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Attachments:	RE Highest Priority DARPA Energy Crops.msg Highest Priority DARPA Energy Crops.msg

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High-Biomass Energy Crops for U.S. Energy Security

Submitted to:

DARPA

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High-Biomass Energy Crops for U.S. Energy Security

VISION: The Texas A&M University System (TAMUS) will create and deliver advanced high yielding energy sorghums and new energy crops through a novel, non-GMO, wide-hybridization technology platform. These unique energy crops will be sustainable, high yielding, widely adapted, drought tolerant, optimized for biofuels and biopower generation, thereby significantly improving U.S. biofuels and biopower production capability and long-term energy security.

REQUIRED RESOURCES: $21M / 5 years: By year: $3M, $3M, $5M, $5M, $5M.

PROJECT DELIVERABLES:

1. Energy sorghum production maximized in locations of national security importance using optimized management practices, harvest logistics, and economic assessment.
2. Energy sorghum hybrids with increased yield and optimized composition for advanced biofuels and biopower generation designed using an integrated genomics-to-energy crop breeding technology platform.
3. Novel wide-hybrid energy crops propagated vegetatively and/or through seed production and an understanding of the genetic basis of wide hybridization.

OVERALL APPROACH: The capacity to sustainably produce a large supply of low cost compositionally optimized biomass is an essential requirement of large-scale economic production of advanced biofuels. Researchers at TAMUS have determined that energy sorghum and wide-hybrids derived by crossing sorghum to cane and other energy grasses can meet the biomass feedstock requirements of the cellulosic-advanced biofuels industry. The proposed R&D plan will accelerate the genetic improvement of energy sorghum, develop novel sorghum/cane wide-hybrids for energy use, deploy these crops in sites of national security importance, and optimize crop management, biomass harvesting and delivery to biorefineries.

NATIONAL SECURITY IMPLICATIONS: Energy sorghum hybrids optimized for geographic regions of national security importance have the capacity to supply lower cost feedstocks with specialized characteristics for advanced biofuels or biopower generation with high GHG offsets. Accelerating the improvement of energy crops will lead to cost decreases, supply increases, better conversion efficiency, reduced competition with food and feed production, and improved life cycle assessment of fuels derived from energy sorghum and wide-hybrids. Successful implementation of these technologies will provide a strategic, sustainable, low-cost source of advanced biofuels and biopower for DOD, and in the longer term could significantly impact the world's supply of energy and transportation fuels.

GOAL 1. Grow and optimize production of current energy sorghum hybrids at sites of importance to national security (Figure 1). TAMUS has created unique high-tonnage drought-tolerant, non-GMO "energy sorghum" hybrids that yield 10-15 dry tons (dT) of lignocellulosic biomass per acre. Current versions of energy sorghum enables biomass delivery to biorefineries at ~$60/dT providing a ~75% GHG offset for advanced biofuels production or ~95% for biopower. Energy sorghum hybrids will be grown at sites of national security interest such as Hawaii and other locations where agronomic management and harvest logistics will be optimized. Hybrid seed will be produced and tested in cooperation with our corporate partner Ceres, a bioenergy crop seed company.

GOAL 2. Build a full-scale integrated genomics-to-breeding technology platform that will accelerate the rate of genetic improvement of energy sorghum and wide-hybrids (Figure 2). Sorghum is one of the most highly productive C4 grass species known, with excellent genetics, a complete genome sequence, and an extensive germplasm collection (~40,000 accessions) that contains valuable genetic diversity for the design and improvement of energy sorghum and other energy crops. TAMUS is creating an advanced Genomics to Breeding Technology Platform based in part on a novel digital genotyping technology that enables the acquisition of detailed profiles of genetic variation of germplasm accessions, gene discovery populations, and breeding materials. Implementation of this technology within an expanded TAMUS energy sorghum and wide-hybrid genomics, breeding, and improvement program will significantly increase the long-term rate of energy crop design and improvement. The goal is to increase energy crop yield to 15-20 dT/acre while optimizing biomass composition to improve conversion efficiency into advanced biofuels and biopower.

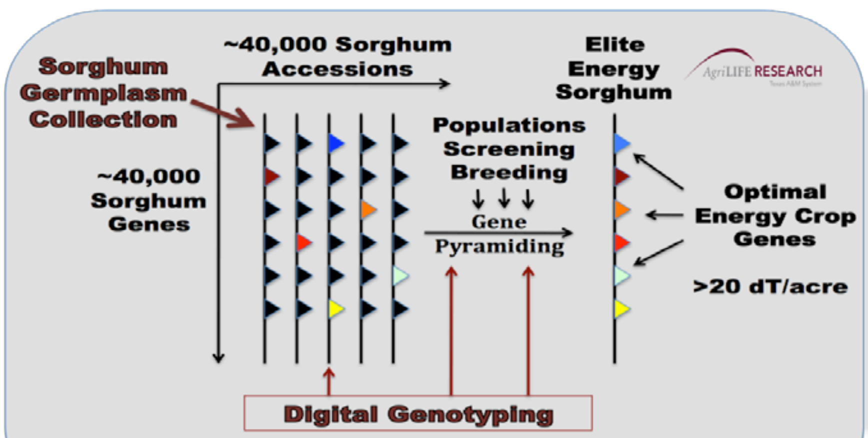


Figure 2. Germplasm/Genomics to Breeding Platform

GOAL 3. Develop next-generation energy crops using novel wide-hybridization technology that enables sorghum to be crossed with energy cane and other energy grasses (Figure 3). Wide-hybridization technology developed at TAMUS allows sorghum to be crossed with sugarcane and other C4 grasses. The resulting intergeneric hybrids have the potential for increased yield, to exhibit unusual hybrid vigor, and to express unique combinations of useful traits from the species being crossed, such as large seed size or drought tolerance from sorghum, with perennial growth, cold tolerance, and/or high nitrogen use efficiency from other C4 grasses such as energy cane or Miscanthus. Revolutionary ramifications are expected: [1] mass-production of hybrid seed from crosses with energy canes (first time ever); [2] 25-90+% cost reduction for planting energy canes; [3] option to grow wide-hybrid energy crops as seed-based crops (*much* more flexible than production based on vegetative propagules); and [4] a suite of newly created next-generation energy grasses for advanced biofuels and biopower generation in diverse agricultural and climatic conditions.

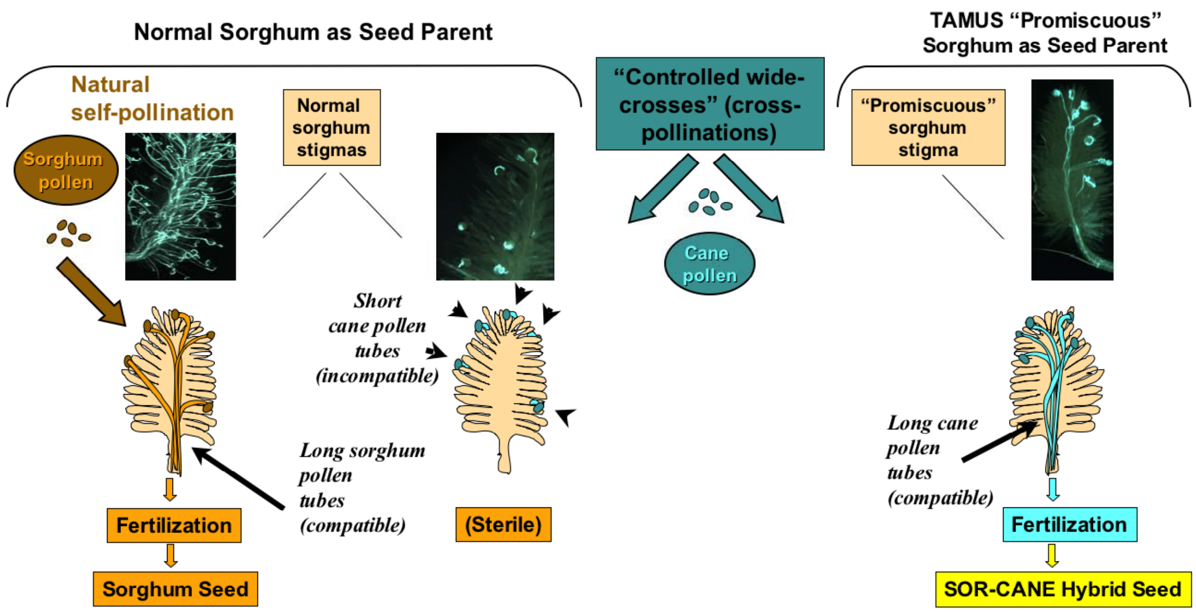


FIGURE 3. Diagrammatic representation of wide-hybridization technology for sorghum (*Sorghum bicolor*): normal sorghum stigmas are highly "self-compatible", i.e., with sorghum pollen (left), but highly incompatible with alien pollen (center), pollen tubes of which are forced to cease growth soon after emergence, thus precluding fertilization. In the specially bred TAMUS "promiscuous" lines of sorghum, the alien pollen is much more compatible (right) resulting in pollen tube growth, which thus permits fertilization, embryogenesis and formation of F1 seed.

IP/LICENSING PLAN: The intellectual property for sorghum wide-hybridization and wide-hybrids is wholly owned by TAMUS. Current energy sorghum hybrids (not wide-hybrids) are being developed in collaboration with Ceres, a commercial energy crop seed company. Ceres has agreed to extend preferred customer status to DOD for its sorghum hybrids and to supply commercial amounts of seed. The results of research funded by DARPA will be protected via both statutory means (e.g. patents, plant variety protection certificate) and contractual means (e.g. distribution of tangible materials under appropriate agreements). Licensing of TAMUS intellectual property will include diligence to ensure licensees will meet both commercial and national security requirements for these novel energy crops.

RESEARCH AND DEVELOPMENT PLAN:

GOAL 1: Optimize production of energy sorghum hybrids in strategic areas to provide feedstocks for biofuels production for military installations.

Deliverables:

1. Energy sorghum hybrids adapted to specific production environments of national security interest.
2. Agronomic guidelines for sustainable production of energy sorghum.
3. Location-specific logistics system for supplying biomass feedstocks to biorefineries that meet product specifications.
4. Economic assessment of dedicated energy sorghum production and biomass delivery to biorefineries at sites of national security interest.

Rationale: Energy sorghum, a C4 grass, is a superior plant species for lignocellulosic bioenergy production due to its ability to produce at least 10-15 dry ton/acre of biomass in good production environments. The development of high yielding energy crops such as energy sorghum is important to the successful establishment of large-scale biofuel production because these crops will (1) reduce the cost of biomass per unit of biofuels produced, (2) reduce competition for land and between food and fuel crops by minimizing bioenergy production footprints, (3) reduce the cost of transporting biomass to biorefineries by increasing the amount of biomass that can be generated near a biorefinery, and (4) reduce carbon emissions per unit of transportation fuel produced generating carbon credits and positive environmental impact. The goal of this objective is to maximize the yield of energy sorghum hybrids in sites of national security interest by screening current and next-generation energy sorghum hybrids for adaptation to specific locations, optimizing crop management, harvesting schedules and the logistics of processing and transport of biomass feedstock to biorefineries. The economics of the integrated energy sorghum production system will also be analyzed.

RESEARCH PLAN:

Objective 1: Identify the best energy sorghum hybrids for specific production environments of national security interest. **(Ceres, Inc)**

Rationale: Energy sorghum hybrids need to be evaluated in multiple environments to identify specific hybrids that are best adapted to each production site, to assess the yield potential in each region, and to identify the factors that limit yield in each environment so they can be minimized through genetic improvement and crop management.

Implementation Plan:

1. Small replicated trials of at least 25 different energy sorghum hybrids in 8-10 environments to assess and identify the optimum genotypes for each adaptation zone. Production test sites will include and focus on sites of current national security interest and regions that are likely to be important in the longer term. As appropriate to environment, selected energycane varieties will be included for relative comparison of performance.
2. Plant Breeding Data to collect includes but is not limited to
    a. Biomass Yield

  b. Composition
  c. Height, Maturity
  d. Disease Reaction
  e. Pest Reaction

Budget: Years 1-2, $175K/year; Years 3-5, $400K/year

Objective 2: Establish sustainable and best management practices for optimum agronomic production of energy sorghum at selected locations of strategic importance. **(Blumenthal)**

Rationale: The genetic potential of elite hybrids or cultivars will be expressed and yield maximized only if best management practices are utilized to optimize the production environment.

Implementation Plan:

1. Large replicated trials of the highest yielding energy sorghum hybrids for agronomic testing at selected locations. Variable factors to evaluate include
   a. plant population
   b. fertility, irrigation requirements
   c. harvest scheduling
   d. hybrid or cultivar
   e. composition
2. In Years 1 and 2, we expect testing in 2 to 3 locations. In Years 3 to 5, additional locations will be established.
3. As appropriate to locations such as Hawaii, energycane cultivars will be planted and evaluated in comparable studies.

Budget: Years 1-2, $175K/year; Years 3-5, $400K/year

Objective 3: Evaluate systems to meet biomass delivery specifications (quantities, moisture content, particle size, total carbohydrate composition, ash content, etc.) for conversion systems. **(Searcy & Thomasson)**

Rationale: Efficient harvest systems that consistently provide acceptable biomass to the processing gate in a timely and desired mode is essential for any biorefinery.

Experimental Plan: Given DOD's desire for drop-in biofuels that can displace significant quantities of petroleum-based JP-8 and JP-5, the most likely conversion techniques will be thermo-chemical in nature, at least in the mid-term future. Without selecting a specific conversion technique, general feedstock characteristics can be anticipated. Thermo-chemical conversion techniques typically require biomass with lower moisture content and smaller, more uniform particle sizes, both of which are significant challenges without applying excessively high levels of energy. All technical challenges to providing the desired feedstock characteristics will be prioritized, and greatest emphasis placed on the most limiting factors.

The following is a five-year plan to assess potential biomass feedstock logistics systems that would be required to meet DOD demands for biofuels. This plan will be closely coordinated with activities under the related objectives, and with DARPA.

Year 1:

a. Obtain from DARPA preferred target locations for biofuels production and consumption and target biomass characteristic specifications. Anticipate three distinct sites to be evaluated during the initial phase of the project. If DOD specs are unavailable, establish target specifications for generic thermo-chemical conversion techniques based on existing techniques such as Fischer-Tropsch and pyrolysis.
b. Assess agricultural infrastructure at the selected sites to determine the potential suite of machinery needed for harvest, storage, transport and preprocessing.
c. Utilize the DOE feedstock logistics simulation model (IBSAL) to assess the impact of regional weather conditions on the operation of potential logistics systems.
d. Identify probable optimum logistics systems for each site evaluated, and challenges to be overcome in each system.

Year 2:

a. Utilizing Texas AgriLife Research regional experiment stations, select locations and harvest periods most capable of simulating the DOD target locations.
b. Establish small-scale (30-50 acre) studies to evaluate of the performance of the identified logistics systems.
c. Evaluate the performance of the logistics systems relative to the established delivery specifications and the anticipated and unanticipated challenges to achieving the desired characteristics.
d. Compare actual and simulated logistics systems performance at each target site based on desired and achieved biomass feedstock characteristics, and identify logistics operations requiring modification or alternative techniques.

Year 3:

a. In consultation with DOD project managers, determine if each target site logistics system testing should continue on a small-scale basis at Texas AgriLife Research centers with revised logistics mechanisms intended to address year 2 identified deficiencies, transition to larger scale/longer harvest period studies, or establish a study in the target site region.
b. Evaluate tested systems in a manner similar to year 2, concentrating on challenges with each site.
c. Comparison of desired and achieved biomass feedstock characteristics under revised logistics systems, identification of logistics unit operations representing greatest opportunity for enhancement of feedstock quality if fundamental changes are made in machines or techniques.

Years 4 & 5:

a. Optimized logistics systems will be evaluated and enhanced in large-scale demonstrations in conjunction with the sorghum hybrids and production technique developed under objective 1 and 2.

Budget: Years 1-2 - $350K/year; Years 3-5 $800K/year

Objective 4: Determine the optimal production harvesting system in alternative regions and prices farmers must be paid to grow a dedicated energy crop. **(Richardson)**

Rationale: It is critical to the project's success to know what the cost of producing biomass feedstocks and the prices (and contracts) farmers will have to be offered to get them to grow a dedicated energy crop. Risk of production and harvesting energy crops must be considered relative to risks of producing traditional crops or breaking out new land to grow energy crops.

Experimental Plan: The results of the field data collection and logistics system simulation will be combined into an assessment of sorghum production, harvest and delivery system. This effort will include details on the equipment, fuel use, labor requirements, and costs of planting, growing, harvesting, handling, and transporting feedstock in order to generate accurate projections of delivered feedstock cost under a commercial scenario. Information about weather risk, soil productivity, and costs of production for traditional crops at each alternative site will be modeled in a Monte Carlo economic model to project risk adjusted net incomes for energy crops and existing food/feed crops. Costs of production and production functions for dedicated energy crops will be modeled to estimate risk adjusted net returns for energy crops using alternative production and harvesting practices. Stochastic efficiency techniques will use the risky net returns for energy crops and traditional crops to estimate utility adjusted net returns for alternative price/contract arrangements to estimate which price/contract will be necessary to induce farmers to grow energy crops. Model results will be validated through interviews with local commercial scale farmers.

Analyses of acreage competition with traditional food/feed crops will be done for alternative areas. For regions with no current crop production, the costs to convert open ground to energy crop production will be estimated. Budgets developed for growing an energy crop under alternative production and harvesting systems will be used to determine the economically optimal production/harvesting system for each study area. Local risk factors are expected to make the optimal production/harvesting system differ across study areas.

Budget: Years 1-2, $225K/year; Years 3-5 $350K/year.

Objective 5: Identify feasible production regions for commercial production of dedicated energy crops at sites of national security interest. **(Richardson)**

Rationale: Many different factors must be considered in identifying production sites. Site selection is a critical factor, as it will directly affect what kind of energy sorghum to produce, crop yields, and the costs of production. Early selection of feasible sites will directly affect the plant breeding program and where field tests will be conducted. Once feasible sites are identified, the basic and agronomic research can proceed to optimize the energy crop's biomass production potential for regions of interest. The site selection criteria must consider many different factors, such as: growing season for sorghum, current production of sorghum in the region, harvesting and storage requirements, proximity to end users, availability of land and irrigation water, rainfall patterns, temperature patterns, competition with existing crops and livestock, population density, and environmental considerations.

Experimental Plan:
1. Identify the DARPA site selection criteria.
2. Identify additional location criteria based on secondary data and experience and assign weights to site selection criteria to form a lexicographic function for ranking alternative sites.
3. Develop models to analyze the possible sites discussed at the meeting plus additional sites that also may meet the site selection criteria.
4. Location studies will be updated in subsequent years as the breeding program produces new varieties that could expand the climatic/resource zones where dedicated energy sorghum can be produced.

Budget: Years 1-2, $75K/year; Years 3-5, $50K/year

***GOAL 2:* Increase the yield and optimize the composition of energy sorghum using an integrated genomics-to-breeding technology platform to accelerate the rate of genetic improvement and design of energy sorghum.**

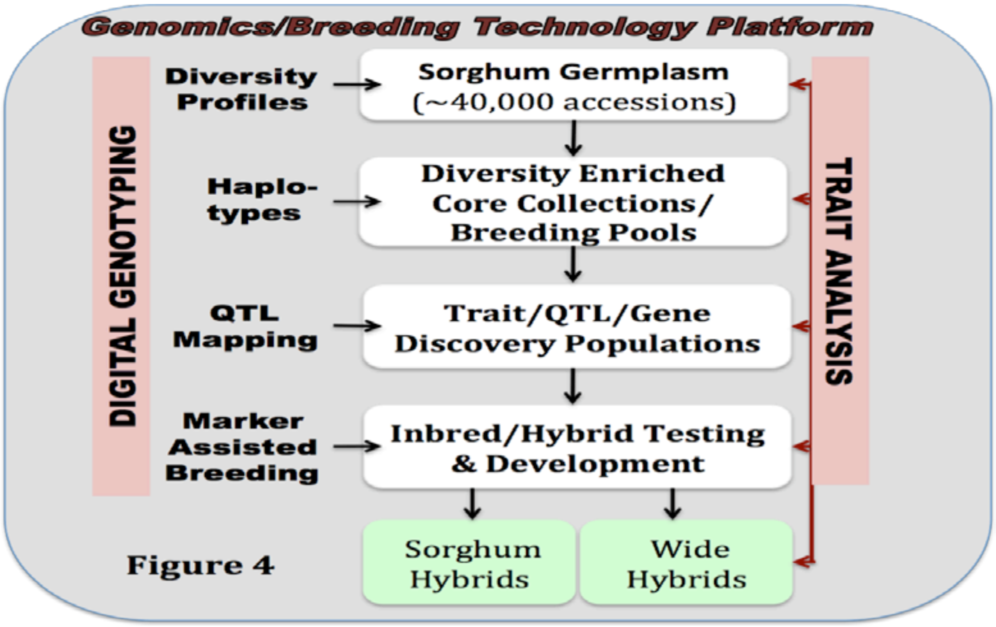
Deliverables:

1. Energy sorghum inbreds and hybrids with increased yield (~15-20dT/acre) and improved composition for conversion processes.

2. A genomics-to-breeding technology platform that will sustain increased rates of energy sorghum improvement.

3. Identification of genes/QTL/markers that control traits important for the design of energy sorghum and other energy crops including wide-hybrids.

Approach: The TAMUS sorghum-breeding program is developing next-generation energy sorghums by screening the sorghum germplasm collection for useful genes/traits and then pyramiding these genes through breeding and marker-assisted selection into elite energy sorghum hybrids (Figure 4). DARPA funding will be used to accelerate the genetic improvement of energy sorghum through development of an advanced Genomics to Breeding Technology Platform.



Digital Genotyping Technology, recently developed at TAMUS, will be used to screen germplasm accessions and breeding lines, create core collections, populations, for QTL/trait to gene mapping, and to carry out genome wide marker-assisted-breeding (MAB) (Figure 5). Implementation of this technology within an expanded energy sorghum and wide-hybrid breeding and improvement program will significantly increase the rate and efficiency of energy crop design.

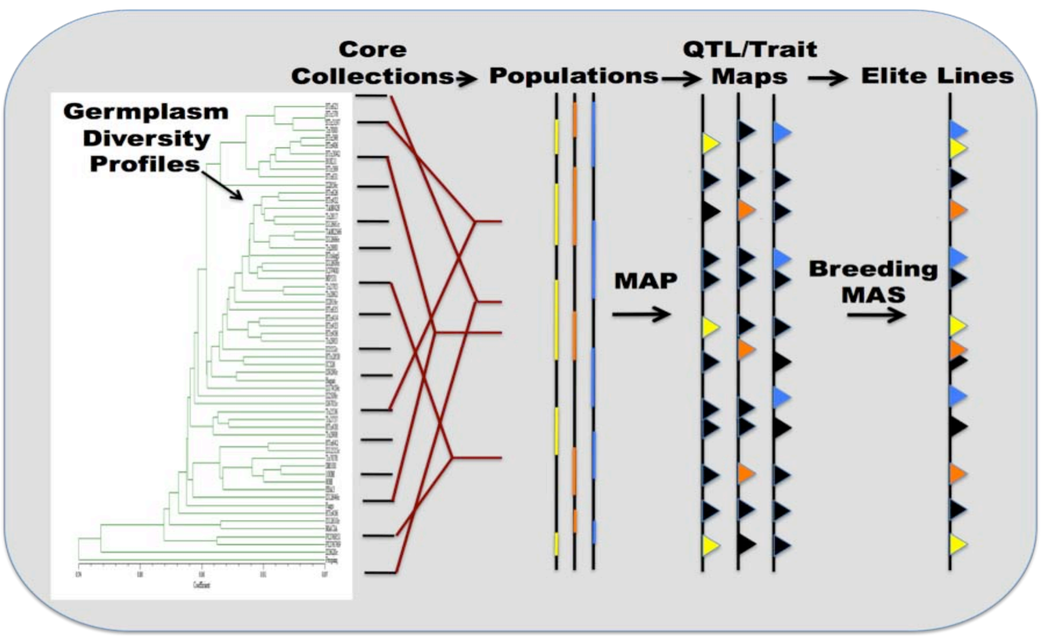


***Figure 5**: Germplasm-mining pipeline that begins by collecting diversity profiles of sorghum germplasm accessions, followed by the creation of core collections, populations, trait/QTL mapping, breeding and marker-assisted selection (MAS).*

RESEARCH PLAN:

Objective 1: Collect diversity profiles of sorghum germplasm accessions and use this information to create core collections of known genetic composition that contain a large portion of the genetic diversity present in the sorghum germplasm collection. **(Mullet, Klein, Rooney)**

Experimental Plan:

1.1 Collect genomic diversity profiles of ~2,500-5,000 accessions per year.
   a. Collect accession phenotypes/genotypes.
   b. Collect diversity profiles (~1,000 markers/genotype)
   c. Process GAII sequence data to identify SNPs, INDELs
   d. Create a diversity database and dendograms.

1.2 Create and genotype enriched core germplasm collections.
   a. Create core collections based on genetic diversity/phenotypes.
   b. Collect 500+ high-resolution genotype profiles of accessions.
   c. Process sequence data, database entry.

Budget: $250K/yr - Yr1/2; $350/yr – Yr 3-5

Objective 2: Create and screen an integrated set of breeding and discovery populations for phenotypes/genotypes/genomic regions that contribute to combining ability, yield, and QTL/genes that modulate key energy sorghum traits. **(Mullet, Klein, Rooney)**

Experimental Plan:

2.1. Create 3-5 PS energy R-line derived populations (F2 to RILs).

2.2. Screen populations for key energy traits.
   a. Biomass yield/partitioning.
   b. Tillering, lodging
   c. Flowering time, height
   d. Composition (NIR)
   e. Conversion efficiency
   f. Drought/salt tolerance

2.3 Map QTL and generate markers for energy trait marker-assisted breeding.

2.4 Search germplasm for optimum versions of target genes/QTL/traits.

Budget: $250K/Yr – Yr1/2; $400K – Yrs 3-5

Objective 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. **(Mullet, Klein, Rooney, Peterson)**

Experimental Plan:

3.1 Construct a large number of crosses among converted energy lines to sample diversity.

3.2 Test-cross/phenotype/genotype/select improved lines.

a. Biomass yield/partitioning.
e. Tillering, lodging
f. Flowering time, height
g. Composition (NIR)
h. Conversion efficiency
i. Drought/salt tolerance

3.4 Conduct selective mapping per se and in hybrids.

3.3 Pyramid beneficial traits in elite pollinator parents.

a. Using phenotypic selection.
b. Using marker-assisted-selection.

3.4 Generate and test hybrids in multiple locations.

Budget: $500K for Years 1, 2; $750K/yr for Years 3-5.

GOAL 3: ***Create revolutionary next-generation energy crops by wide hybridization of diverse sorghums with sugarcanes and other energy grasses.***

Deliverables:

1. Elite intergeneric hybrids individually selected for vegetative propagation and biofuel production characteristics; diversity that expands production range.
2. Assessments of values and production ranges for the elite intergeneric hybrids.
3. Parental lines that optimize production of intergeneric F1 or BC1F1 seed for seed-based cultivation of heterotic "sor-canes" and other wide-hybrids, and/or self-fertile synthetic polyploids.
4. Genetic types that expand the ease and scope of wide-hybridization among C4 grasses for deriving additional biofuel crops.

Approach: Because the highest yields of plant biomass are derived from exceptional C4 grass hybrids, *the most energy and cost-effective plant-based biofuel strategies will rely on C4 hybrids.* TAMUS recently established technology that revolutionizes the range and facility of wide-hybridization between sorghum and other C4 grasses, including sugarcanes. TAMUS is ***uniquely*** capable of creating extremely large numbers of genetically unique intergeneric hybrids that have *never* been produced before. These have the potential for [1] strong vegetative hybrid vigor, [2] unique combinations of useful traits from the species and genotypes being crossed, [3] high capacities for clone-based production, like sugarcane, *and* [4] large enough seed sizes to allow for production of "wide-hybrid" canes from seed-based plantings. Potential trait combinations include resistances to multiple diseases, pests, drought, heat, cold, salinity, and soil deficiencies, perennial or annual growth, high nitrogen-use efficiency. This DARPA project will expectedly lead to a suite of novel next-generation energy grasses for advanced biofuels and biopower generation, the revolutionary benefits of which will include significantly higher biofuel yields, greater diversity of bio-fuel crops available to DARPA and other bio-fuel producers, expanded geographic, climatic and environmental ranges usable for biofuel production (thereby reducing impact on food production), and a seed-based system for producing cane-like intergeneric crops which eliminate costly vegetative plantings (up to $1,000/acre).

Research Plan

Objective 1: Create large numbers of individually distinct sorghum/energycane hybrids. **(Rooney, Stelly, Gould/Sugarcane Breeder)**

Rationale: Evaluation of current wide-hybrids indicates that "elite" hybrids (top 2%) offer great promise as biofuel feedstocks. If grown clonally as biofeedstocks using methods that rely on vegetative propagules, as for sugarcane, only the very best wide-hybrids will be used for biofuel production. The best hybrids will be obtained most rapidly by screening very large numbers of progeny from very diverse pollen parents. The best hybrids will be advanced for use in crop testing and hybrid development.

Experimental Plan:

1. Hybridization of Tx3361 sorghum (and other *iap* derivative lines) with energycane in crossing greenhouses in Weslaco and/or College Station, Texas.
2. Produce at least 10,000 intergeneric seed annually
3. Selectively establish at least 1,000 seedlings in field nurseries in College Station for evaluation.
4. Through five years, screen at least 50,000 hybrids. Select hybrids will be propagated at the end of the season for use in other objectives.

Budget: $150K/yr, Years 1-2; $200K/yr, Years 3-5

Objective 2: Multi-location screening of wide-hybrids to identify elite and unique hybrids. **(Rooney, Beaumont, Weslaco)**

Rationale: The performance of intergeneric hybrids will be affected by interactions of genotype and environment (GxE). Given the possibility that subsequent production may be best served by selection directly in each area of subsequent use, we will grow and select early-generation wide-hybrids in multiple locations that are expected to be biofuel production sites. Data and selections from these sites will collectively reveal site-specific and site-nonspecific trait and/or genetic tendencies that foster improved selection subsequently.

Experimental Plan:

1. Intergeneric hybrids will be grown as transplanted seedlings or direct-seeded seedlings in three different production environments (College Station, Weslaco and Beaumont). One of two general approaches will be used to distribute materials to multiple locations destined to serve as biofuel production test sites.
2. F_1 seedlings selected in breeding nurseries will be vegetatively propagated and distributed clonally for replicated evaluation at the test sites.
3. Wide-hybrid F_1 seed identified as to parentage will be distributed to the multiple-location sites, for either direct seeding or greenhouse-based germination and subsequent transplanting; in this case, results from multiple locations would be

collated according to parentage, not individual genotypes (i.e., family-based selection).

Budget: $150K/yr, Years 1-2; $200K/yr, Years 3-5

Objective 3: Determine agronomic performance and conduct pilot-scale testing of intergeneric hybrids. **(Rooney, Gould/Sugarcane Breeder, El-Hout, Blumenthal)**

Rationale: Agronomic characterization of intergeneric hybrids will define the best management practices for these new crops, their relative strengths, and the best genotypes for commercialization. This information will be used to identify clones to advance for research objective 4.

Experimental Plan:

1. Years 1-2:
    a. Hybrids selected in Objective 1 or 2 will be clonally propagated and planted in multiple locations (Weslaco, College Station, and Beaumont, Texas).
    b. In select locations, two complete trials will be planted; one will be grown under full irrigation and the second will be produced under limited irrigation to fully assess the level of drought tolerance.
    c. Evaluation for at least three years to determine adaptation, perenniality and biomass yield. All trials will have comparative checks of bioenergy sorghum and energycane.
2. Years 3-5:
    a. Continue additional testing, as described in Years 1-2.
    b. In years 3-5, the elite lines will be clonally propagated for advanced testing in a larger number of locations; including but not limited to sites of importance to DARPA and DOD production.

Budget: $150K/yr, Years 1-2; $300K/yr, Years 3-5

Objective 4: Develop seed-based propagation systems for sorghum/energycane hybrids **(Rooney, Gould/Sugarcane Breeder, Stelly)**

Rationale: Seed-based planting of an energycane-like crop could reduce planting costs by >90% and greatly increase production flexibility, and attractive feature for both producer and biofuel plant feedstock production facilities. Current data indicate that *major* strides in seed production and hybrid performance will be obtainable through improvements of both parents, i.e., breeding of improved sorghum seed parents and concomitant selection among energycane pollen parents.

Experimental Plan:

1. Sorghum Seed Parental Line Development (*iap*)
    a. *iap* sorghum with high stem sugar
    b. enhanced disease resistance
    c. improved drought tolerance
    d. production of A/B lines

2. Energycane Pollinator Parent Screening and Development
    a. assess seed set of different energycane pollinators on Tx3361 or other *iap* sorghums
    b. select energycane pollinators based on pollen viability, Tx3361 seed set, and progeny performance
    c. selectively breed energycane specifically for pollination of *iap* sorghum genotypes
    d. test selected derivatives from breeding for enhance pollination of *iap* sorghum genotypes
3. Cytological Manipulations of Pollinator Parent to Produce Uniform Commercial Hybrids
    a. recover an amphiploid of each elite sorghum/energycane hybrid
    b. test fertility of derived line
    c. test hybridization of derived line to sorghum (4.1a)
    d. derive lines that are good pollinators and genetically stable

Budget: $400K/year, Years 1-2; $600K/year, Years 3-5

Objective 5: Molecular characterization of *iap* and other important genes that influence intergeneric hybridization and recombination, and parental and intergeneric hybrid genotypes. (**Klein, Mullet)**

Rationale: Characterization of the *iap* gene will facilitate further selection of promising parental genotypes for producing intergeneric crosses. In this case, marker-assisted selection will expedite and economize the development of genetic combinations of *iap* with other genes that enhance production, performance and quality of wide-hybrids. Mapping of a trait-controlling gene at high resolution is often key to its identification, which may well stimulate the discovery and utilization of similar genes in other species. Such advances could lead to additional advances in US biofuel feedstock production.

Experimental Plan:

a. Evaluate a BC1F1 mapping population (N=300) for the *iap* phenotype.
b. Genotype the BC1F1 mapping population to the ~0.3 cM resolution.
c. The *iap*-containing segment between most closely flanking markers will be searched for genes that have the expected function.
d. Candidate genes will be sequenced from Tx3361 and wild type and validated bioinformatically and experimentally as the gene that enables intergeneric hybridization.
e. DNA markers linked to *iap* alleles will be developed for use in the wide-hybridization breeding program.
    a. "Digital Genotype" parental lines and select progeny.
    b. Analyze the Digital Genotypes to determine the sizes and extents of genome admixture in the hybrids.
    c. "Digital Genotype" selected cane genotypes that are tested for efficacy in wide-hybridization, and determine if the genotyping technology can enhance parental line selection.

Budget: $150K/yr, Years 1-2; $200K/yr, Years 3-5

BUDGET SUMMARY: Total Costs

Task	Year 1	Year 2	Year 3	Year 4	Year 5	Totals
1.1	$175,000	$175,000	$400,000	$400,000	$400,000	$1,550,000
1.2	$175,000	$175,000	$400,000	$400,000	$400,000	$1,550,000
1.3	$350,000	$350,000	$800,000	$800,000	$800,000	$3,100,000
1.4	$225,000	$225,000	$350,000	$350,000	$350,000	$1,500,000
1.5	$75,000	$75,000	$50,000	$50,000	$50,000	$300,000
	$1,000,000	$1,000,000	$2,000,000	$2,000,000	$2,000,000	$8,000,000
2.1	$250,000	$250,000	$350,000	$350,000	$350,000	$1,550,000
2.2	$250,000	$250,000	$400,000	$400,000	$400,000	$1,700,000
2.3	$500,000	$500,000	$750,000	$750,000	$750,000	$3,250,000
	$1,000,000	$1,000,000	$1,500,000	$1,500,000	$1,500,000	$6,500,000
3.1	$150,000	$150,000	$200,000	$200,000	$200,000	$900,000
3.2	$150,000	$150,000	$200,000	$200,000	$200,000	$900,000
3.3	$150,000	$150,000	$300,000	$300,000	$300,000	$1,200,000
3.4	$400,000	$400,000	$600,000	$600,000	$600,000	$2,600,000
3.5	$150,000	$150,000	$200,000	$200,000	$200,000	$900,000
	$1,000,000	$1,000,000	$1,500,000	$1,500,000	$1,500,000	$6,500,000
Total	$3,000,000	$3,000,000	$5,000,000	$5,000,000	$5,000,000	$21,000,000

From:	Helms, Adam
To:	wlr@tamu.edu; stelly@tamu.edu; Mullet, John E.; ssearcy@tamu.edu; jwrichardson@tamu.edu; jmgould@ag.tamu.edu; pklein@tamu.edu
Cc:	Simpson, Shay; Spurlin, Shayna; Nelson, Michelle; Bridges, Brenda; Giroir, Brett; Avant, Bob; McCutchen, Bill
Subject:	Highest Priority: DARPA Energy Crops
Date:	Monday, September 28, 2009 2:58:09 PM
Attachments:	DARPA Energy Crops Assigned Tasks.doc TIGM DTRA Milestones and Deliverables.pdf Budget Instructions.doc RR FedNonFed Budget 2010.xls
Importance:	High

Good Afternoon:

Please find attached the proposal for the DARPA Energy Crops proposal. I have updated each objective to include assigned PI's. There are several things we need to accomplish to submit this proposal to DARPA by October 9th.

1. Please submit your budgets by close of business October 1 to myself and Shayna Spurlin. Shayna prepared the budget template (attached) with instructions for entering information – if you have any questions, please refer them to her (sfspurlin@tamu.edu). If you have any capital equipment or lab supplies, we will need a detailed list of items to be purchased as well as a quote for the expenses. We will send an example budget justification out ASAP.
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If you have any questions, please contact me at your earliest convenience.

Best,

Adam

Adam Helms
AgriLife Research Corporate Relations
979-255-0752 (mobile)
979-458-2677 (office)

From: Avant, Bob [mailto:bavant@tamu.edu]
Sent: Sunday, September 27, 2009 12:54 PM
To: McCutchen, Bill; wlr@tamu.edu; stelly@tamu.edu; Mullet, John E.; ssearcy@tamu.edu; jwrichardson@tamu.edu; jmgould@ag.tamu.edu; pklein@tamu.edu
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Subject: RE: Highest Priority: DARPA

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512/422-6171 (Cell)
bavant@tamu.edu
http://agbioenergy.tamu.edu

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To: McCutchen, Bill
Cc: Pollard, Claudia
Sent: Sun Sep 27 08:28:24 2009
Subject: RE: DARPA UPDATE

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I will be happy to meet multiple times in the next 2 weeks to get this done

Brett P. Giroir, MD
Vice Chancellor for Research,
The Texas A&M University System;
Research Professor, Dwight Look College of Engineering;
Adjunct Professor, The Bush School of Government and Public Service;
200 Technology Way, Suite 2043
College Station, Texas 77845-3424
Phone: 979-458-6054
Fax: 979-458-6044

From: McCutchen, Bill
Sent: Friday, September 25, 2009 5:33 PM
To: Schuerman, Peter L.; Ellison, Mark M.; Howell, Bill; Diedrich, Guy
Cc: Giroir, Brett; Avant, Bob
Subject: Fw: DARPA UPDATE

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To: Rooney Bill <wlr@tamu.edu>; John Mullet (jmullet@tamu.edu) <jmullet@tamu.edu>; stelly@tamu.edu <stelly@tamu.edu>; James Richardson (jwrichardson@tamu.edu) <jwrichardson@tamu.edu>; 'Gould Mike' <jmgould@tamu.edu>; Steve Searcy (ssearcy@tamu.edu) <ssearcy@tamu.edu>; (pklein@tamu.edu) <pklein@tamu.edu>
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Thanks again for all of your hard work and dedication, and no doubt that this request from DARPA is very positive news – no guarantees yet, but good news.

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Bill F. McCutchen, Ph.D.
Associate Director
Texas AgriLife Research
Texas A&M University System

113 Jack K. Williams Administration Building
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979-845-8488 Tel
979-458-4765 Fax
bmccutchen@tamu.edu

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Cc:	Simpson, Shay; Spurlin, Shayna; Nelson, Michelle; Bridges, Brenda; Giroir, Brett; Avant, Bob; McCutchen, Bill
Subject:	RE: Highest Priority: DARPA Energy Crops
Date:	Monday, September 28, 2009 3:08:24 PM
Attachments:	Sample Budget Justification.doc

Attached is the sample budget justification. Please use this format – it will assist us in the final assembly of this proposal.

Best,

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RR Budget Fed/NonFed

Category	Salary	Year 1 Person Months Request	Year 1 Fed	Year 1 Total	Salary	Year 2 Person Months Request	Year 2 Fed	Year 2 Total	Salary	Year 3 Person Months Request	Year 3 Fed	Year 3 Total	Salary	Year 4 Person Months Request	Year 4 Fed	Year 4 Total	Salary	Year 5 Person Months Request	Year 5 Fed	Year 5 Total	Total Project Fed	Total Project Total
A. Sr. Personnel																						
PI:		0	$ -	$ -	0	0	$ -	$ -	0	0	$ -	$ -	0	0	$ -	$ -	0	0	$ -	$ -		
Fringe Benefits			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
B. Other Personnel																						
Research Associate:	0	0	$ -	$ -	0	0	$ -	$ -	0	0	$ -	$ -	0	0	$ -	$ -	0	0	$ -	$ -		
Fringe Benefits			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Graduate Research Assistant (50%)	0	0	$ -	$ -	0	0	$ -	$ -	0	0	$ -	$ -	0	0	$ -	$ -	0	0	$ -	$ -		
Fringe Benefits			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Undergraduate Student Labor			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Fringe Benefits			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Total Personnel Costs			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -	$ -	$ -
C. Equipment																						
Itemize			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Itemize			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Total Equipment			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -	$ -	$ -
D. Travel																						
Itemize			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Itemize			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Total Travel			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -	$ -	$ -
E. Participant Support Costs																						
Itemize			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Itemize			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Total Particpant Support Costs			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -	$ -	$ -
F. Other Direct Costs																						
1. Materials and Supplies			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
2. Publication Costs			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
3. Consultant Services			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
4. ADP/Computer Services			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
5. Subawards/Consortium/Contractual Costs			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
6. Equipment or Facility Rental/User Fees			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
7. Alterations and Renovations			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
8. Other Costs			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Tuition (GRA)			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Itemize			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Itemize			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Itemize			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -		
Total Direct Costs			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -	$ -	$ -
G. Total Direct Costs			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -	$ -	$ -
H. Indirect Costs/F&A																						
@ 46.5% MTDC			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -	$ -	$ -
I. Total Project Costs			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -			$ -	$ -	$ -	$ -

Note

1) Fringe Benefits are estimated per the FY 10 procedures:
   - Faculty/Staff: 17.1% of salary plus group health benefits of $494/month/FTE
   - GRA: 9.7% of salary plus group health benefits of $199/month
   - Undergrad/Wage Labor: 9.7% of salary only

2) If Subcontracts are involved calculation must be modified to exempt each subcontract in excess of $25K

Budget Justification
A total of $21,000,000 is requested from the DARPA to support this proposed project to develop … over a 5-year project life. This document describes the budget requests for each year of the proposed 5-year project.

Personnel
No salary support is requested for the Principal Investigator or any of the other faculty from Texas A&M University or Texas AgriLife Research and Extension. Salary and fringe benefits for the Principal Investigator, Travis Miller, and each of the named faculty members are being covered by existing contracts.

Because the proposed research program involves a complex set of research plots and larger scale plantings, a large number of different genotypes and agronomic conditions, and will be conducted at multiple locations simultaneously, a relatively large technical support staff will be required.

Support is requested for a Postdoctoral Research Associate (to be named) for each year of the project. Starting monthly salaries for this individual is estimated at $3,333. The postdoctoral research associate will assist the PI and other faculty with aspects of germplasm collection, selective plant breeding operations, field tests, and large-scale planting objectives. This includes designing experiments, establishing research plots, collecting data, analyzing data, drafting reports and publications, identifying and reporting inventions, and working with assistants such as technicians, graduate students, and undergraduate students. Specifically, the postdoctoral associate will conduct ELISA based assays to determine ricin concentration on an individual seed basis from M3 and M4 mutant populations. This individual will also use Real Time PCR to determine if the ricin gene(s) are being expressed in selected plants. This process will allow us to identify 50 to 100 plants that have low toxin phenotypes prior to genomic analysis. Salary increases at a rate of 3% annually while insurance increases at an anticipated rate of 10% annually

Support is also requested for two Ph.D. graduate students (to be named) and two M.S. students (to be named). Starting monthly salaries for the graduate students ranges from $1,500 to $1,833, which is typical compensation for graduate students at Texas A&M University. These students will be supervised by participating faculty in all aspects of the research, including assisting with: (1) collecting and characterizing germplasm with phenotypic and genotypic data; (2) identifying heterotic groups and combining abilities among jatropha parents; (3) evaluating breeding lines and progeny for capsule disease; (4) conventional and molecular genetic studies on mutant plants with low toxin phenotypes to determine both the mode and the specific site of gene silencing – allowing us to sexually hybridize selected mutants to derive segregating lines with even lower levels of ricin toxins. Additionally, they will support efforts related to genetic testing, breeding, field studies, data collection and analysis, and presentation of study results. All graduate students will be encouraged to submit abstracts for presentation of study results at regional and national meetings of relevant professional societies. They will also be recognized as authors on any journal manuscripts they assist with.

The budget also includes funds for 10 undergraduate student workers (to be named) throughout the project. Students will work approximately 20-40 hours per week, over the life of the project. This will provide valuable research experiences for the students, who will provide additional personnel to assist with all laboratory and field research functions of the research program, including economic and environmental impact assessments.

Fringe benefits are estimated for faculty and staff positions based on 17.6% of salary plus group health insurance benefits at $471 per person per month. Fringe benefits for undergraduate students are estimated based on 10.1% of salary.

Project Costs – Personnel: $1,257,506

Permanent Equipment

This budget includes funds for several pieces of permanent equipment needed to complete the project. This equipment will be purchased in Years 1 and 2 of the project and will include a tractor and implements ($40,000 in Year 1), a dehuller – needed to separate hulls from seed for oil content analysis – ($12,000 in Year 2), and a truck and trailer – needed to transfer equipment and personnel to and from testing locations – ($40,000 in Year 1).

Project Costs – Permanent Equipment: $92,200

Travel

Funds are requested for travel for the investigators to attend planning meetings, annual professions meetings to present study results, and for personnel to conduct the extensive field work at the various test locations planned for this project. Funds for domestic travel support will provide $1,000 in each year to supervise planting and harvesting at offsite locations which include sites at Vernon, TX; Uvalde, TX, Manhattan, KS (KSU); and Starkville, MS (MSU). In year 1, $1,000 will be used for travel to facilitate research coordination, and in all years, $5,000 is reserved for the PIs, Research Associate, and Graduate Research Assistants to attend professional meetings. It will also support primarily in-state travel to visit nursery locations and breeding sites in South Texas.

Project Costs – Travel: $120,000

Other Direct Costs

Materials and Supplies – Costs of materials and supplies are estimated at $66,000 for Year 1, and $59,000 each for Years 2 and 3. Materials and supplies include funds needed for maintenance and repair of equipment, field materials, laboratory supplies, growth media, greenhouse supplies, fertilizer, herbicides, insecticides, tools, safety equipment, office supplies, materials for greenhouse propagation, harvesting supplies, seed packaging, chemical and laboratory materials necessary to conduct approximately 5,000 ELISA assays and 500 RT-PCR analyses in each year of the study. Additional materials will be used to grow seedlings in the greenhouse and mature plants in the field,

Project Costs – Materials and Supplies: $185,000

Subawards/Consortium Partners: Chevron Technology Ventures – Funds for the Chevron Technology Ventures efforts in this project are described fully in a separate budget justification attached to this application. A brief summary is provided below.

Fifty percent support is requested for the technical personnel involved in the project. The Program Manager, 15% time commitment, will provide technical over site for the project with some expert technical support. The four year budgeted cost for salary for this position is $58,458 in requested DARPA support, $58,458 in Chevron match ($116,916 total) with fringe support requested from the DARPA being $40,826, and Chevron match $40,826 ($81,651 total). The hydrotreating PI, 80% time commitment, will provide primary technical expertise to the overall hydrotreating work to be completed. The four year budgeted cost for salary for this position is $201,367 in requested DARPA support, $201,367 in Chevron match ($402,735 total) with fringe support requested from the DARPA being $115,786, and Chevron match $115,786 ($231,572 total). One Senior Hydrotreating Technician, 50% time commitment, will operationalize the hydrotreating technology. The four year budgeted cost for salary for this position is $125,855 in requested DARPA support, $125,855 in Chevron match ($251,709 total) with fringe support requested from the DARPA being $72,313, and Chevron match of $72,313 ($144,626 total). One Technician, 50% time commitment, will provide support to the PI and Senior Technician in scaling the technology for the project. The four year budgeted cost for salary for this position is $95,121 in requested DARPA support, $95,121 in Chevron match ($190,242 total) with fringe support requested from the DARPA being $52,792, and Chevron match $52,792 ($105,584 total). A Process Engineer, 15% time commitment, will have responsibility for biomaterial preprocessing, queuing, and logistics processes. The four year budgeted cost for salary for this position is $37,756 in requested DARPA support, $37,756 in Chevron match ($75,513 total) with fringe support requested from the DARPA being $21,710, and Chevron match $21,710 ($43,420 total).

No federal support is being requested for travel costs associated with this project ($12,740).

Material and Supply costs for this project include consumables required to support operation of hydrotreating micro-unit and pilot unit as well as disposables such as single use safety equipment. DARPA support being requested for the four years of the project ($396,269) with Chevron match of $167,756 ($564,025 total).

Facility user fees for the pilot hydrotreating unit are expected to be approximately $28,000 per month for the four-year life of the project. In year two of the project, 6 months of use are expected. In years three and four 12 months of use are expected. DARPA support requested for this expense is $420,000 with Chevron match of $420,000 for the total cost of $840,000.

Other miscellaneous costs associated with the projects for which DARPA support is requested for the four years of the project include equipment configuration ($71,000 DARPA support, $94,000 Chevron match, $165,000 total) and shipping of biomass

($14,210 DARPA support, $8,120 Chevron match, $22,240 total). DARPA support is being requested for 100% of the materials and supplies requested for the four-year project ($83,654). The materials and supplies include the costs of spares and disposables for the use and maintenance of the Catalytic Bed Reactor.

In house Chemical Analysis, including quality analysis and compositional analysis of conversion products will be conducted. DARPA support is requested in the amount of $153,178 with Chevron match of $94,078 ($247,256 total).

Other miscellaneous costs associated with the projects for which DARPA support is requested for the full four years of the project include Facility Rental ($28,937), outsourcing of Chemical Analysis for biomass feedstocks ($5000), Disposal of Charred Biomass ($13,388), Disposal of Waste Oil ($23,847), Shipping of Biomass ($8,367).

Project Costs – Partner: $4,199,542

Equipment and Facility Rental: A total of $36,000 is requested over the life of the project so support rental of equipment and facilities. This includes rental of farmland for large-scale plantings, equipment required to perform field operations such as transplanting seedlings and applying fertilizer and pesticides under non-conventional, experimental agronomic conditions.

Project Costs – Equipment/Facility Rental: $36,000

Tuition: Funds for graduate student tuition are included at $34,008 in Years 1-3 of the project. The amount is based on information about current tuition and fees at Texas A&M University.

Project Costs – Tuition: $102,024

Cooperator/Grower/Production Costs. Costs associated with the production of castor plots are estimated to total $37,000 in years 1-3 of the project. These costs include operational costs of cooperative growers associated with preparing fields, planting seedlings, applying fertilizer, irrigation, weed control, insect and disease control applications, and harvesting.

Project Costs – Cooperator/Grower/Production Costs: $37,000

Analyses. Costs associated with the analyses of various jatropha and castor samples are estimated to total $35,100 in years 1-3 of the project. These costs include testing for quantity and quality of jatropha and castor oil samples, as well as genetic analysis to aid in determining molecular sequencing.

Project Costs – Analyses: $35,100

Project Costs – Other Direct Costs: $4,594,666

***Project Costs – Total Direct Costs*: $6,064,172**

Indirect Costs

Indirect Costs are calculated at 46.5% of the modified total direct costs, which is the rate negotiated through our cognizant agency the Department of Health and Human Services. A copy of our current rate agreement is included with this proposal.

Project Costs – Indirect Costs: $788,457

Total Project Costs: $6,852,629

Task 1:

Milestones and Metrics:

1. We will scale WT clones to grow and differentiate in 96-well plates. We will demonstrate that greater than 90% of wells differentiate into tissue types as defined below.
2. Undifferentiated ES cells will be defined by morphology under a light microscope and expression of markers specific for pluripotency including Oct4 and alkaline phosphatase.
3. Neurons will be defined by expression of neuronal markers: Lhx3 and Isl 1/2.
4. EBs will be defined by morphology, size, and cell number.
5. Gut epithelia will be defined by Cdx2 expression and spontaneous contractions. Lung epithelia will be defined by Q-RT-PCR for SP-C and TTF-1.

Deliverables: We will provide a report with complete methods for growth and differentiation of mouse embryonic stem cells in 96-well plates for use by the government and its contractors.

Cost: $513,407

Task 2:

Milestones and Metrics:

1. We will demonstrate a dose response curve, minimally including LD5 to LD95, over ≥ 1 log concentration of agent, in tissue types under examination.
2. We will identify and QC at least 3 mutant clones as positive controls for each agent. Positive controls will be defined by a ≥2X shift in the dose response curves for each agent.

Deliverables:

1. We will generate a report detailing complete assay protocols and all data generated.
2. As desired by DTRA, we will transfer the positive control clones to a DoD cell bank for use by the government and its contractors.
3. We will provide the government an option for TIGM to generate knockout mouse models using these resistance genes, for use by the government and its contractors as models for BW resistance.

Cost: $1,744,030

Task 3:

Milestones and Metrics:

1. We will identify at least 10 novel genetic targets that mitigate toxicity for the agents tested. Mitigation is defined as a ≥2X shift in the dose response curve for each agent.

Deliverables:

1. We will generate a complete report detailing all data on identified genetic targets, as well as genetic alterations that do not result in mitigated toxicity.
2. As desired, we will transfer clones mitigating toxicity to a DoD cell bank for use by the government and its contractors.

Cost: $3,304,226

Task 4:

Milestones and Metrics:

1. We will demonstrate ≥ 1 siRNA per target that shows ≥ 90% *in vitro* knockdown of target mRNA expression as assayed by quantitative RT-PCR.
2. We will evaluate siRNAs for in vitro activity (resistance induction) against agents under examination in the tissue types screened.
3. If candidate siRNAs demonstrate a ≥2X shift in the dose response curve for a given agent in vitro, we will proceed to in vivo evaluation.
4. Efficacy goals for in vivo siRNA studies will be evaluated by a shift in the lethality curve of ≥2X.

Deliverables:

1. We will generate a complete report detailing protocols, all data generated, sequences of siRNAs, and quantitative RT-PCR primers, probes, and assays.
2. All materials and protocols will be made available to the government and its contractors.

Cost: $436,189

Task 5:

Milestones and Metrics:

1. We will develop 2-4 homozygous knockout mouse lines per agent, based on novel targets identified in previous tasks.
2. All knockout lines will be clinically phenotyped. (These data will be relevant for any therapeutic developed by TMTI against this target for any current or newly identified infectious diseases, irrespective of efficacy of the target in this model).
3. A validated target will be defined by achieving a knockout mouse line with ≥ 1 log shift in the dose response curve to that agent.

Deliverables:

1. We will generate a complete report detailing protocols and all data generated including all clinical phenotyping data and resistance data.
2. Mice will be made available for use by the government and its contractors as models for BWA resistance. As desired frozen sperm or embryos for each mouse line will be transferred to the DoD bank/repository.

Cost: $1,921,538

Task 6:

Milestones and Metrics:

1. We will establish differentiation protocols such that ≥ 90% of wells show differentiation into dendritic cells.
2. Dendritic cells will be identified by morphology and immunohistochemical analyses.
3. We will demonstrate a dose response curve, minimally including LD5 to LD95, over ≥ 1 log concentration of agent, for Brucella in tissue types under examination.
4. We will identify and QC at least 3 mutant clones as positive controls for Brucella. Positive controls will be defined by a ≥2X shift in the dose response curve for Brucella.

Deliverables:

1. We will provide a report with complete methods for growth and differentiation of mouse embryonic stem cell in 96-well plates for use by the government and its contractors.
2. As desired by DTRA, we will transfer the positive control clones to a DoD cell bank for use by the government and its contractors.
3. We will provide the government an option for TIGM to generate knockout mouse models using these resistance genes, for use by the government and its contractors as models for BW resistance.

Cost: $1,161,931

Task 7:

Milestones and Metrics:

1. We will identify at least 10 novel genetic targets that mitigate toxicity for the agents tested. Mitigation is defined as a ≥2X shift in the dose response curve for Brucella.

Deliverables:

1. We will generate a complete report detailing all data on identified genetic targets, as well as genetic alterations that do not result in mitigated toxicity.
2. As desired, we will transfer clones mitigating Brucella toxicity to a DoD cell bank for use by the government and its contractors.

Cost: $745,292

Task 8:

Milestones and Metrics:

1. We will demonstrate ≥ 1 siRNA that shows ≥ 90% *in vitro* knockdown of target mRNA expression as assayed by quantitative RT-PCR.
2. We will evaluate siRNAs for in vitro activity (resistance induction) against Brucella in the tissue types screened.
3. If candidate siRNAs demonstrate a ≥2X shift in the dose response curve for Brucella in vitro, we will proceed to in vivo evaluation.
4. Efficacy goals for in vivo siRNA studies will be evaluated by a shift in the lethality curve of ≥2X.

Deliverables:

1. We will generate a complete report detailing protocols, all data generated, sequences of siRNAs, and quantitative RT-PCR primers, probes, and assays.
2. All materials and protocols will be made available to the government and its contractors.

Cost: $585,130

Task 9:

Milestones and Metrics:

1. We will develop 2-4 homozygous knockout mouse lines based on novel targets identified in previous tasks.
2. All knockout lines will be clinically phenotyped.
3. A validated target will be defined by achieving a knockout mouse line with ≥ 1 log shift in the dose response curve to Brucella.

Deliverables:

1. We will generate a complete report detailing protocols and all data generated including all clinical phenotyping data and resistance data.
2. Mice will be made available for use by the government and its contractors as models for BWA resistance. As desired frozen sperm or embryos for each mouse line will be transferred to the DoD bank/repository.

Cost: $1,689,378