DARPA-Energy Crops Milestones, Deliverables & Metrics

Task 1.1: Identify the best energy sorghum hybrids for specific production environments of national security interest.

Milestones:

1. Meet with DARPA to get their site and region prioritization criteria
2. Identify locations for energy sorghum testing
    a. Sites of known strategic importance for crop testing immediately (Hawaii and West Texas)
    b. Regions where broad adaptation of hybrids can be delineated for potential biomass production within the US
3. Align biomass production regions with appropriate testing sites
4. Secure appropriate trial locations, prep fields, plant and establish sorghum (and energycane as appropriate) trials
5. Harvest sorghum (and energycane) trials

Deliverables:

1. Identify optimal hybrids for a given location or region
2. Generate report summarizing yield results
3. Provide data/feedback from trialing other aspects of the improvement program

Metrics

18 months –

1. Successful establishment and harvest of 1st year trials in at least 6 locations; report generated; successful establishment of 2nd year trials in 10 locations

36 months –

1. 3 years of yield data in trial locations identifying optimal hybrid(s) for biomass production in the respective site/region

Cost 1.1:

Year 1	Year 2	Year 3	Year 4	Year 5	Total
300,000	300,000	350,000	350,000	350,000	1,650,000

<u>Task 1.2:</u> Establish sustainable and best management practices for optimum agronomic production of energy sorghum at selected locations of strategic importance.

Milestones:

1. The effects of plant population on yield and quality of bioenergy sorghums will be evaluated in east, central, and south Texas. (years 1+2).
2. The effects of nitrogen nutrition on yield and quality of bioenergy sorghums will be evaluated in east, central, and south Texas. (years 3+4).
3. A large scale cropping systems experiment will be established and maintained for five years. The experiment is a factorial combination of tillage practices (no-till; strip-till; conventional tillage) and crop rotations (continuous bioenergy sorghum; bioenergy sorghum – corn; bioenergy sorghum – corn – corn). Long term cropping systems performance and sustainability will be characterized. (years 1-5)
4. A large scale experiment investigating the contribution of cool-season legumes to the nitrogen economy of bioenergy sorghum will be established and maintained for five years. The effects of double-cropping legumes such as burr medic and rose clover on N economy and cropping system performance will be contrasted to mineral N nutrition only. (years 1-5).

Deliverables:

1. Optimal plant population for bioenergy sorghums will be determined.
2. Optimal N nutrition for bionergy sorghums will be determined.
3. Effects of and interaction between cropping practices (tillage, crop rotation, inclusion of legumes in the cropping system) on bioenergy sorghum production will be quantified and documented.
4. Nitrogen contribution and non-N rotational effect of cool-season legumes on bioenergy sorghum cropping systems will be quantified and documented.
5. Best management practices (tillage, crop rotation, plant population, N nutrition) for bioenergy sorghum cropping systems will be developed.

Metrics:
18 Months

1. One year of plant population trials conducted and second year in progress. Data from year one analyzed.
2. Large scale cropping systems experiment (tillage x crop rotation) established. First year's data collected. Second year of experiment under way.
3. Large scale cropping scale experiment investigating the contribution of cool-season legumes to the nitrogen economy of bioenergy sorghum established. First year's data collected. Second year of experiment under way.

36 Months

1. Population trial experiments concluded. Data analyzed and final report on this objective assembled.
2. Experiments on effects of nitrogen nutrition on yield and quality of bioenergy sorghums established.
3. Large scale cropping systems experiment (tillage x crop rotation) conducted for three years. Data collected for three years and analyzed for year one and two. Progress report for year one and two assembled.
4. Large scale cropping scale experiment investigating the contribution of cool-season legumes to the nitrogen economy of bioenergy sorghum conducted for three years. Data collected for three years and analyzed for year one and two. Progress report for year one and two assembled.

Cost 1.2:

Year 1	Year 2	Year 3	Year 4	Year 5	Total
216,069	218,363	447,512	452,579	458,030	1,792,493

Task 1.3: Enhance logistics systems to meet biomass feedstock specifications (quantities, moisture content, particle size, total carbohydrate composition, ash content, etc.) with acceptable cost and maximum energy gain.

Milestones and Deliverables:

Task 1.3.1

1. Best current unit operations machines and processes are defined for each of the DOD target biofuels production sites. (18 months)
2. Probability of working days is established at each location, and used to determine minimum stored stocks at each location during the harvest season. (18 months)
3. Establish a base line of delivered cost and energy gain performance for the best current technology systems with energy sorghum at the target locations. (18 months)

Task 1.3.2

1. IBSAL modeling is modified to incorporate an option to select energy sorghum as a biomass source. (18 months)
2. Functional relationships in each unit operation element will be evaluated and modified as necessary for accurate prediction with energy sorghum. Empirical relationships obtained for energy sorghum will be incorporated to enhance simulation accuracy. (36 months)
3. The IBSAL unit operation element library will be expanded to include elements for all operations developed under task 1.3.3. (36 and 60 months)

Task 1.3.3

1. Unit operations with the greatest potential for reducing cost and increasing energy gain will be identified and prioritized for development. (18 months)
2. High priority unit operations will be evaluated with design function analysis techniques to identify functions that could be enhanced for use with the unique properties of energy sorghum. (18 and 36 months)
3. Modifications to unit operations will be made in collaboration with manufacturers. (36 and 60 months)
4. Continuous improvement of the modified designs will be made through field evaluation and refinement in response to performance limitations. (36 and 60 months)

Metrics:
18 Months

1. An improved logistics system will be identified for each of the target locations
2. Provide energy sorghum feedstocks to a conversion facility at a year-round average cost of $60/dry Mg
3. Logistics energy gain of 25. These performance metrics will be predicted using the modified IBSAL modeling environment as modified for energy sorghum.

36 Months

1. Logistics systems will be demonstrated at the two evaluation locations
2. Provide energy sorghum at the desired product specifications at a year-round average cost of $50/dry Mg
3. Logistics energy gain of 33.

Cost 1.3:

Year 1	Year 2	Year 3	Year 4	Year 5	Total
252,948	416,206	855,241	850,987	740,733	3,116,115

Task 1.4: Determine the optimal production harvesting system in alternative regions and prices farmers must be paid to grow a dedicated energy crop.

Milestones:

1. Develop budgets for producing energy sorghums in alternative study areas using best management systems (BMPs) and alternative harvesting systems. Information from Goal 1, Tasks 1, 2, and 3 will be inputs into the budget development process.

2. Develop budgets for crops presently being grown in the alternative locations. This will involve developing representative farm panels and a rep farm data base for use in a whole farm simulator.
3. Develop a Monte Carlo simulation model to estimate the probability distributions for growing energy sorghum and competing crops in the alternative study areas.
4. Determine the price/contract that will most likely have to be offered to farmers to persuade them to grow energy sorghum in each of the alternative study areas using alternative management practices and harvesting systems.

Deliverables:

1. Report with budgets for different varieties of energy sorghum grown under alternative production management and harvesting systems by study area.
2. Report on the riskiness of producing different varieties of energy sorghum, by study area, and the risk associated with its net returns.
3. Report showing the budgets and net returns for competing crops on commercial size farms in each study area.
4. Report showing the riskiness of net returns for energy sorghum and competing crops in each study area.
5. Report identifying the economically optimal variety/production system/harvesting system for each study site.
6. Report summarizing the types of contracts and price levels that will likely persuade farmers to grow energy sorghum in the study areas, rather than current crops being produced.

Metrics:

18 Months

1. The total cost of production for energy sorghum must be less than the maximum economic price a cellulosic ethanol plant can pay for cellulose.

36 Months

1. The risk adjusted net returns for energy sorghum must be greater than the risk adjusted net returns for at least one crop local farmers are producing.
2. The contract price for energy sorghum that induces farmers to grow it must be less than the maximum economic price a cellulosic plant can pay for cellulose.

Cost 1.4:

Year 1	Year 2	Year 3	Year 4	Year 5	Total
224,999	225,000	341,785	350,000	350,000	1,491,784

Task 1.5: Identify feasible production regions for commercial production of dedicated energy crops at sites of national security interest.

Milestones:

1. Meet with DARPA to get their site selection criteria.
2. Use secondary data regarding land resources, crop production, climate and strategic fuel need sites to identify feasible sites to commercially grow energy sorghum given DARPA's criteria.
3. Develop an economic/geophysical model to evaluate the economic feasibility of alternative sites suggested by DARPA.

Deliverables:

1. Report summarizing the meeting with DARPA to identify their site selection criteria.
2. Report in year 1 on the analysis of feasible sites for commercially producing sufficient fuel to supply strategic needs.
3. Reports in years 2-5 will be an update of feasible areas for commercially growing energy sorghum as new varieties and management/harvesting systems are developed and optimized.

Metrics:
18 Months

1. Proceed if we can identify economically feasible production regions based on the criteria specified by DARPA, expected yields for new varieties of energy sorghum, and environmental production requirements for energy sorghum.

36 Months

1. Proceed if new varieties of energy sorghum have demonstrated that they can be produced at economically feasible yields in production regions based on DARPA's criteria for location of production regions.

Cost 1.5:

Year 1	Year 2	Year 3	Year 4	Year 5	Total
75,000	75,000	50,000	50,000	50,000	300,000

<u>Task 2.1</u>: Collect digital genotyping diversity profiles of ~16,000 sorghum germplasm accessions to enable more efficient and comprehensive utilization of germplasm for trait/gene discovery and energy sorghum breeding.

Milestones:

1. DNA will be extracted from 2,000 sorghum accessions per year (Yrs 1, 2) and 4,000 accessions per year in Years 3-5 and placed in an archive.
2. DNA diversity profiles will be collected at DG-1 depth (~10,000 unique sequences/genome) from 2,000 accessions (Yrs 1, 2) and 4,000 accessions (Yrs 3-5) and at DG-2 depth (~100,000 unique sequences/genome) from 200 accessions (Yrs 1, 2) and 400 accessions (Yrs 3-5).
3. Processed sequence data from each GAII run will be added to a database on approximately a monthly basis.
4. The genetic relationships among accessions will be determined once per year using hierarchical clustering programs that implement UPGMA (Unweighted Pair Group Method with Arithmetric mean) methodology.

Deliverables:

1. An archive of DNAs from ~16,000 sorghum accessions will be created.
2. An archive of ~10,000 unique DNA sequences ~36bp in length acquired from ~16,000 sorghum accessions will be created enabling the construction of core germplasm collections and parental lines for breeding and population construction based on sequence-based genetic diversity.
3. An archive of ~100,000 unique DNA sequences ~36bp in length acquired from ~1,600 sorghum accessions will be created enabling the visualization of graphical genotypes and iterative mining of the collection for alleles of genes that control traits important for energy sorghum.
4. A database and displays showing the extent and distribution of genetic variation present in 16,000 accessions of the sorghum germplasm collection.

Metrics:

18 Months

1. DNA extracted from 3,000 sorghum accessions and archived.
2. Digital Genotyping diversity profiles collected from 2,000 accessions.
3. @ ~10,000 unique sequences/genome.
4. Processed DNA sequence profiles archived in a database.
5. Genetic relationships among 2,000 accessions characterized using hierarchical clustering programs that implement UPGMA (Unweighted Pair Group Method with Arithmetric mean) methodology.
6. High-resolution genotypes collected from 200 accessions.

7. @ ~100,000 unique sequences/genome.

36 Months

1. DNA extracted from an additional 5,000 accessions (8,000 total) and archived.
2. Digital Genotyping diversity profiles run on an additional 4,000 accessions.
3. Genetic relationships among 6,000 accessions analyzed.
4. High-resolution genotypes collected from an additional 500 accessions.
5. Tools for visualization of high-resolution graphical genotypes developed.

Cost 2.1:

Year 1	Year 2	Year 3	Year 4	Year 5	Total
250,000	251,708	346,199	349,806	352,288	1,550,001

Task 2.2: Create and screen core sorghum germplasm collections and an integrated set of breeding and discovery populations for phenotypes, genotypes, and genomic regions that contribute to yield, and QTL/genes that modulate key energy sorghum traits

Milestones:

1. A core germplasm collection will be established for photoperiod sensitive energy sorghum and populated with 100 accessions by the end of Year 1, 400 by Year 3 and 1,600 by Year 5, based on accession phenotypes and genotypes. A second core germplasm collection comprised of up to 100 'converted' energy sorghums will also be established.
2. Seed stocks for core germplasm collections will be created to enable these materials to be screened for a range of traits and in different environments.
3. Core collections will be screened for variation in traits relevant to energy crops including but not limited to biomass yield, duration of vegetative growth (flowering time), height, lodging resistance, composition (NIR), conversion efficiency, stem traits, and response to drought stress.
4. Two populations based on parental genotypes that capture genetic and trait diversity in the sorghum germplasm collection will be created each year and advanced to RILs.
5. Genetic loci (QTL) that modulate energy traits will be mapped in one energy sorghum population in Year 2 and two energy sorghum populations in Years 3-5.
6. DNA markers for marker-assisted selection (MAS) will be identified in Years 3-5 based on QTL mapping and used in the energy-sorghum breeding program starting in Years 4-5.

Deliverables:

1. Core collections of sorghum germplasm accessions that contain a large portion of the genetic and trait diversity present in the sorghum germplasm collection.
2. Information about the extent of energy trait variation in core collections relevant to the production of improved versions of energy sorghum.
3. Ten populations segregating for energy traits that enable QTL mapping, DNA marker and gene discovery.
4. Information about the location and complexity of genetic loci (QTL/genes) that modulate key energy traits in sorghum.
5. DNA markers useful for marker-assisted breeding of energy sorghum.

Metrics:

18 months

1. A core energy sorghum germplasm collection will be established and populated with at least 100 photoperiod sensitive energy sorghum and/or converted energy sorghum accessions based on phenotypes and Digital Genotyping diversity analysis.
2. Seed stocks for accessions in the Core Collection will be created to enable these materials to be screened for a range of traits and in different environments.
3. One hundred accessions from the Core Collection will be screened in the field for variation in traits relevant to energy crops including but not limited to biomass yield, duration of vegetative growth (flowering time), height, lodging resistance, composition (NIR), stem traits, and response to drought stress.
4. One hundred accessions from the Core Collection will be screened in the greenhouse for photoperiod sensitivity (as days get shorter in the fall).
5. Screens for stem traits (anatomy, lignin content, length density), nitrogen use efficiency, and root traits related to lodging resistance/drought/salt tolerance will be developed.
6. Two populations that capture genetic and trait diversity present in the sorghum germplasm collection will be created and advanced towards RILs (to F5 status).

36 months

1. The energy sorghum Core Collection will be expanded to 400 accessions.
2. Four hundred additional accessions (200/summer) will be screened for energy traits (same as above) in the field.
3. Four hundred additional accessions will be screened for photoperiod sensitivity and other traits in the greenhouse in the fall each year.

4. Four additional discovery populations will be started and all populations advanced towards RILs.
5. Fifty genotypes will be screened in the greenhouse/growth chamber for nitrogen use efficiency and root traits related to lodging resistance, drought/salt tolerance.
6. Initial energy trait QTL analysis on one population will be carried out.

Cost 2.2:

Year 1	Year 2	Year 3	Year 4	Year 5	Total
249,477	249,539	396,091	400,456	404,437	1,700,000

Task 2.3: Develop and test advanced inbred lines and hybrids for yield, adaptation, and traits that contribute to the value and overall utility of energy crops.

Milestones:

1. Development of populations and inbred lines
2. Phenotypic evaluations of populations and inbred lines for important traits
3. Detection of QTLs and identification of genes that affect important traits
4. Development of and selection in breeding populations for enhanced productivity of sorghum bioenergy hybrids

Deliverables:

1. 4 RIL populations for further research and development
2. Phenotypic evaluations of these 4 RIL populations in at least 2 environments for important agronomic and composition traits
3. QTLs for important agronomic and composition traits, the relative importance of each, their locations and sizes.
4. Breeding populations and improved parental lines that have enhanced biomass yield and improved compositional profiles for bioenergy sorghum hybrids.

Metrics:

18 Months

1. 2 populations (P1, P2), completed and ready for evaluation
2. At least 10 breeding populations developed and available for selection.

36 Months

1. Remaining 2 populations (P3, P4), completed and ready for evaluation.
2. A total of 40 breeding populations developed and in selection for enhanced bioenergy productivity and composition.

2. Evaluation of the first 2 populations (P1 and P2) in at least 2 environments, for QTL analysis.
3. Initial QTL mapping will be completed

Cost 2.3:

Year 1	Year 2	Year 3	Year 4	Year 5	Total
499,999	500,000	750,000	727,680	727,680	3,205,359

Task 3.1: Create revolutionary next-generation energy crops by wide hybridization of diverse sorghums with sugarcanes and other energy grasses. Create 10,000s of individually distinct sorghum/energycane hybrids. Subtasks a, b, and c will follow similar timelines.

Milestones:

1. Establishment of pipeline for producing large numbers of sorghum/energycane hybrids: >10,000/yr
2. Hybridization of [redacted] sorghum with other perennial bioenergy grass species.

Deliverables:

1. Large numbers (30,000) intergeneric sorghum/sugarcane hybrids for breeding and evaluation.
2. The identification of additional grass genera and species that are cross-compatible with [redacted] sorghum and might be used to create novel bioenergy crops.

Metrics:

18 Months

1. Production of at least 5,000 seed/seedlings of sorghum/sugarcane hybrids and this will demonstrate scalability and feasibility of production
2. Attempt intergeneric hybridization of at least 4 different species of grasses with [redacted] sorghum. Assess the potential recovery of hybrids.
    a. If none, attempt four others.
    b. If some, then move to evaluation of produced hybrids.

36 Months

1. Production of at least 20,000 seed/seedlings of sorghum/sugarcane hybrids.
2. Attempt hybridization of 4 different species of grasses
   a. If successful, complete evaluation of initial hybrids for potential.
   b. If initial was not successful, attempt 4 other grass species and if unsuccessful, discontinue task.

Cost 3.1

Year 1	Year 2	Year 3	Year 4	Year 5	Total
176,202	150,668	208,889	204,130	204,158	944,047

Task 3.2: Multi-location screening of wide-hybrids to identify elite and unique hybrids.

Milestones:

1. Establishment of Breeding/Evaluation Nurseries to identify intergeneric hybrids that have potential for further development
2. Identification of elite and unique sorghum/sugarcane hybrids that enhance performance.

Deliverables:

1. Elite intergeneric hybrids that can be used as a bioenergy crop species.

Metrics:

18 Months

1. Establishment of three screening nurseries and screening at least 80% of the seed (4000) produced in Task 3.1.

36 Months

1. Establishment of three screening nurseries and screening at least 80% of the seed (16,000) produced in Task 3.1.
2. Identification of the top 1% of hybrids from the 18-month establishment (~40 hybrids).

Cost 3.2

Year 1	Year 2	Year 3	Year 4	Year 5	Total
150,050	149,996	199,909	199,468	200,599	900,022

<u>Task 3.3:</u> Determine agronomic performance and conduct pilot-scale testing of intergeneric hybrids.

Task 3.3a: Production of elite sorghum/sugarcane hybrids for agronomic testing (from task 3.2)

Milestones:

1. Selected hybrids will be clonally propagated and planted in strategic locations.

Deliverables:

1. Quantities of elite intergeneric hybrids that can be used for replicated agronomic testing.

Metrics:

18 Months

1. Establishment of increase nurseries of the early advanced material (at least 20 clones)

36 Months

1. Distribution of early increases to agronomic cooperators
2. Establishment of increase nurseries of the advanced material (120 clones).

Task 3.3b: Determine agronomic performance and conduct pilot-scale testing of intergeneric hybrids – *College Station*

Milestones

1. Replicated experiments (one irrigated and one rainfed) will be established at College Station. Agronomic performance of clonally planted intergeneric hybrids will be evaluated. The studies will be conducted for five years. New intergeneric hybrids will be included in the trials as soon as sufficient seedstock will come available. Performance of intergeneric hybrids will be compared to energycane and bioenergy sorghum. (years 1-5)
2. Replicated experiments will be established under rainfed and irrigated conditions at College Station. The effects of varying row widths and nitrogen nutrition on performance of intergeneric hybrids will be evaluated. (years 3-5)

Deliverables:

1. Report describing the comparison of genotypes and identification of superior varieties for commercialization under irrigated and rainfed conditions, respectively.
2. Report describing the maximum agronomic production potential for superior genotypes under irrigated and rainfed conditions, respectively.

3. Determine optimal row widths for production of intergeneric hybrids under irrigated and rainfed conditions, respectively.
4. Determine optimal nitrogen nutrition for production of intergeneric hybrids under irrigated and rainfed conditions, respectively.
5. Report identifying clones with superior traits for study and utilization under Task 3.4.

Metrics:

18 months

1. Agronomic performance trials of clonally planted intergeneric hybrids established. First year's data collected. Data from year one analyzed. Second year of experiment under way.

36 months

1. Agronomic performance trials of clonally planted intergeneric hybrids conducted for three years. Three year's data collected. Data from year one and two analyzed. Progress report for year one and two assembled.
2. Trial on the effects of varying row widths and nitrogen nutrition on performance of intergeneric hybrids established. First year's data collected.

Task 3.3c: Determine Agronomic Performance and Conduct Pilot Scale Testing of Intergeneric hybrids - *Weslaco*

Milestones:

1. Establish a nursery in Weslaco using vegetative material from promising intergeneric hybrid genotypes and energy cane varieties.
2. Establish replicated trials at two locations near Weslaco to evaluate the relative productivity of promising intergeneric hybrid and energy cane genotypes.
3. Establish replicated trials to evaluate agronomic production potential and input requirements and develop best management practices for promising genotypes.
4. Establish replicated trials to determine the performance of promising genotypes at locations of interest to DARPA and DOD.

Deliverables:

1. Superior varieties for potential commercialization are identified.
2. Best management practices for planting and growing intergeneric hybrids for maximum production and minimal inputs are identified and described.
3. Maximum agronomic production potential for superior genotypes is determined and documented..

4. Genotype x location interactions that will facilitate selection of genotypes for production in various regions and guide future genotype development are identified and described.
5. Clones with superior traits for study and utilization under Task 3.4 are identified and available for subsequent studies.

Metrics:

18 months

1. Nursery established at Weslaco site, growth and other data collected for nursery specimens. (see year 2, quarter 1-3 on timeline)

36 months

1. Data from 1st year productivity trials analyzed, replicated agronomic and productivity trials are planted. Replicated variety trials are planted at DARPA/DOD specified location.

Cost 3.3:

Year 1	Year 2	Year 3	Year 4	Year 5	Total
149,986	220,055	300,187	301,139	302,147	1,273,514

Task 3.4: Develop seed-based propagation systems for sorghum/energycane hybrids. Three subtasks are necessary to meet the goal of this project.

Milestones:

1. Creation of breeding crosses among sorghums to enhance productivity of crosses between sorghum and sugarcane. Derivation of new seed parent lines for use in hybrids.
2. Creation of breeding crosses among sugarcanes to enhance productivity of crosses between sorghum and sugarcane. Derivation of new sugarcane pollinator clones specifically selected for enhanced pollen compatibility with [redacted] sorghum.
3. Creation of new sorghum, sugarcane, and/or hybrid types that enable mass-production of large seeds containing intergeneric hybrids exhibiting seedling vigor and uniformity.

Deliverables:

1. Improved [redacted] sorghum seed parent lines.
2. Improved sugarcane pollinator parental lines.
3. New or modified sorghums, sugarcanes or hybrids that enhance feasibility of seed-based hybrid cane production.

Metrics:

18 Months

1. Hybridization of [redacted] sorghum with other sorghum types (with unique traits) and derivation of segregating populations
2. Identification of sugarcane genotypes that are superior pollinators for hybridization with [redacted] sorghum
3. Creation of tetraploid [redacted] sorghums; possibly also chromosome-doubled canes and/or intergeneric hybrids.

36 Months

1. Selection and initial testing of new sorghum seed parental lines.
2. Creation of segregating (F1) sugarcane populations by hybridizing the best sugarcane pollinators of [redacted] sorghum. Identification of more canes that are good pollinators.
3. Initial assessment of tetraploid [redacted] sorghums for wide-cross and self fertility. Analogous assessments of chromosome-doubled canes or intergeneric hybrids, if available.

Cost 3.4

Year 1	Year 2	Year 3	Year 4	Year 5	Total
635,249	509,363	700,308	691,186	692,493	3,228,599

Task 3.5: Molecular characterization of [redacted] and other important genes that influence intergeneric hybridization and recombination, and parental and intergeneric hybrid genotypes.

Milestones:

1. Phenotypes will be collected on individuals from a BC1F1 population segregating for the ms3 and [redacted] traits. Approximately 150 individuals will segregate as ms3/ms3 and these will be phenotyped using current cytological methods (Yr 1).
2. DNA will be extracted and digital genotype analysis (DGA) performed on individuals from the BC1F1 population as well as the parents to confirm and refine the genetic map location of the [redacted] gene. DGA profiles will be collected at DG-1 depth (~20,000 unique sequences/genome) from the parents and ~12 [redacted] and 12 [redacted] progeny (Yrs 1, 2).
3. Up to 10 different maize pollen sources will be examined on sorghum [redacted] and [redacted] testers to improve efficacy of [redacted] phenotypic analysis (Yr 1).
4. An additional mapping population consisting of ~1000 progeny for high resolution mapping of the [redacted] gene will be constructed (Yrs 1-3) to delimit the locus to less than 0.1cM for candidate gene identification.

5. Progeny from the high resolution mapping population will be phenotyped and DNA extracted for genotypic analysis around the [redacted] locus (Yrs 3, 4).
6. The completed sorghum genome sequence (818Mbp) will be searched in the [redacted] locus for any potential genes with the expected function (Yrs 4, 5).
7. Sequence analysis of genomic DNA and cDNA of potential [redacted] candidate genes (~3-4 genes total) will be performed between the mapping parents and candidate gene identification will be confirmed using bioinformatic and experimental (RT-PCR and/or mRNA sequence analysis) approaches (Yrs 4, 5).
8. DGA at DG-1 and DG-2 (~125,000 unique sequences/genome) depths will be tested on cane and sorcane intergeneric hybrids (Yr 2) and if successful DNA will be extracted from ~1200 intergeneric hybrids for additional analysis (Yrs 3-5). DGA will be performed on all 1200 individuals at DG-1 depth and on a subset of 150 individuals at DG-2 depth to identify additional markers linked to [redacted] alleles for use in the intergeneric hybridization breeding program and to determine the sizes and extents of genome admixture in intergeneric hybrids (Yrs 3-5).

Deliverables:

1. Development of an improved screening protocol for identification of [redacted] genotypes.
2. Construction of a mapping population (~1000 individuals) and development of DNA markers for high resolution mapping of the [redacted] gene.
3. Identification of the [redacted] gene in sorghum leading to an understanding of the mechanism involved in intergeneric hybridization and IP capture.
4. An archive of DNA from ~1200 intergeneric hybrids.
5. An archive of ~20,000 unique 36bp sequences from 1200 intergeneric hybrids and ~125,000 unique 36bp sequences from 150 intergeneric hybrids.
6. Identification of ~1000 additional DNA markers for utilization within the wide hybrid breeding program to facilitate the screening of new hybrids as they are developed. Identification of ~10,000 markers among 150 intergeneric hybrids allowing for visualization of haplotype variation among them.

Metrics:

18 Months:

1. Confirmation and refinement of the genomic location of the [redacted] gene.
2. Development of a rapid phenotypic screen for identification of individuals with the [redacted] genotype for use in subsequent fine mapping activities.

36 Months:

1. Construction of an additional fine mapping population consisting of ~1000 progeny for use in high resolution of the mapping of the [redacted] gene.
2. Refined resolution of the [redacted] gene down to less than 0.1cM (~3-4 genes).

3. Development of DGA methodology for cane and intergeneric hybrids (i.e. sorcane).

Cost 3.5

Year 1	Year 2	Year 3	Year 4	Year 5	Total
149,839	149,885	199,601	199,784	200,417	899,526