheno-Perez et al., 1999; Moquel et al., 2001; Urias Lugo et al., 2005). The (HD) sorghum genotypes have a modified endosperm matrices with invaginated kafirin protein bodies that do not surround the starch granules and restrict hydration. As such, the grain

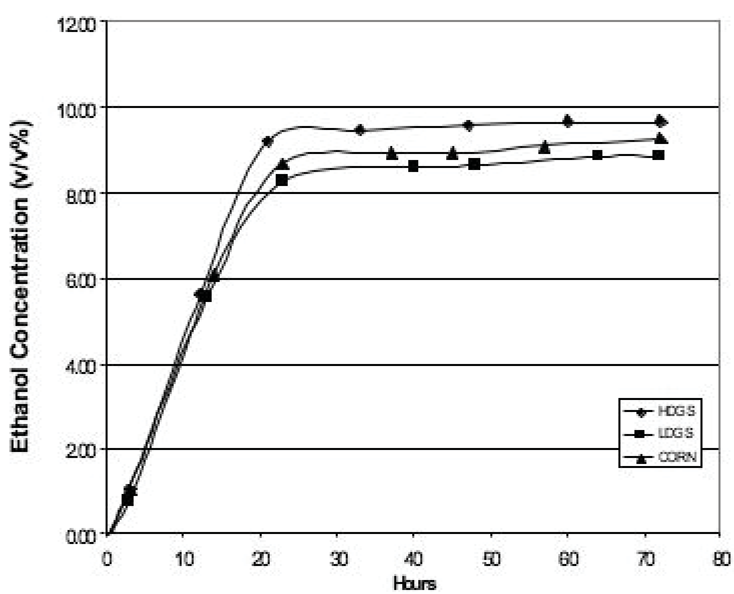


**Figure 2. Change in ethanol level during 72 hrs of simultaneous hydrolysis and ethanol fermentation of high digestible grain sorghum (HDGS) (TX635/P850029), low digestible grain sorghum siblings (LDGS) (TX635/P850029) and Corn.**

starch gelatinizes at lower temperatures, in less time, and produces significantly more ethanol (Capareda, D. Hays, W. Rooney, Figure 2). Second, the proteins present have improved feed value with higher bioavailability and 60% more lysine, similar to high lysine corn lines (Weaver et al., 1998). Waxy sorghum genotypes and cultivars exist with little or no linear starch (amylase) and a highly-branched amylopectin starch content. Waxy sorghums and their counterparts in other cereals require significantly less energy input for gelatinization and have improved malting (Moheno-Perez et al., 1999; Moquel et al., 2001; Urias Lugo et al., 2005) and feed quality properties. We have found similar significant reductions in gelatinization temperatures in HD protein endosperm lines (not shown). Due to the similar and complementary properties that the HD and waxy endosperm traits confer, the PI and collaborators in the U.S. and Africa (J. Taylor, Cereal Chemist, University of Pretoria, South Africa) believe combining both traits into a single sorghum cultivar will have ideal end-use attributes for malting & bio-ethanol conversion and high value grain distillers feed products with improved nutritional value coming from the high HD lysine content. We believe HD-waxy sorghums will also have added marketability in terms of improved nutritional value for traditional and fast cook sorghum based food and snack products. Crosses between advanced HD and waxy lines have been made and segregating lines will be screened for lines possessing both the HD and waxy endosperm traits combined or only the HD or waxy traits using MAS and NIR sorting technologies. These lines will be advanced along with low digestible siblings for bio-ethanol analysis in the near future

## Networking Activities

D. Hays traveled to Pretoria, South Africa in October 2004 to present a talk on the use of biotechnology in the development of new high nutritional quality sorghum varieties at the White Food Sorghum Workshop at the University of Pretoria. Collaborations with Medson Chisi, Sorghum Breeding, Golden Valley Research Station, Zambia, and John Taylor, University of Pretoria were developed at this meeting on priorities for testing potential food, fuel, and brewery products that could be developed from the modified endosperm HD lines. The PI has maintained regular phone contact with John Taylor and supplied HD lines to him for analysis of brewing functionality. The PI also meets regularly with the William Rooney (TAM-220c) to discuss priorities for breeding HD -waxy sorghums, screening germplasm for the HD and waxy traits using MAS and protease assays, and increasing advanced lines for bio-ethanol, and food and feed product research studies in collaboration with Dr. Sergio Capareda (Dept. of Biological and Agricultural Engineering, Texas A&M University) and Lloyd Rooney (TAM-226).

## Publications and Presentations

### *Workshops Meetings/Invited Presentations*

Hays DB, Portillo O, Finlayson S, Cepeda S, Rooney WL. 2007. Designer sorghums: Analysis of sorghum genotypes with modified endosperm matrices for optimized low energy input ethanol production. National Sorghum Producers and Southern Seed Trade Association. Santa Ana Pueblo, New Mexico. presentation.

Robbins A, Hays DB. 2005 Expression Quantitative Trait Loci Mapping Grain Mold Resistance in Sorghum. American Society of Agronomy, Salt Lake City, Utah. Presentation.

Dirk B Hays. 2004. Sorghum Biotechnology: Combinability of high grain digestibility with grain mold resistance, USA-AID-INTSORMIL, White Food Sorghum Workshop, University of Pretoria, Pretoria, South Africa.

Dirk B. Hays, 2004 Using biotechnology to develop resistance in cereals to pathogens with extant diversity, Department of Plant Pathology, Texas A&M University.

# Development and Enhancement of Sorghum Germplasm with Sustained Tolerance to Biotic and Abiotic Stress


## Project PRF 207
## Gebisa Ejeta
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Dr. Ketema Belet, Pearl Millet Breeder, EARO, Ethiopia
Mr. Zenbaba Gutema, Sorghum Breeder, EARO, Ethiopia


### Summary


Breeding sorghum varieties and hybrids for use in developing countries requires proper recognition of the major constraints limiting production, knowledge of germplasm, and an appropriate physical environment for evaluation and testing. Successful breeding efforts also require knowledge of mode of inheritance and association of traits that contribute to productivity as well as tolerance to biotic and abiotic stresses. Research and germplasm development activities in PRF-207 attempt to address these essential requirements.

PRF-207 addresses major biotic and abiotic constraints (drought, cold, grain mold, and other diseases) that limit productivity of sorghum in many areas of the world. Over the years significant progress has been made in some of these areas. Superior raw germplasm have been identified, mode of inheritance established, chemical and morphological traits that contribute to productivity as well as to tolerance to these stresses have been identified. Selected gene sources have been placed in improved germplasm background, some of which have already been widely distributed.


### Major Achievements

#### *Promote Economic Growth*

Within the six-year life of this project we have learnt more about the inheritance and individual mechanisms of resistance to grain mold and tolerance to drought. A number of lines, populations, and hybrids will be made available for public release and distribution. Several seed parents were developed and scheduled for release to the US seed industry in 2002.

Improved hybrids and varieties developed in this project have been distributed for testing at various locations in collaboration with a number of NARS. These cultivars have been widely tested in several agroecologies and show excellent adaptation and promise. The potential benefits from these cultivars and others that are now in the pipeline should be substantial.

The activities of this project and the overall INTSORMIL efforts in Sudan have demonstrated that sustained support and focused research efforts would produce tangible and useful results. A similar deliberate effort has begun in Ethiopia and may be expanded to Eritrea and Kenya as well. We have shown that effective utilization of research-generated technologies would in return bring due recognition to scientists and research programs, and generate increased national support for agricultural research.

Benefits arising from germplasm are often tangible and easy to recognize. Figures on acreage, volume of seed distribution, and number of farmers using improved germplasm can tell the success story sufficiently well.

### *Improve Nutrition*

We conducted experiments that provided key understanding for the basis of improved digestibility in sorghum and utilized this information towards the development of high digestible sorghum lines. High digestible sorghum variants associated in progenies derived from the high lysine-breeding program are being developed for food.

We more recently started evaluation of hybrids derived from sorghum-inbred lines with improved agronomic characteristics but with the brown midrib trait that exhibits increased forage digestibility.

### *Increase Yield*

We assessed genetic variation and relationships among seedling vigor traits in sorghum. Estimates of heritability and the significant additive genetic variances obtained indicated that selection for vigor and stand establishment would be effective as long as superior sources of the trait are available.

We evaluated the role of various phenolic compounds in sorghum kernels on early season seedling vigor and showed that effective selection for seedling vigor can be done by selecting sorghum kernels with high concentration of certain phenols.

We conducted specific studies in attempting to understand the genetic and physiological basis of drought tolerance using a mix of both traditional and molecular approaches.

We also conducted several studies in elucidating the basis of grain mold resistance in low and high tannin sorghums. Specific studies were undertaken in determining the role of physical and chemical kernel properties associated with mold resistance, and in assessing the nature of specific phenolic compounds that contribute to grain mold resistance.

We conducted observations relative to identification and characterization of sorghum genetic variants in glycinebetaine accumulation and their role in tolerance to drought and salinity stresses. We discovered GB protects cellular components from injury caused by dehydration. We established that GB accumulation in sorghum is inherited as a single recessive gene.

We employed molecular marker assisted selection to introgress the excellent cold tolerance observed in Chinese sorghums with the agronomic adaptation of our standard cultivars.

### *Improve Institutional Capacity*

We provided graduate and non-graduate education of U.S. and LDC scientists in the area of plant breeding and genetics. We also contribute to human capital development by providing training opportunities and sponsoring program visits and international conferences for some LDC partners. We developed liaison and facilitate effective collaboration between LDC and U.S. sorghum and millet scientists. We worked towards developing interdisciplinary research teams with clearly defined research agenda with NARS scientists in selected countries. We have encouraged and facilitated positive institutional changes in research, extension and seed programs of collaborating countries involved in sorghum and millet research and development. We promoted the value of agricultural research as a catalyst for change and development, the recognition of improved seed as an essential input and the role of the private sector entrepreneurial approach to seed production and distribution

## Objectives, Production and Utilization Constraints

- To identify and characterize traits associated with nutritional quality improvement and with increased levels of drought and grain mold resistance in sorghum.
- To assess the physiological role of the staygreen trait in sorghum.
- To elucidate mechanisms of resistance to drought and grain mold.
- To study the inheritance of phenotypic, physiological, chemical, and molecular traits associated with nutritional quality and resistance to early season cold, drought and grain mold.
- To devise breeding approaches and methods for effective exploitation of useful traits for effecting improved nutrition, cold and drought tolerance and grain mold resistance in sorghum.
- To characterize genomic differences among botanical races and germplasm pools of sorghum.
- To extensively evaluate hybrid combinations developed from sorghum inbred lines with the brown midrib trait.
- To develop sorghum varieties and hybrids with improved yield potential and broader environmental adaptation.
- To diversify the germplasm base of improved grain sorghum cultivars through introductions.
- To introgress useful traits found in landrace and wild sorghum germplasm into adapted cultivars.
- To maintain a strong seed parent breeding program (elite and diverse food-type seed parents) for both US and LDC collaborators.
- To generate and release populations, varieties, and hybrids with high yield, resistance to diseases, grain quality attributes, and increased forage digestibitibility and biofuel conversions characteristics for public and private seed programs.
- To assemble unique sorghum germplasm, and to encourage and facilitate free exchange of germplasm between US and LDC scientists and institutions.
- To assess applicability of various statistical and DNA fingerprinting technologies for evaluating genomic similarity or for discerning genetic diversity of sorghum and millet germplasm pools.

## Research Findings and Project Output

We developed a better understanding of drought stress in sorghum by identifying and characterizing associated traits that contribute to stress tolerance. We employed this approach in the study of the role of osmoregulatory compounds such as glycine-betaine (GB) in drought stress and salinity tolerance. We discovered that GB protects cellular components from injury caused by dehydration. We established that GB accumulation in sorghum is inherited as a single recessive gene. We demonstrated the value of well-structured genetic populations for phenotypic and genotypic characterization leading to mapping of molecular markers associated with the important loci conditioning drought tolerance in sorghum.

We have generated an array of sorghum germplasm populations to study the genetics of drought tolerance in sorghum. We have evaluated drought tolerance of recombinant inbred lines under different moisture levels in dryland in Mexico. We identified putative molecular markers associated with drought tolerance.

Upon large-scale screening we found that sorghums from China exhibit excellent level of seedling cold tolerance. From our permanent recombinant inbred lines generated from a cross between a Chinese kaoliang and an African caudatum we identified key molecular markers associated with seedling cold tolerance. We employed molecular marker assisted selection to incorporate the excellent cold tolerance observed in Chinese sorghums with the agronomic adaptation of our standard cultivars. Introgression of seedling cold tolerance from Chinese landraces into high yielding lines using molecular markers was initiated. Key markers were identified and validated. Two new populations of sorghum were synthesized for the purpose of verification.

Newly derived mold resistant breeding lines will be evaluated at several locations to provide essential data to support release. Hybrids with mold resistant seed parents have been generated and new mold resistant parental lines will be released upon supportive evaluation data evaluated in two years. Data collected will be analyzed to support planned. Mold resistant seed parents and new sets of early maturing lines were available for release in 2002.

We conducted experiments that provided key understanding for the basis of improved digestibility in sorghum and utilized this information towards the development of high digestible sorghum lines. New and improved high digestible sorghum lines generated via longterm breeding and selection in cooperation with Dr. Bruce Hamaker will be further evaluated both for agronomic characteristics as well as for in vitro digestibility. High digestible sorghum variants associated in progenies derived from the high lysine-breeding program are being developed for food. Brown midrib mutants with reduced lignin have been introgressed into improved sorghum cultivars for commercial exploitation.

New sorghum inbred lines with improved agronomic characteristics but with the brown midrib trait that exhibits increased forage digestibility and biofuel conversion characteristics will be extensively evaluated in hybrid combinations.

We have developed a large array of food grain seed parents (A&B) lines in the last few years. These seed parents are currently under evaluation in test crosses that have been distributed to Niger, Mali, Sudan and Ethiopia. Seed increase of entries in this set has been completed and their release was planned before the 2002 crop season.

Several nurseries on drought, mold, and food grain experimental hybrids will be distributed for evaluation in cooperating NARS and the performance of these material will be assessed. This has been an on-going project that will continue as long as support is available. It has been an effective way for distribution and sharing of improved germplasm.

A study on introgression of useful genes from elite landraces into improved temperate lines has been initiated through a modified conversion program. The procedure utilizes conventional backcrossing into an improved food-grain population accelerating the rate in which useful genetic stocks are obtained in diverse genomic background. We extracted and selfed progenies which were evaluated for per se performance and for combining ability. Diverse germplasm generated from this project will be released for wide distribution.

We considered the possible role of DNA fingerprinting technology in discerning genomic similarity among and within races of sorghum. We thus conducted major studies on assessing genetic diversity using both phenotypic characters and molecular markers. In a collaborative study we analyzed genetic diversity among sorghums from Sudan.

We have also embarked on DNA marker assisted introgression of landraces and wild relatives to increase efficiency of transferring genes of value from exotic to improved sorghum lines. This is a longer-term project on increasing efficiency of breeding methods.

We are collaborators on a study that is currently underway to assess potential gene flow between and among sorghum species under natural conditions in native environments of sorghum in Africa.

Characterization of germplasm for drought, cold tolerance and grain mold resistance, as well as identification of QTL associated with these traits continues to be undertaken as major research efforts in the program.

Array of elite food-grain type seed parents with excellent food quality and yield potential and others with improved maturity and disease resistance have been developed and will be released. Drought resistant inbred lines developed from intercrossing germplasm selected in Sudan and Niger into U.S. germplasm will be released. Genetic stocks of unique gene sources will also be shared with LDC and U.S. users on a regular basis. Stay green lines developed in a seed parent background that combines excellent food grain characteristics with enhanced productivity and drought tolerance will also be available for distribution.

## Description of Methods of Work Used

The research efforts of PRF-207 are entirely interdisciplinary. The on-campus research at Purdue is in close collaboration with colleagues in several departments. We undertake basic research in the areas of biotic and abiotic stresses where a concerted effort is underway in elucidating the biochemical and genetic mechanism of resistance to these constraints. Field and laboratory evaluations of sorghum and millet germplasm are coordinated, the results from one often complimenting the other. In addition, there have been collaborative research efforts with colleagues in Africa where field evaluation of joint experiments are conducted.

Our germplasm development and enhancement program utilizes the wealth of sorghum and millet germplasm we have accumulated in the program. Intercrosses are made in specific combinations and populations generated via conventional hybridization techniques, through mutagenesis, or through tissue culture in vitro. Conventional progenies derived from these populations are evaluated both in the laboratory and in the field at West Lafayette, Indiana for an array of traits, including high yield potential, grain quality, as well as certain chemical constituents that we have found to correlate well with field resistance to pests and diseases. We also evaluate our germplasm for tropical adaptation and disease resistance during the off-season at the USDA Tropical Agricultural Research Center at Isabella, Puerto Rico. Selected progenies from relevant populations are then sampled for evaluation of specific adaptation and usefulness to collaborative programs in Sudan, Niger, and more recently Mali. Evaluation of the drought tolerance of our breeding materials have been conducted at Lubbock, Texas in collaboration with Dr. Darrell Rosenow, in a winter nursery at Puerto Vallarta, Mexico, as well as the University of Arizona Dryland Station at Yuma, Arizona, and several locations in Africa. Over the years, assistance in field evaluation of nurseries has also been provided by industry colleagues particularly at Pioneer Hi-Bred and DeKalb Genetics

The training, networking and institutional development efforts of PRF-207 have been provided through graduate education, organization of special workshops and symposia as well as direct and closer interaction with research scientists and program leaders of NARS and associated programs. Much of the effort in this area has been primarily in Sudan and Niger, with limited activity in Mali and some in Southern Africa through SADC/ICRISAT.

## Networking Activities

We continue to provide an array of sorghum germplasm from our breeding program to NARS in developing countries. Our germplasm is provided in either a formally organized nursery that is uniformly distributed to all collaborators that show interest or upon request by a national program of specific germplasm entries or groups from or germplasm pool. Germplasm was distributed to cooperators in seven countries in 2000-01. Three new *Striga* resistant varieties of sorghum from our program in 2001 were recommended for commercial cultivation to two African countries, one in Tanzania, and two in Ethiopia. Germplasm was distributed to cooperators in 20 countries in 2002. Sorghum germplasm from our program was sent to Ethiopia, Kenya, Tanzania, Eritrea, Niger, and Mali in 2003. Germplasm was distributed to cooperators in 10 countries in 2004.

Technical assistance was provided to sorghum and millet research programs in Sudan, Kenya, Uganda, Eritrea and Ethiopia, Tanzania (Horn of Africa) on a regular basis, and to Niger and Mali in West Africa on a request basis. PI additional effort will be placed on firming up plans for operating a regional network for INTSORMIL in the Horn of Africa in association with ICRISAT and ASARECA.

Two U. S. students are currently supported by PI and are in the middle of their graduate degree programs. An Ethiopian student from EARO has just started a graduate program, and before the end of the year, a student each from Tanzania and Nigeria will start graduate program in the project. PI also hosts a Fulbright fellow from Burkina Faso and anticipates short-term training for scientists from Eritrea, Ethiopia, Sudan and Tanzania with support from other agencies. Effort will be made to assist in the procurement of support for collaborators from other donors.

Major linkages with several NARS have been established. Effective collaborative linkages have been put in place both with U.S. and LDC partners. DNA marker studies for drought tolerance as well as for germplasm classification have been accomplished in collaboration with Dr. Peter Goldsbrough (Purdue, Horticulture dept.). The germplasm development effort will continue to be in cooperation with programs in Ethiopia, Eritrea, Uganda, Kenya, Sudan as well as Niger and Mali. The pool of germplasm in PRF-107 has shown good adaptation in both West and East Africa. Sorghum breeders in each of the above countries have been partners in both the make up of synthesized populations as well as the evaluation and collection of data. This project has a good record of joint publications with both LDC and U.S. collaborators. In addition, germplasm is freely exchanged among collaborating partners and others. The PI has excellent ties with the sorghum breeders in Niger, Sudan, and Mali and recently formalized linkages with sorghum breeders in Ethiopia and Kenya.

Effective interdisciplinary teams have been put in place in Ethiopia, Eritrea, Kenya, Uganda, and Sudan. Specific research agendas engaging teams of scientist in each country or prime site will be developed in consultation with host country collaborators.

With the revival of ECARSAM as a regional network for sorghum and millet scientists in the Eastern and Central African countries, we hope to channel all our regional networking efforts through this network. Experience in the use of hybrids with successful production and distribution of hybrid seeds will be shared with neighboring countries. We hope to develop regional nurseries of drought tolerant lines and hybrids from germplasm thoroughly evaluated in Sudan and Ethiopia which will most likely be useful in other Horn of Africa countries. Germplasm, sufficiently tested in NARS experiment stations, will be made available to participating NGO and PVO to enable wider testing and demonstration in farmers' fields. Collaborative interdisciplinary projects involving agronomists, breeders, and plant protection scientists will be established.

Techniques in breeding for drought tolerance which have resulted in unique drought tolerant genotypes will be promoted and transferred. Parental lines and hybrids tested for stability and productivity will be made available. The PI will work closely with national program scientists to obtain resources for on-farm demonstration and extension of improved germplasm and package of technologies deriving from collaborative research efforts. The assistance of NGOs and PVOs will be solicited when possible.

## Publications and Presentations

### *Refereed Papers*

Knoll J.E., N. Gunaratna and G. Ejeta. 2007. QTL analysis of early-season cold tolerance in sorghum. Theor Appl Genet. (in press).

Knoll J.E. and G. Ejeta. 2007. Marker-assisted selection for early-season cold tolerance in sorghum: QTL validation across populations and environments. Theor Appl Genet (in press).

Knoll J.E. and G. Ejeta. 2007. Marker assisted selection in sorghum. In: Tuberosa R, Varshney RK (eds.) Genomics-Assisted Crop Improvement. Springer-Verlag (inpress).

Tesso, T., G. Ejeta, A. Chandrashekar, C.-P. Huang, A. Tandjung, M. Lewamy, J.D. Axtell and B.R. Hamaker. 2006. A novel modified endosperm texture in a mutant high-protein digestibility/high-lysine grain sorghum (Sorghum bicolor (L.) Moench). Cereal Chemistry 83(2): 194-201.

### *Book Chapters*

Joel, D.M., Y. Hershenhorn, H. Eizenberg, R. Aly, G. Ejeta, P.J. Rich, J.K. Ransom, J. Sauerborn and D. Rubiales. 2007. Biology and management of weedy root parasites. in: J. Janick (ed.) Horticultural Reviews, Vol. 33. John Wiley & Sons, Inc. Hoboken, NJ. pp. 267-350.

### *Invited Presentations*

Knoll, J. and G. Ejeta. 2005. Identification and validation of QTL for early-season cold tolerance in sorghum. Invited graduate student poster presented at American Seed Trade Association meeting, Chicago, IL, USA, 7-9 December, 2005.

Tesso, T., I. Kapran, C. Grenier, A. Snow, P. Sweeney, D. Marx, J. Pedersen, G. Bothma and G. Ejeta. 2005. Potential of crop-to-wild gene flow in sorghum in Ethiopia and Niger: A geographic survey. Poster presented at NCWSS/Crop Gene Flow meeting. Kansas City, MO, USA, 14-15 December, 2005.

Saballos, A., G. Ejeta and W. Vermerris. 2006. Development of brown midrib sweet sorghum as a biomass crop. Oral presentation (1b-04) given at Symposium on Biotechnology for Fuels and Chemicals hosted by the Oak Ridge National Laboratory, Nashville, TN, USA, 30 April to 3 May, 2006.

Saballos Espinal, A., W. Vermerris and G. Ejeta. 2006. Genetic enhancement of sorghum for biomass conversion. Oral presentation (#57-1) given at ASA-CSSA-SSSA International Meetings, Indianapolis, IN, USA, 12-16 November, 2006.

Gutema, Z.W., J. Knoll, J. Santini and G. Ejeta. 2006. Genetic analysis of early season cold tolerance in sorghum. Oral presentation (#57-3) given at ASA-CSSA-SSSA International Meetings, Indianapolis, IN, USA, 12-16 November, 2006.

# Enhancing the Utilization of Grain Sorghum and Pearl Millet through the Improvement of Grain Quality via Genetic and Nutrition Research

## Projects

**KSU 220A - Mitchell Tuinstra, Kansas State University**
**KSU 220 B- Joe Hancock, Kansas State University**
**TAM 220C - William Rooney, Texas A&M University**
**TAM 220D - Clint Magill, Texas A&M University**

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### Summary

The marketing and utilization of sorghum grain often has been limited by lower grain quality and feeding value compared to other cereals. Our research project attempts to address this weakness through plant breeding to develop elite varieties and hybrids with improved nutritional and grain quality traits and through development and transfer of animal feed and production technologies to developing countries.

### Improve Nutrition and Increase Yield

One objective of our project was to develop sorghum varieties and hybrids with enhanced grain quality characteristics. Large-seeded sorghum genotypes with enhanced feed-value and grain-quality characteristics were identified via animal feeding trials. The genes for improved nutritional value were incorporated into local and improved genetic backgrounds. These varieties are being evaluated for adaptation and production characteristics through collaboration with colleagues in Western and Southern Africa and Central America.

Additionally, much of the historical effort in development of food-grade sorghum varieties has focused on selecting for improved grain mold resistance. Grain mold resistance is so important that molecular markers are being developed to more efficiently manipulate this trait in breeding populations. Questions have emerged that ask whether selection of genotypes that produce grains resistant to microbial and fungal attack may inadvertently have reduced digestibility by enzymes of the digestive tracts in livestock and humans. Poultry feeding trials were conducted to compare the nutritional value of grain produced from mold resistant and susceptible genotypes in different environments. These trials demonstrated that selection for mold resistance had minimal and inconsistent effects on the nutritional value of sorghum grain for broiler chicks.

### Improve Institutional Capacity

The focus of our training program was to build human capital among research institutions in developing countries. In West

Africa, technology transfer efforts were initiated through interaction with Dr. Salissou Issa, Head of the Animal Husbandry Unit at the INRAN Rainfed Crops Program in NIGER. These efforts included farm visits and feeding trials to demonstrate the relative feeding value of local and improved sorghum varieties in comparison to traditional corn-based feed rations. After completion of a Poultry Field Day and formation of the Nigerien Poultry Growers Association, Salissou transferred to Kansas State to pursue a Ph.D. degree. His program included course work and some research experience in the U.S. with much of his research efforts designed to demonstrate the feeding value of sorghum grain via well designed experiments "on-site", back at his research institute in Niamey. Other graduate students and visiting scientists with interest in crop improvement, crop utilization, and molecular biology were hosted for short-term and graduate training at Kansas State University and Texas A&M University. The research projects of these student were strongly multidisciplinary and provided opportunities for collaboration with investigators from other departments and universities. Finally, our U.S. investigators presented seminars to feed manufacturers, poultry producer groups, and research scientists in Niamey, Burkina Faso, and Senegal to promote the use of sorghum grain in animal feeds.

In Central America, our training program was focused on the transfer of technology to allow development and utilization of improved sorghum and pearl millet cultivars for animal feeding and human food. A key component of our technical assistance efforts was a series of seminars given to livestock and poultry producers in El Salvador and Nicaragua on the use of sorghum grain as animal feed. Additionally, efforts in Central America included development of human capital via collaboration on an research project at the agricultural university of Nicaragua in Managua (UNA). For this project, ties were formed with the Feed Plant Manager (Francisco Baltodano) and Director of Livestock Operations (Miguel Rios) at the university and the Executive Director (Francisco Vargas) of the National Sorghum Growers Association (AMPROSOR). The university facilities were renovated to allow conduct of chick feeding experiments that demonstrated the excellent feeding characteristics of sorghum grain (when properly milled) compared to imported corn. Of equal importance was that these experiments were used as the Senior Project for four undergraduate students in the Department of Animal Science at UNA. On another front, participation in the LANCE/RAPCO Short Courses for Animal Nutrition and Feed Manufacturing was a vital component of our technology efforts in Central America.. This week-long short course was held each year in Costa Rica and included participants (professionals in nutrition, feed plant management, quality control, and ingredient procurement) from Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, the Dominican Republic, Columbia, Venezuela, Peru, and Ecuador. In the course, attention was given to issues (real and perceived) that limit expanded use of sorghum as a feedstuff for animal producers throughout Latin America.

## Promote Economic Growth

Past breeding efforts have significantly enhanced yield potential of sorghum in semi-arid regions of the world. However, little attention has been focused on feed value and grain quality in these production environments. Tan-plant sorghum hybrids with improved drought tolerance are being developed to address this problem. In the United States, food-grade hybrids are now commercially available in all maturity groups. These hybrids are high-yielding and well-adapted to dryland and limited-irrigation environments and will undoubtedly increase the value of sorghum as a human foodstuff. As for feed value, poultry and egg production is rapidly increasing throughout the developing world. These industries are providing increased market opportunities for sale of grain not consumed by humans. In many semi-arid environments, sorghum is the logical cereal grain for use in manufacturing feed. However, sorghum is not always considered the feedstuff of choice because of fear about tannins that decreased feed intake (and, this growth rate) and nutrient digestibility. Numerous laboratory and animal feeding experiments were conducted in the United States, West Africa, and Central America to demonstrate and quantify the value of local and improved sorghum grains for use in feed manufacturing. Of particular importance was demonstration of the impacts of various manufacturing processes (such as fine-grinding) on feed value of the grain for layers and broilers. These experiments conclusively demonstrated that when properly milled, sorghum grain was similar to corn as a feedstuff in diets for broilers and layers used for meat and egg production.

## Objectives, Production and Utilization Constraints

### *Objectives*

- Identify and map genes associated with improved grain and feed quality characteristics
- Develop robust biotechnology tools for tagging genes that contribute to grain mold resistance and enhanced nutritional value
- Develop high-yielding sorghum cultivars with improved feed quality and local adaptation characteristics by conventional plant breeding and marker-assisted selection technologies
- Provide technology transfer and technical assistance in promoting the use of improved sorghums and millet in poultry feeding in the developing regions of Africa and Central America.

### *Production and Utilization Constraints*

New entrepreneurial opportunities for production of animal feeds, meat, and eggs in developing countries are needed to move sorghum and millet from subsistence crops to value-added commodities. Thus, our research efforts were focused to address food quality and feed efficiency traits that would make sorghum the cereal of choice in diets for humans and livestock.

Components of feed quality are usually defined in terms of animal performance or metabolizable energy value. Thus, in our project we integrated laboratory assays for feeding quality, traditional plant breeding, and biotechnology techniques to develop elite hybrids and cultivars with improved nutritional and grain quality traits. These genetic resources were transferred to developing countries with modern poultry production practices to facilitate development of new markets for sorghum and millet. Recognition of the true nutritional value of grain sorghum by animal producers lead to greater poultry production and productivity in regions of the world where hunger and poverty are major issues.

## Research Findings and Project Output

### *Use of Sorghum for Poultry Feed*

Maize-based feed is common in many areas of Africa and Central America because the grain is cheap and readily available. In semi-arid environments, sorghum represents a more logical choice for use in manufacturing feed. However, animal producers generally will not feed sorghum-based diets because of misconceptions about the feed value and tannin content of the grain. In most cases, these concerns are unfounded because improved sorghum cultivars are low in tannin and have feed-value nearly equal to maize. Thus, to demonstrate the nutritional value of sorghums, chick growth and metabolism assays were conducted and the nutritional quality of sorghum was determined and compared to that of maize.

Our first objective was to compare sorghum genotypes with different grain morphologies and compositional characteristics to determine how these traits were related to variation in poultry feed quality. Extensive testing indicated that large seeded sorghum genotypes such as KS115, with their greater protein and fat content, had greater nutritional value and higher yield potential (Kriegshauser et al., 2006). At the time of this writing, KS115 was being used in combinations with locally adapted landrace varieties from West and Central Africa to produce lines and hybrids having large seed size and good local adaptation.

An additional consideration is that feed manufactures in Africa are likely to use grains of landrace varieties as well as improved sorghum cultivars for production of poultry feed. Many landrace sorghum varieties produce grain with a pigmented testa that has condensed tannins. Although tannins are known anti-nutritional factors that can depress growth performance in chicks, pigs, and rats, it is not known how much tannin can be tolerated in the diet before animal performance suffers. Furthermore, it is known that tannins from different origins (tea, grapes, coco) have different potencies leaving the possibility that some sorghums might contain tannins of little or no nutritional consequence. So, sorghum treatments were created by mixing decorticated endosperm from a non-tannin sorghum with blends of bran from tannin and non-tannin sorghum varieties. A broiler chick feeding trial indicated a 20% decrease in average daily gain as tannin bran (from Shanqui Red) in the diet was increased. In subsequent experiments, tannin and non-tannin brans were blended to produce tannin concentrations ranging from 0 to 5.44% CE (where % CE = catechin equivalents/100 mg of grain DM). Rate of gain was decreased by 8% as tannin concentration was increased from 0 to 5.44% CE. However, the responses were mostly quadratic and suggested that some tannin (up to 1.35% CE) was tolerated by growing broiler chicks without loss in growth performance and/or nutrient utilization.

To further investigate the possible variability in the negative effects of tannins, broiler and layer trials were conducted in Niger. The control diet had corn that was imported from Nigeria (a common practice among Nigerien poultry producers) with fishmeal and peanut cake used as the primary protein supplements. Sorghum was used to replace the corn on a wt/wt basis so that treatments were: 1) corn-based control; 2) a locally adapted sorghum landrace (Mota Galmi) with a pigmented testa and 0.3 mg % CE; and 3) an improved (white-seeded, food-grade) sorghum variety (IRAT204) with no detectable tannins. In the broiler feeding trial, ADG and ADFI were greater ($P < 0.001$) for chicks fed corn than the sorghums but carcass weight, carcass yield, and carcass fat were not different ($P > 0.35$) among the various treatments. However, more close scrutiny of the data indicated that the locally-adapted landrace was superior in nutritional value to the improved food-grade sorghum and comparable in nutritional value to imported corn. In a second experiment, Harco layers were evaluated for 18-months to determine the effects of the sorghum varieties on egg production. No differences in growth rate were detected among birds fed the corn and sorghum treatments in the first 126 days of the experiment. However, birds fed the sorghum-based diets took fewer days to come into production ($P < 0.007$), ate more feed ($P < 0.02$), and produced more eggs ($P < 0.001$) than birds fed the corn-based diet There were no differences in egg weight and egg:feed ratio among birds fed corn and the sorghums. In summary of these experiments, it can be argued that some sorghums are equal or superior to corn as a feedstuff in diets for broilers and layers reared in West Africa.

A final need for chick feeding experiments resulted from questions concerning the effects of selection for mold resistance in sorghum grain without concern for whether resistance to microbial/fungal attack might also cause resistance to attack by digestive enzymes. A chick experiment was designed with diets based on grain produced from mold resistant and susceptible genotypes grown in different environments. Analyses of the data indicated no consistent differences among locations and sorghum type for growth performance or nutrient utilization. Thus, selection for mold resistance had minimal and inconsistent effects on the nutritional value of sorghum grain for broiler chicks. Our data demonstrated that sorghum breeders should continue with current efforts to improve mold resistance in sorghum without fear of reducing nutritional value of the resulting grain.

### *Marker Assisted Selection for Grain Mold Resistance*

In previous work, five quantitative trait loci (QTL) were detected for grain mold resistance from Sureño in the recombinant inbred line progeny from the cross of RTx430 x Sureño. In recognition of these findings, a critical component of the KSU220 project was to determine the efficacy of marker assisted selection (MAS) when used to improve grain mold resistance in sorghum.

Five sorghum populations were developed. In each population, Sureño was used as the grain mold resistant parent with one of five elite parental lines (Tx430, Tx436, Tx2903, Tx635, and Tx631). From each cross, F2 progeny were selected based on maturity and short plant height. Molecular markers associated with QTL for grain mold resistance originating in Sureño were then used to determine if their presence enhanced selection for grain mold resistance in these populations. The QTL genotypes of the 87 F4 lines were determined using simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP) markers. The F4:5 lines also were evaluated for grain mold resistance in replicated trials in eight diverse environments in South and Central Texas during the summer of 2002. The effects of each allele from Sureño were determined across and within all five populations, within individual environments, and in each population x environment combination. The results indicated that while these alleles confer additional grain mold resistance in the progenies derived from RTx430 x Sureno, their applicability beyond the mapping population is limited. This fact reduces their potential usefulness in applied sorghum breeding programs and suggests that additional molecular characterization of this trait is needed.

Other efforts at MAS for improved grain quality focused on development of robust, easily-scored markers for known genes and QTL involved in grain mold resistance. Several sorghum defense response genes that are activated in immature florets were identified in response to inoculation with Curvularia lunata and Fusarium thapsinum. The timing and level of mRNA induction was detected using real-time PCR to identify genes with optimal regulation characteristics.

A gene that confers resistance to anthracnose (*Colletotrichum gramincola*) in sorghum cultivar SC748 was tagged with flanking PCR-based markers. These markers permit the efficient incorporation of the gene into otherwise high yielding cultivars that lack resistance, even in the absence of high disease pressure. As other anthracnose resistance genes are tagged, it also will be possible to combine or "stack" multiple genes for resistance based on the easily detected DNA-based markers. Gene stacking is expected to provide much more durable resistance to pathogens, but is virtually impossible without the use of marker assisted selection. Another anthracnose resistance gene present in cultivar SC155 has been tagged with two AFLP markers that also show polymorphism in the mapping parents, allowing the marker to be pinpointed to a location on sorghum linkage group "B". Nearby SSR markers are being evaluated to identify those suitable for use in marker-assisted selection.

### *Enhancing Protein Digestibility*

Sorghum breeding and utilization programs have identified several sorghum variants that possess unique and in some cases desirable grain quality traits. One such trait is enhanced protein digestibility which allows greater utilization of both protein and starch in the grain. This characteristic has obvious benefits for an array of end uses; however, if varieties with these mutations are to be grown, they must possess suitable levels of grain mold resistance. Two accessions with enhanced protein digestibility were evaluated in South Texas and Central America and grain mold susceptibility in these lines were increased compared to locally produced checks. To determine if susceptibility was because of increased protein digestibility or other grain based factors, these lines were crossed to grain mold resistant lines and progeny were selected for grain mold resistance. In cooperation with TAM230, these lines were screened to determine if any possessed increased digestibility. Within the group of 25 lines, six had greater protein digestibility. To determine if grain mold resistance was better in these lines, they, along with comparable lines with normal digestibility were grown in multi-location trials in 2005 and 2006. Comparisons indicated that enhanced protein digestibility can be selected in genotypes with improved levels of grain mold resistance (when compared to the original accessions). These improvements seemed to result from changes in pericarp, mesocarp, and endosperm characteristics that likely serve to protect the endosperm from degradation. None of the lines were highly resistant and it is not known if commercially acceptable levels of resistance could be developed in these materials. These populations do provide the basis for further study and development.

### *Striga Resistance*

Researchers attending the 2004 and 2006 West Africa Regional Workshop in Burkina Faso indicated that *Striga* infestations were one of the most important regional constraints to sorghum production. It has been estimated that 3.5 million ha of agricultural lands in West Africa are infested by *Striga*. Thus, we developed a new low-biotech strategy for managing *Striga* infestations in sorghum.

Seeds from an experimental sorghum hybrid (ATx623 x Tailwind) with resistance to acetolactate synthase (ALS) inhibiting herbicides were treated with varying rates of imazapyr (0.018, 0.037, 0.075 mg ai seed-1) and metsulfuron (0.003, 0.006, 0.012 ai seed-1). These treatments were compared to an untreated control group in field trials in Cinzania, Mali and Konni, Niger in 2005 and 2006. *Striga* infestation was variable in Mali and no significant differences were detected among treatments. However, in Niger significant differences were detected among herbicide treatments. *Striga* emergence was delayed by up to 20 days in plots produced from herbicide treated seeds with eight-fold reductions in *Striga* infestation in herbicide treated plots compared to the control. These studies suggested that herbicide seed treatments may provide another tool for suppressing or delaying *Striga* parasitism in sorghum. Improved food- and feed-grade sorghum varieties that integrate this low-biotech approach with host-plant resistance to *Striga* are being developed for evaluation in integrated *Striga* management (ISM) crop production systems.

## Products and/or Impacts

### *Poultry Production in West Africa*

Numerous laboratory and production trials were conducted in the United States, West Africa, and Central America to demonstrate and quantify the value of local and improved sorghum grains for use in animal feed. Feed manufacturing technologies such as fine-grinding were evaluated as tools to further improve the feed value of grain for meat and egg production. Poultry producers from each region were invited to participate in a review of these research projects. In Niger, this dialogue led to the formation of the Nigerien Poultry Producers Cooperative.

### *Sorghum variety and hybrid development*

Kansas State University developed and distributed seven new parent lines and 19 germplasm lines to seven commercial seed companies in the United States and other countries. These lines are being evaluated in commercial breeding programs and one parent currently is being used in commercial hybrid seed production. Additionally, Texas A&M University released two sets of sorghum germplasm (Tx2912-2920 and Tx2921-Tx2928) that were made available to sorghum improvement programs throughout the world.

From 2001 to 2004, the PIs in our project coordinated the United States Tan Plant Hybrid Trial that was designed to evaluate commercially available tan plant sorghum hybrids for agronomic adaptation and grain quality parameters. The test was grown at multiple locations in Kansas and Texas. These trials indicated that commercial food-grade hybrids are now available in all maturity groups. These hybrids are high-yielding and well-adapted to dryland and limited-irrigation environments.

Germplasm exchange activities included distribution of elite parent lines, testcross hybrids, high-feed value breeding lines, and forage sorghums. These materials were distributed to national program scientists for evaluation in Niger, Mali, Ghana, Senegal, Zambia, and Nicaragua.

### *Herbicide Seed Treatments: A new tool for* Striga *management in sorghum*

As explained earlier in this report, a low-biotech strategy was developed for managing *Striga* infestations in sorghum. This strategy involves use of low-dose ALS-inhibiting herbicide seed coating applied to ALS-herbicide tolerant sorghum varieties. This tolerance trait is being incorporated into sorghum varieties and hybrids adapted for production in different regions of Africa in combination with host-plant resistance genes for *Striga*. Kansas State University is working with the agricultural chemical industry to register one or more herbicides for this use.

## Description of Methods of Work Used

Collaborative research efforts in Africa and Central America were supported through short and long-term training programs, germplasm exchange and evaluation, and basic research support activities. These research efforts were conducted in three regional programs including West Africa, Southern Africa, and Central America.

Crop improvement efforts to develop cultivars adapted to environments in West Africa, Southern Africa, and Central America utilized elite varieties and cultivars that were adapted to each of the regions. The lines used to create these populations were selected through evaluation of elite U.S. and host-country germplasm in the targeted region. This material was evaluated in the target region in conference with collaborating plant breeders. Improvement efforts in Western and Southern Africa were focused on development of early-maturing, drought-tolerant cultivars and hybrids that incorporate *Striga* resistance. Efforts in Central America were on improved food-type and Macio Criollos cultivars. These efforts were focused on development of photoperiod sensitive hybrids using Ma5 and Ma6.

The underlying objective for research to identify and map genes related to grain quality was to develop a better understanding of the genetic control of important quality traits. This greater understanding then would allow us to generate genetic markers to be used by sorghum improvement programs in the immediate future. Combining these traits into one genotype will be a significant challenge that can be facilitated by use of molecular technology. Development of these technologies should enhance the efficiency of combining grain quality factors (e.g., feed quality characteristics and grain mold resistance) into varieties with high yield potential. Mapping populations were developed and characterized in cooperation with collaborators at domestic and international sites. These populations were genotyped in laboratories in the U.S. using various of genetic markers.

Technical assistance and technology transfer efforts in poultry production and nutrition were facilitated through workshop and short course activities as well as feeding trials and demonstrations. The feeding trials specifically were designed to compare feed value of corn- and sorghum-based poultry diets in West Africa and Central America. Replicated trials to evaluate performance of broiler and/or layer chickens were conducted in Niger and Nicaragua from 2003-2007.

## Networking Activities

Workshops, conferences, and meetings to support extensions services, NGOs, regional networks, international agencies, USAID missions and others

INTSORMIL Principal Investigators (PI) Conference, Addis Ababa, Ethiopia – 2002

ASA-CSSA-SSSA – 2002, 2003, 2004

Cursos Regionales en Produccion Animal (RAPCO Short Courses), Costa Rica – 2003, 2004, 2005, 2006, 2007

Sorghum Improvement Conference of North America (SICNA) – 2003, 2005, 2007

USDA Sorghum Germplasm Committee – 2003, 2004, 2005, 2006, 2007

American Seed Trade Association (ASTA) – 2003, 2004, 2005, 2006

INTSORMIL Western Africa Regional PI Workshop, Ouagadougou, Burkina Faso – 2004, 2006

INTSORMIL Central American Regional Workshop – 2002

United States Great Plains Sorghum Conference – 2004, 2005, 2006

NSF Sorghum Genomics Workshop –2004

INTA/CINA/INTSORMIL Sorghum Utilization Conference, Managua, Nicaragua - 2005

Integrating New Technologies for *Striga* Control: Towards Ending the Witchhunt, Addis Ababa Ethiopia – 2006

USAID/INTSORMIL West Africa Sorghum in Poultry Diets Conference, Saly, Senegal -2007

West African Workshop on Hybrid Sorghum and Millet, ICRISAT, Samanko, Mali – 2007

## Germplasm and Research Information Exchange

Kansas State University developed and distributed seven new parent lines and 19 germplasm lines to seven commercial seed companies in the United States and other countries. Texas A&M University released two sets of sorghum germplasm (Tx2912-2920 and Tx2921-Tx2928) in 2002. These germplasms were made available to sorghum improvement programs throughout the world.

The PIs were active members of the USDA Sorghum Germplasm Committee (SGC) from 2002 to 2007. The SGC funds and coordinates public and private germplasm research in the United

States including evaluation and seed increase of all photoperiod insensitive sorghum lines from the U.S. collection and development, seed increases, and characterization of a sorghum association panel (300 genotypes) for use in gene association studies.

Elite parent lines, testcross hybrids, high-feed value breeding nurseries, *Striga* resistant varieties, and forage sorghums were distributed to national program scientists for evaluation in Niger, Mali, Ghana, Senegal, Zambia, El Salvador, and Nicaragua in each year from 2003 to 2007.

The PIs coordinated the United States Tan Plant Hybrid Trial. This project was designed to evaluate commercially available tan plant sorghum hybrids for agronomic adaptation and grain quality parameters from 2001-2004. The test was grown at multiple locations in Kansas and Texas.

Assistance given to collaborating scientists with research equipment, supplies, and/or other support.

The PIs supported the development and/or expansion of sorghum breeding and poultry production research in West Africa, Southern Africa, and Central America. Support for these programs included direct financial support from this project as needed (e.g., pollinating bags, cameras, computers and other equipment, graduate training, purchases of chicks and feed ingredients, etc). Additionally, PIs from this project participated and presented in numerous workshops (sponsored by INTSORMIL, USAID, UNA, INRAN, INTA, CINA, ICRISAT, et al.) in each region.

An e-mail interactive group of scientists interested in the use of biotechnology has been established. These scientists participate from national laboratories in Mali, Burkina Faso, Nigeria, Ghana, and Senegal. Small equipment items required for DNA extraction, PCR amplification, and gel separation have been provided for the use of IER associates in Mali. Pass through funds have been provided for use in Burkina Faso and Nigeria, primarily for permitting disease surveys to be made and for providing samples for use in DNA comparisons.

## Publications

### Journal Articles

Bean S.R., O.K. Chung, M.R. Tuinstra, J.F. Pedersen, and J. Erpelding. 2006. Evaluation of the single kernel characterization system (SKCS) for measurement of sorghum grain attributes. Cereal Chemistry 83:108-113.

Brown, P.J., P.E. Klein, E. Bortiri, C. Acharya, W.L. Rooney, and S.K. Kresovich. 2006. Inheritance of inflorescence architecture in sorghum. Theor. Appl. Genetics 113: 931-942.

Kriegshauser T.D., M.R. Tuinstra, and J.D. Hancock. 2006. Variation in nutritional value of sorghum hybrids with contrasting seed weight characteristics and comparisons with maize in broiler chicks. Crop Science 46: 695-699.

Kuhlman, L.C., D.R. Pring, W.L. Rooney, and H.V. Tang. 2006. Allelic frequency at the Rf3 and Rf4 loci and the genetics of A3 cytoplasmic fertility restoration in converted sorghum lines. Crop Sci. 46:1576-1580.

Perumal, R., T. Isakeit, M. Menz, S. Katilé, E-G. No, and C.W. Magill. 2006. Characterization and genetic distance analysis of isolates of Peronosclerospora sorghi using AFLP fingerprinting. Mycological Res. 110:471-478.

Price, H. J., G.L. Hodnett, B.L. Burson, S.L. Dillon, D.M. Stelly, and W.L. Rooney. 2006. Genotype dependent interspecific hybridization of sorghum bicolor. Crop Sci. 46 2617-2622.

Rodriguez-Herrera R., R.D. Waniska, W.L. Rooney, C.N. Aguilar, and J.C. Contreras-Esquivel. 2006. Antifungal proteins during sorghum grain development and grain mold resistance. Plant Pathology J. 154:565-571.

Rodríguez-Herrera, R., R. A. Frederiksen, W. L. Rooney, I. Kollo, C. N. Aguilar-González, and A. Quero-Carrillo. 2006. Grain molding fungi association in food type sorghum kernels and their effects on germination. Plant Pathology Journal. 5: 221-227.

Sabry, A.B., D. Jeffers, S.K.Vasal, R. Frederiksen, and C.W. Magill. 2006. A region of maize chromosome 2 affects response to downy mildew pathogens. Theor. Appl. Genet. 113:321-330.

Singh, M., K. Chaudhary,, H.R. Singal, C.W. Magill, and K.S.Boora. 2006. Identification and characterization of RAPD and SCAR markers linked to anthracnose resistance gene in sorghum [Sorghum bicolor (L.) Moench]. Euphytica 149:179-187.

Viswanathan, A., A. Sankaralingum, R.P. Thakur, D. Hess, S. Sivaramakrishnan, and C.W. Magill. 2005. Sequence of ITS-2 from pearl millet downy mildew samples. International Sorghum and Millets Newsletter 46: 123-125.

Wu X., R. Zhao, D. Wang, S.R. Bean, P.A. Seib, M.R. Tuinstra, M. Campbell, and A. O'Brien. 2006. Effects of amylose, corn protein and corn fiber contents on production of ethanol from starch rich media. Cereal Chemistry 83: 569-575.

Wu X., R. Zhao, S.R. Bean, P.A. Seib, J.S. McLauren, R.L. Madl, M.R. Tuinstra, M.C. Lenz, and D. Wang. 2007. Factors impacting ethanol production from sorghum. Cereal Chemistry 84:130-136.

Xie X.J., Y. Teh, S. Liang, P.A. Seib, and M.R. Tuinstra. 2006. Wet-milling of grain sorghum of varying seed size without steeping. Starch/Staerke 58: 353-359.

### Abstracts

Feoli, C., S. Issa, J.D. Hancock, T.L. Gugle, S.D. Carter, and N.A. Cole. 2007. Effects of adding saturated fat to diets with sorghum-based distillers dried grains with solubles on growth performance and carcass characteristics in finishing pigs. Accepted for presentation at the National Meetings of the American Society of Animal Science, San Antonio, TX.

Feoli, C., J.D. Hancock, C. Monge, T.L. Gugle, S.D. Carter, and N.A. Cole. 2007. Digestible energy content of corn- vs sorghum-based distillers dried grains with solubles in finishing pigs. Presented at the Midwest Sectional Meetings of the American Society of Animal Science, Des Moines, IA.

Feoli, C., J.D. Hancock, C. Monge, T.L. Gugle, S.D. Carter, and N.A. Cole. 2007. Effects of corn- and sorghum-based distillers dried grains with solubles on growth performance and carcass characteristics in finishing pigs. Presented at the Midwest Sectional Meetings of the American Society of Animal Science, Des Moines, IA.

Feoli, C., J.D. Hancock, M.C. Herrera, G.M. Herrera, M.J. Ríos, F. Vargas, and S.C. Mason. 2007. Nutritional value of corn versus sorghum when ground through different screen sizes and used in diets for broiler chicks. Accepted for presentation at the National Meetings of the Poultry Science Association, San Antonio, TX.

Issa, S., J.D. Hancock, M.R. Tuinstra, I. Kapran, and S. Kaka. 2007. Effects of sorghum variety on growth and carcass characteristics in broiler chicks reared in West Africa. Accepted for presentation at the National Meetings of the Poultry Science Association, San Antonio, TX.

Issa, S., J.D. Hancock, M.R. Tuinstra, I. Kapran, and S. Kaka. 2007. Effects of sorghum variety on growth and subsequent egg production in layers reared in West Africa. Accepted for presentation at the National Meetings of the Poultry Science Association, San Antonio, TX.

Katilé, S.O., P. Ramasamy, L.K. Prom, W.L. Rooney, and C.W. Magill. 2007. Defense gene response to sorghum grain mold using real time PCR analysis. Sorghum Improvement Conference of North America, Albuquerque, NM.

Monge, C.R., J.D. Hancock, C. Feoli, R.C. Kaufman, M.R. Tuinstra, S.R. Bean, S. Beyer, and B.P. Ioerger. 2007. Effects of tannin concentration on nutritional value of sorghum grain in broiler chicks. Accepted for presentation at the National Meetings of the Poultry Science Association, San Antonio.

Monge, C.R., J.D. Hancock, C. Feoli, R.C. Kaufman, M.R. Tuinstra, S.R. Bean, S. Beyer, and B.P. Ioerger. 2007. Effects of tannins from different sorghums on nutrient utilization in broiler chicks. Accepted for presentation at the National Meetings of the Poultry Science Association, San Antonio, TX.

Ramasamy P., P. Nimmakayala, U.K. Reddy, S. Erattaimuthu, E-G. No, L.K. Prom, D.G. Luster, and C.W. Magill. 2007. Simple sequence repeat markers distinguish among pathotypes of sorghum downy mildew (*Peronosclerospora sorghi*) and related species. Sorghum Improvement Conference of North America, Albuquerque, NM and the 2007 International Plant and Animal Genome Conference, San Diego http://www.intl-pag.org/15/abstracts/PAG15_P03b_146.html

Ramasamy, P., M.A. Menz, P.J. Mehta, S. Katile, L.A. Gutierrez Rojas, R.R. Klein, P.E.Klein, L.K.Prom, J.A. Schlueter, W.L. Rooney and C.W. Magill. 2007. Molecular mapping of Cg1, a gene for resistance to anthracnose (*Colletotrichum sublineolum*) in sorghum. Sorghum Improvement Conference of North America, Albuquerque, NM and the 2007 International Plant and Animal Genome Conference, San Diego, http://www.intl-pag.org/15/abstracts/PAG15_P05d_371.html

# Germplasm Enhancement for Resistance to Insects and Improved Efficiency for Sustainable Agriculture Systems

## Project TAM 223
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### Summary

#### *Increase Yield*

To develop new genetics for cultivars and hybrids a number of sorghum lines were evaluated for resistance to several abiotic and biotic stresses. The objective was to identify, characterize, and utilize the genetic diversity of sorghum to develop sorghum germplasm, parental lines, or varieties with resistance to multiple stresses including insects, disease, and drought. Primary insect pests are the greenbug (*Schizaphis graminum*), sorghum midge (*Stenodiplosis sorghicola*), and sugarcane aphid (*Melanaphis sacchari*). Concurrent selection is practiced for the following diseases: sorghum downy mildew (caused by *Peronosclerospora sorghi* (Westan and Uppal) Shaw), head smut (caused by (*Sphacelotheca reiliana* (Kuhn) Clinton), anthracnose (caused by *Colletotrichum graminicola* (Cesati) Wilson), zonate leaf spot (caused by (Gloeocercospora sorghi Bain and Edgerton), bacterial leaf streak (caused by *Xanthomonas holcicola* (Elliot) Star and Burkholder), bacterial leaf stripe (caused by *Pseudomonas andropogoni* (E.F. Smith) Stapp), rust (caused by caused by *Puccinia purpurea* Cooke) and charcoal rot (caused by *Macrophomina phaseolina* (Tassi) Goid). Selections were made to incorporate the stress resistance into food type sorghum with improved grain mold/weathering resistance.

### Promote Economic Growth and Improve Nutrition

Sorghum varieties or hybrids with multiple stress resistance increase the potential to produce a consistent supply of high quality grain for household or off-farm end-use industry. Parental lines A/BTx642 through A/BTx645 contain several valuable traits including stay-green, lodging resistance, and grain mold/weathering resistance. Seventeen biotype E greenbug/disease resistant germplasms and 17 biotype E/I greenbug resistant germplasms were approved for release. The lines are tan plant with wide adaptation and contain genes for resistance to several diseases. Tan plant red or white grain sorghum hybrids with multiple stress resistance and high yield potential may help increase utilization of sorghum in new or non-traditional uses. Forty-nine converted exotic cultivars broaden the genetic base of temperately adapted germplasm available to sorghum breeders. Tx3301 through Tx3360 possess a high level of resistance to sorghum downy mildew. The lines can be used as hybrid parents or as a source of useful genes to develop food type sorghums.

### Improve Institutional Capacity

Served as co-chair of one U.S. Ph.D. student in the Texas A&M University Department of Entomology and as co-chair of one Mali Ph.D. student at Texas Tech University. Served on the

M.S. and Ph.D. committees of a Mali student and two M.S. international students at Texas A&M University.

## Objectives, Production and Utilization Constraints

### *Project Objectives*

- Obtain and evaluate germplasm for resistance to arthropod pests and other abiotic or biotic stresses including drought and selected diseases.
- Develop and release high grain yield, agronomically improved sorghums resistant to selected insects and other biotic or abiotic stresses.
- Develop and release high grain yield sorghums with multiple stress resistance and improved grain quality traits.
- Utilize molecular biology to increase understanding of plant traits for stress resistance.

### *Sorghum Production Constraints*

Sorghum production is constrained by less than desired yield, and biotic (insect pests and disease pathogens) and abiotic (primarily pre- and post-flowering drought) stresses that reduce yield, grain and forage quality, lower value and amount of grain or forage available to cash markets, and government policy. Sorghum with genetic resistance to stress will increase ecological fitness and reduce pesticide use while increasing productivity and profitability. Grain weathering reduces grain quality, marketability and utilization. Grain with traits suitable for end-use processing will improve marketability, profitability, and increase demand for grain. As new technologies that render solutions to existing problems are deployed new biotic stresses and production constraints may occur.

Improved plant genetics for adaptation, stress resistance, and quality are needed to meet the demands of increased food production and provide high quality grain for commercial end-uses. Integrated cropping systems with improved plant genetics should be easy to implement, economically profitable, and environmentally sustainable. Plant stress occurs as concurrent multiple events, and while research can be conducted on individual stresses hybrids or cultivars must have resistance to multiple stress(es). Development of multiple stress resistant crops is a continual effort in response to a dynamic evolving cropping agro-ecosystem.

## Research Methods, Findings and Project Output

### *Research Methods*

Crop improvement programs requires a knowledge of plant genetics, field nurseries with the capability of providing adequate selection pressure to evaluate populations for important traits, and a multi-disciplinary team to research multiple traits. Pedigree breeding supplemented with backcrossing for specific traits can efficiently incorporate desired genes into new lines. Field nurseries replicated over locations and years expose segregating populations to multiple environments and lead to identification of those genotypes with the best combination of genes for the trait(s) of interest. For combining ability evaluation advanced selections are crossed to A-line testers (for R-lines) or sterilized (A1 cytoplasm) and then crossed to R-line testers (for B-lines).

Host country research is supported through short-term training, graduate education, germplasm exchange and evaluation, site visits, and research in Texas, Southern Africa, and Central America. Southern Africa activity is focused on incorporating sugarcane aphid resistance, disease resistance, adaptation, and improved grain end-use traits into new cultivars. Central America research in Nicaragua in for with sorghum midge resistance, drought resistance, disease resistance, adaptation, and end-use traits. In the U.S. important traits include biotic (disease, insect, grain mold/weathering) and abiotic (drought) stress resistance, and increased yield. Collaboration with other projects facilitates studies on inheritance, resistance mechanisms, molecular mapping, and marker-assisted selection.

Germplasm is evaluated for resistance to insects in field nurseries or greenhouse facilities. Suitable germplasm from other programs, exotic lines, and converted exotic sorghum lines is crossed to elite resistant germplasm or to germplasm with superior traits. The goal is to develop a multiple stress resistant genotype with improved end-use traits. For insects important in host countries but not in the U.S., germplasm is selected for adaptation, grain yield potential, and disease resistance in nurseries in sub-tropical South Texas. The germplasm is provided to host country collaborators in replicated trials for insect resistance and agronomic evaluation under the local production system.

## Research Findings and Project Output

Five sets of germplasm were released. Parental lines A/BTx642 through A/BTx645 (A1 cytoplasm) have with purple plant color. A/BTx642 (originally A/B35) has excellent stay-green, charcoal rot, and lodging resistance. A/BTx643 (B35 derivative) has higher grain yield potential but a lower level of stay-green. A/BTx644 has excellent pre-flowering drought tolerance and a low level of stay-green and lodging resistance. A/BTx645 has excellent grain mold/weathering resistance and produces hybrids with high test-weight grain. Tx2945 through Tx2961 are tan plant types with excellent resistance to biotype E greenbug and several diseases including grain mold/weathering. Tx2962 through Tx2978 are resistant to biotype E and I greenbug. Several lines possess a high level of disease resistance. Forty-nine converted exotic cultivars broaden the genetic base of temperately adapted germplasm available. Tx3301 through Tx3360 possess a high level of resistance to sorghum downy mildew.

A Ph.D. problem compared the efficiency of marker-assisted selection versus traditional selection methodology for greenbug (*Schizaphis graminum* (L.) Rondani) resistance and post-flowering drought tolerance (stay-green). Ninety-eight recombinant inbred line populations (RILs) were derived from a cross between BTx642, a post-flowering drought resistant but pre-flowering drought susceptible line, and Tx7000, a post-flowering drought susceptible but pre-flowering drought resistant line. Two hundred and seventy four restriction fragment length polymorphic (RFLP) markers were used to identify the main-effect and epistatic QTLs. Highly significant ($P<0.05$) differences were detected between the parents and among the RILs for grain yield, drought susceptibil-

**Table 1. Sugarcane aphid damage rating, grain yield, and grain mold rating of selected entries in the sugarcane aphid test at Potchefstroom and Burgershall, South Africa and Mt. Makulu, Zambia**

| PEDIGREE | Potchefstroom[†] | Burgershall[†] | Greenhouse[‡] | Grain Yield Potchchefstroom | Grain Yield Mt. Makulu | Grain Mold[§] |
|---|---|---|---|---|---|---|
| | | | | –kg/ha-- | - -kg/ha-- | |
| (CE151*TAM428)-LG8-BG1-LG1 | 2.3 | 1.7 | 1.0 | 5390 | 4008 | 3.8 |
| (Macia*TAM428)-LL9 | 1.3 | 1.7 | 1.0 | 4430 | 6401 | 3.5 |
| (SV1*Sima/IS23250)-LG15-CG1-BG2 -BGBK-LBK | 2.0 | 2.3 | 1.0 | 4290 | 3090 | 4.0 |
| (Segaolane*WM#322)-CG1-BGBK-C CBK-LBK | 3.0 | 2.3 | 1.0 | 4260 | 5503 | 3.2 |
| ((6BRON126/87BH8606-6*GR107-90 M46)*CE151)-LG2-CG1-BG2-BG1-C G1-CABK | 3.7 | 2.7 | 1.7 | 4030 | 4977 | 4.0 |
| (Town*EPSON2-40/E#15/SADC)-LG 1-BGBK-CCBK-LBK | 3.3 | 2.3 | 1.3 | 4020 | 1936 | 4.5 |
| (SDSL89426*6OB124/GR134B-)-LG 5-CCBK-CCBK-LBK | 2.7 | 2.3 | 1.7 | 4000 | 4651 | 3.8 |
| (EPSON2-40/E#15/SADC*A964)-CG 3-BGBK-CCBK-LBK | 3.7 | 2.7 | 5.0 | 3870 | 2747 | 3.3 |
| (CE151*TAM428)-LG15-LG1-BG1-B GBK-LBK | 2.3 | 2.3 | 1.3 | 3760 | 4644 | 3.8 |
| (EPSON2-40/E#15/SADC*A964)-LG 2-CG1-BG1-BG2-CGBK | 3.3 | 2.7 | 3.0 | 3550 | 3336 | 3.3 |
| (6OB128/(Tx2862*6EO361)*CE151)-LG16-CG1-LGBK-LG2-LBK | 2.3 | 1.7 | 1.3 | 3440 | 2079 | 4.0 |
| Kuyuma | 4.3 | 3.3 | 3.0 | 3410 | 5943 | 4.2 |
| (Macia*TAM428)-LL2 | 1.0 | 1.3 | 1.0 | 3320 | 3302 | 3.7 |
| WM#177 | 1.0 | 1.0 | 1.7 | 3270 | 4268 | 3.5 |
| (6BRON161/((7EO366*Tx2783)-HG5 4)*CE151)-LG1-BGBK-CCBK-LBK | 1.3 | 1.0 | 1.0 | 3250 | 5435 | 3.8 |
| (Marupantse*TAM428)-HM7*CA1-C G1-CA3 | 3.3 | 3.3 | 1.7 | 3230 | 1262 | 3.7 |
| (Macia*GR128-92M12)-HM20-CA2-CG1-CGBK | 1.7 | 2.0 | 1.0 | 3220 | 3968 | 3.8 |
| (6OB128/(Tx2862*6EO361)*CE151)-LG27-LG1-BG1-LG1-CGBK | 3.0 | 2.3 | 1.3 | 3200 | 1117 | 3.3 |
| PRGC/E#69414 | 1.7 | 2.0 | 1.0 | 3180 | 4815 | 4.2 |
| (96AD34/6BRON116/5BRON131/(80 C2241*GR108-90M30)-HG46-*WM# 177)-CG2-BG1-LG1-CGBK | 3.3 | 2.0 | 1.0 | 3180 | 4086 | 3.3 |
| CE151 | 2.0 | 3.3 | 2.0 | 3110 | 2921 | 4.5 |
| TAM428 | 1.7 | 2.0 | 1.3 | 3090 | 4586 | 3.8 |
| Sima (IS23250) | 1.7 | 1.3 | 1.0 | 2970 | 7082 | 3.8 |
| WM#322 | 1.7 | 1.3 | 1.3 | 2650 | 3186 | 4.0 |
| FGYQ353 | 2.7 | 2.0 | 1.0 | 2590 | 2687 | 3.3 |
| Ent.62/SADC | 1.3 | 1.3 | 2.0 | 2480 | 3988 | 4.2 |
| SDSL89426 | 2.7 | 1.7 | 1.7 | 2310 | 3571 | 4.0 |
| Segaolane | 5.0 | 5.0 | 4.3 | 670 | 5040 | 3.3 |
| Macia | 5.0 | 5.0 | 3.3 | 620 | 2866 | 3.7 |
| Mean | 3.2 | 2.9 | 2.4 | 2350 | 3689 | 3.6 |

[†]Rated on a scale of 1 = 0-10% plant necrosis or plant tissue covered by aphids, 2 = 11-25%, 3 = 26-50%, 4 = 51-70%, 5 = 71-90%, up to 6 = 91-100% plant necrosis or plant tissue covered by aphids.
[‡]Rated on a scale of 1 = no aphids present, 2 = light infestation and no dead leaves, 3 = moderated infestation and no dead leaves, 4 = high infestation and many dead leaves, up to 5 = majority of plants dying.
[§]Rated on a scale of 0 = no grain mold present to 5 = grain mold on all kernels with significant grain deterioration.

ity index, grain yield loss percentage, and grain yield geometric mean. Low drought susceptibility index did not always indicate higher grain yield but generally indicated lower grain yield loss percentage. The MAPMAKER/QTL analysis detected 11 main-effect QTLs for grain yield under water stress and non-stress conditions with 7 on linkage groups A and F. Three main-effect QTLs collectively explained 48.2% of the phenotypic variation in grain yield. Linkage group A contained a QTL (Gya.2) strongly associated (21.0%) with sorghum grain yield under one stress environment. Among the QTLs identified under non-stress environments, 1 QTL (Gyf.2) located on linkage group F was consistent across locations.

**Table 2. Grain yield, midge damage rating, and days to 50% anthesis, for selected entries in the Midge Line Test at Santa Rosa, Nicaragua, and Corpus Christi and Lubbock, TX**

| | Yield | Midge Damage Rating | Days to 50% Anthesis | | Plant Height | | |
|---|---|---|---|---|---|---|---|
| Designation | Santa Rosa | Corpus Christi† | Santa Rosa | Lubbock | Santa Rosa | Corpus Christi | Lubbock |
| | Kg/ha$^{-1}$ | | | | ----------cm---------- | | |
| (Tx2883*(Tx2864*(Tx436*(Tx2864* PI550607))))-PC1-SM1-CM2-SM2-CM2-CABK-CMBK | 6002 | 2.5 | 65 | 69 | 142 | 130 | 86 |
| (Tx2883*(Tx2737*(Tx436*(Tx2783* PI550607))))-PC4-SM1-CM2-SM2-CG2-CABK-CMBK-CGBK | 5194 | 5.0 | 60 | 68 | 151 | 140 | 112 |
| (Tx2880*(86EO361*(Tx2880*PI550 607)))-PC2-PR6-LG7-CG3-CM2-CM2-CGBK-CMBK-CG2 | 5144 | 4.5 | 62 | 69 | 152 | 128 | 122 |
| (Tx2880*(86EO361*(Tx2880*PI550 607)))-PC1-PR10-LG34-CG2-CM3-CG1-BGBK-CABK-CG2 | 5066 | 7.5 | 69 | 68 | 154 | 118 | 110 |
| (Tx2880*(86EO361*(Tx2880*PR55 0607)))-PC1-PR10-LG34-CG1-CG1-CG2-CMBK-BGBK-CG1 | 4784 | 8.5 | 58 | 71 | 147 | 110 | 117 |
| (Tx2883*(Tx2737*(Tx2783*PI55060 7))))-PC2-SM3-CM1-SM1-LGBK-CABK-CABK | 4609 | 2.0 | 59 | 70 | 148 | 118 | 100 |
| (Tx2783*(Tx2737*(Tx436*(Tx2783* PI550607))))-PC1-SM2-CM1-SM1-CMBK-CABK-BGBK-CGBK | 4223 | 5.5 | 66 | 70 | 139 | 122 | 122 |
| (91CC515*MR114-90M11)-SM4-LMBK-CM1-SM2-SM1-HM1-CMBK-CMBK | 3943 | 2.0 | 62 | 69 | 121 | 105 | 105 |
| (7ML54/7BRON132/((IS2549C*Tx2 767)*Tx2876)*MB108B)-SM3-SM1-CM1-CM1-CMBK | 3852 | 2.5 | 64 | 70 | 148 | 135 | 130 |
| 00MLT165/01MLT156/(PM12713*T x2880)-CM5-CM3- | 3709 | 3.5 | 58 | 72 | 136 | 115 | 107 |
| (Tx2883*(Tx2737*(Tx436*(Tx2783* PI550607))))-PC1-SM2-CM1-SM2-SM2-CABK-BGBK-CMBK | 3626 | 4.0 | 64 | 70 | 122 | 120 | 99 |
| (Tx2880*(Tx2880*(GR108-90M24*(Tx2862*(Tx430*(Tx2862* PI550607))))))-PR1-SM1-CM1-CM2-LGBK-BGBK-BGBK | 3556 | 4.0 | 59 | 72 | 157 | 120 | 105 |
| (Tx2880*(Tx2880*(Tx2864*(Tx436* (Tx2864*PI550607)))))-PR3-SM6-CM3-CM1-CM2-CABK | 3430 | 3.5 | 60 | 69 | 131 | 108 | 107 |
| Tx2880 | 3392 | 2.9 | 67 | 69 | 141 | 100 | 94 |
| (Tx2880*(Tx2880*(Tx2864*(Tx436* PI550607)))))-PR2-LG24-CG2-CG1-CG1-CA1-CMBK | 3333 | 2.0 | 67 | 70 | 118 | 115 | 115 |
| (Tx2880*(GR127-90M39*(Tx2862*(Tx2864*PI550607 ))))-PC1-SM1-SM1-CM2-CG2-BGBK-CABK | 3303 | 7.0 | 67 | 69 | 125 | 118 | 105 |
| (Tx2883*(Tx2864*(Tx436*(Tx2864* PI550607))))=PC1-LG4-CG2-CM1-CM2-CABK-BGBK | 3263 | 3.0 | 65 | 69 | 118 | 115 | 86 |
| (Tx2880*(Tx2880*(Tx2864*(Tx436* (Tx2864*PI550607)))))-PR3-SM6-CM3-CM3-CG3-BGBK-CABK | 3130 | 5.0 | 58 | 68 | 148 | 113 | 105 |
| MEAN | 2249 | 4.3 | | | | | |
| LSD.05 | 550 | 1.8 | | | | | |

†Rated on a scale of 1 = 0-10% damaged kernels, 2 = 11-21%, up to 9 = 80-100% damaged kernels.

Performance of BC2 to BC4 generations from introgressing stay-green into greenbug resistant lines was evaluated in 217 back-cross lines from 9 populations. No progeny or parental line was as stay-green as the resistant check and only four lines were classified as stay-green. The populations generally produced more grain than the parental lines. Most progeny were resistant to biotype E greenbug and slightly resistant to susceptible to biotype I. In general, lines with a combination of multiple QTLs were resistant to moderately resistant to both greenbug biotypes. QTL9 was more linked to resistance to both greenbug biotypes than QTL2, which was found linked to biotype E.

The performance of backcross generations from introgressing stay-green QTLs into elite sorghum lines was evaluated in 150 BC2 to BC4 progenies from 5 populations. Only one line could be classified as stay-green as the resistant check. There was no relationship between stay-green QTL analysis and field phenotypic ratings. Some progenies with stay-green QTLs were considered stay-green susceptible based on the field reaction.

A Ph.D. study was conducted to determine if grain yield of an adapted line can be increased by introgressing exotic cultivar genes. A BC2 derived line (DLs) population between a recurrent parent (Tx2783) and an exotic donor parent (SC170-14E) was developed. One hundred forty two DL pollinators were used to create derived line hybrids (DLHs). Grain yield (GY) and yield component analysis indicated highly significant differences among DLs and DLHs within and across environments. Several DLs and DLHs produced significantly higher GY than their parental lines, parental line hybrids and the commercial check. Grain yield was poorly correlated with later maturity while taller genotypes increased grain yield. Genetic analysis of GY and its components revealed 62 QTLs: grain yield (14), maturity (9), plant height (11), panicle exsertion (10), panicle length (9), stand after emergence (3), panicles harvested per plant (3) and 1000 seed weight (3). DLs and DLHs had 7 common QTLs for GY across environments. Fourteen QTLs positively affected GY: 3 were from DP and 11 from the RP.

The sugarcane aphid is a sorghum insect pest throughout Southern Africa. Resistance sources including TAM428, CE151, WM#177, Sima (IS23250), SDSL89426, FGYQ336 were crossed to locally adapted cultivars and to elite Texas lines. Segregating populations were selected in semi-tropical South Texas for plant height, foliar disease resistance, head smut resistance, grain yield potential, and lodging resistance. Evaluation for pest resistance and local adaptation was conducted at the ARC-GCI in Potchefstroom and the Burgershall Station near Hazyview, South Africa or Gaborone, Botswana. The test was evaluated at Mt. Makulu, Zambia for grain mold resistance. Segaolane (local susceptible check) was rated at 4.3 and TAM428 (resistant check) was rated at 1.3 (Table 1). Forty-seven entries and 36 entries respectively at Potshefstroom and Burgershall were highly resistant (one or two). In a greenhouse seedling stage trial 67 entries were highly resistant (rated at one or two). Data analysis led to the conclusion that many express seedling stage resistance and consistent resistance over locations.

The Potchefstroom and Mt. Makulu trials were harvested for grain yield. Sixty entries produced more grain than the mean. Eleven experimental entries with the highest grain yield produced more grain than the best local check (Kuyuma). Of the 60 entries that produced more grain than the mean, 26 are highly resistant to sugarcane aphid in field (rating 2.3 or less) and greenhouse (rating 1.7 or less) trials. The three entries with the highest grain yield also possess excellent resistance to sugarcane aphid. At Mt. Makulu forty-three entries produced more grain yield than the test mean (3689 kg/ha). Genotype x environment interaction was apparent with only a few entries ranking high for grain yield at both locations.

Sorghum midge is the most ubiquitous insect of sorghum and poses a production risk in many areas. Genetic resistance can provide a low cost, stable, and durable measure of control. Primary research emphasis is to develop varieties for host country cropping systems with tan plant color, white grain, disease resistance, drought tolerance, 1.5 meters in height with a moderate level of sorghum midge resistance. Primary collaboration is with the Nicaragua INTA program. Partial results from a grown at Corpus Christi and Lubbock, Texas and Santa Rosa, Nicaragua are shown in Table 2. The midge damage rating of 4.3 indicated a moderate population density of sorghum midge at anthesis. Several entries sustained less than 30% yield loss. Sufficient midge were not present during anthesis at Santa Rosa, Nicaragua to evaluate the trial for midge damage and the yield (kg/ha$^{-1}$) is a good indication of performance in a tropical environment. Despite a low test mean (2249 kg/ha$^{-1}$) many entries produced significantly (LSD.05 = 550 kg/ha$^{-1}$) more grain that the test mean. Data analysis led to the conclusion that it is possible to select for a moderate level of sorghum midge resistance with moderate to high grain yield potential. Several of the lines were selected for continued evaluation.

## Networking Activities

### *Germplasm exchange*

- Germplasm was distributed as requested to private companies and to countries including but not limited to: Mali, Senegal, Ghana, Nicaragua, El Salvador, Guatemala, South Africa, Botswana, Zimbabwe, Zambia and Mozambique. The All Disease and Insect Nursery (ADIN) was evaluated domestically and internationally.
- Germplasm previously developed and released by this project is used by private seed companies.

### *Technology transfer*

Assisted the Zambia national program to develop and publish a AProduction Guide for Sorghum and Pearl Millet in Zambia@.

### *Other Cooperators*

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## Publications and Presentations

### *Refereed Journal*

Peterson, G.C. 2007. Registration of A/B Tx639, A/B Tx640 and A/B Tx641 midge-resistant sorghum inbred lines. Crop Sci. 47:458-459.

Peterson, G.C., K. Schaefer and B.B. Pendleton. 2007. Registration of Tx2962 through Tx2978 biotype E and I greenbug resistant sorghum germplasm lines. Crop Sci. 47:453-455.

### *Dissertations and Thesis*

Teme, N. 2007. Molecular marker analysis of quantitative trait loci in BC2 derived population influencing heterosis in grain sorghum. Ph.D. dissertation. Texas Tech University, Lubbock, TX.

### *Miscellaneous Publications*

Damte, T., B.B. Pendleton, L.K. Almas, and G.C. Peterson. 2006. Farm-level return on use of midge-resistant sorghum hybrid. International Sorghum and Millets Newsletter 47:101-102.

Dykes, L., L.W. Rooney, and G.C. Peterson. 2006. Comparison of total phenol and antioxidant activity levels of lemon-yellow and red pericarp sorghums. In Proc. of the 2007 AACC International Annual Meeting. San Antonio, TX. Oct. 7-10, 2006.

Teme, N., D.T. Rosenow, G.C. Peterson and R.J. Wright. 2007. Analysis of QTLs Improving Heterosis of Grain Yield in Sorghum (Sorghum bicolor (L.) Moench). In Proc. of the 2007 Joint Grain Sorghum and SICNA Conference. Albuquerque, NM. Jan. 14-16, 2007.