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Subject:	DARPA GOAL 3
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Attachments:	Goal 3.docx

David:

Attached is my first attempt at Goal 3, which is the wide hybridization work. A couple of thought for both you and the group when considering this draft.

First, I've limited the wide hybrids to sorghum/energycane and sorghum/miscanthus and for the breeding (later objectives) it is focused solely on the sorghum/sugarcane work. The reason for this is to streamline the project and give it some focus based on the information that we have at this time. While we may have success with other grass species (ie, corn), we can pursue opportunities elsewhere for them in the future as appropriate.

Second, I've not yet put numbers to the categories. I wanted to get this to you for your input and editing. I'll be thinking about funds today and if you have thoughts please include them in the revision.

Third, I've not listed other faculty and don't plan on it in this draft. I would like to establish that after this appears more likely to be funded. If I'm wrong on that please let me know. I have listed the role of the weslaco station (and other as appropriate) in the draft.

Fourth, the molecular characterization is important, and I need help in fleshing this out. Hopefully between John, Trish and Dave you can help.

Finally, I received the overview document that John sent out this morning, but I've not included this in that and don't think we should until we have this section in a more finished phase.

Now, I'm onto Goal 1.

Regards,

bill

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<u>Goal 3:</u> Develop next generation energy crops using novel wide hybridization technology that enables sorghum to be crossed with energy cane and other energy grasses.

Rationale: Wide-hybridization technology developed at TAMUS allows sorghum to be crossed with sugarcane and other C4 grasses that can increase yields by 20% or more. The resulting intergeneric hybrids have the potential to exhibit strong hybrid vigor and 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 energycane or miscanthus. This technology, when fully developed, has the potential to allow mass-production of hybrid seed from crosses with energy canes (first time ever). This innovation would reduce the cost of planting an energycane-type crop by at least a factor of ten, providing for the <u>first</u> time seed-based (or vegetative) propagation of wide hybrid energy crops, and potentially generating a novel suite of next generation energy grasses for advanced biofuels and biopower generation.

Project Objectives:

1. Production and selection of elite wide hybrids of sorghum/energycane and sorghum/miscanthus
2. Agronomic evaluation of intergeneric hybrids using vegetative propagated hybrids.
3. Use elite hybrids for introgression of desirable traits into both sorghum and energycane or miscanthus
4. Development of seed-based propagation systems for sorghum/energycane hybrids.
5. Molecular Characterization of iap and other important genes that influence intergeneric hybridization and recombination.

Deliverables:

1. Elite intergeneric hybrids with improved phenotypic performance for a variety of traits including but not limited to drought tolerance, sugar accumulation, and adaptation range.
2. Assessment of the value and range of production of these intergeneric hybrids.
3. Production of sorghum, energycane and miscanthus derivatives with traits derived from the complementary donor species.
4. Development of parental lines that optimize seed-based production of sorghum/energycane and/or sorghum/miscanthus hybrids.

Research Plan

Objective 1: Production and selection of elite wide hybrids of sorghum/energycane and sorghum/miscanthus

Rationale: The basis for the wide hybridization is the creation of intergeneric hybrids between sorghum with both energycane and miscanthus. Once large numbers of these hybrids are produced, these hybrids can be selected for elite performance and selected hybrids will be advanced for use in crop testing, and as a source for further breeding and

development (Figure 1). Evaluation of current hybrids indicates that elite hybrids comprise <5% of all currently produced hybrids. Consequently, screening of large numbers of hybrids is needed to identify the most promising germplasm.

Implementation Plan: Sorghum line [redacted] and other iap derivative lines will be hybridized in large numbers in special crossing greenhouses in Weslaco and College Station, Texas. On an annual basis, at least 10,000 seedlings will be produced for evaluation, with at least 1,000 of these seedling transplanted to the field in College Station for evaluation and selection. By year 3, production of at least 30,000 hybrid seed are produced and directly seeded into the field for selection. Select hybrids will be propagated at the end of the season for use in objectives 2-4.

Budget: $_____/year

$_____/year for hybridization

$_____/year for hybrid evaluation nursery

Objective 2: Agronomic evaluation of intergeneric hybrids using vegetatively propagated hybrids.

Rationale: The agronomic characterization of wide hybrid progeny is critical to clearly identify which traits are expressed in these hybrids and what their value is relative to existing sorghum and energycane bioenergy crops. This baseline information is also necessary to identify important clones to advance for research objectives 3 and 4.

Implementation Plan: Hybrids selected from Objective 1 will be vegetatively propagated through stem cuttings. Once enough clonal "seed" stock is available, replicated trials of these materials will be planted in multiple locations to assess productivity in different environments and for different phenotypic traits. At a minimum, replicated trials will be established in Weslaco and College Station, Texas. In both 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. Trials will be evaluated for at least three years to determine adaptation, perenniality and biomass yield potential. All trials will have comaparative checks of bioenergy sorghum and energycane. In year 3, the elite lines will be identified and propagated for advanced testing in a larger number of locations; including but not limited to sites of important to DARPA and DOD production.

Budget: $_____/year

$_____/year for evaluation - Weslaco

$_____/ year for evaluation – College Station

Objective 3: Use elite hybrids for introgression of desirable traits into both sorghum and energycane or miscanthus

Rationale: Introgression breeding provides a viable mechanism to transfer agronomically important traits across species using traditional approaches that are not GMO. In addition, many of these traits are quantitatively inherited and are difficult if not impossible to transfer using transgenic approaches. Thus, the iap wide hybrid program provides a viable "bridge" through which these traits can move across generic boundaries that were not possible previously. Potential lines expressing valuable traits that were identified in Objectives 1 and 2 will serve as the basis for work in Objective 3.

Implementation Plan: Selected hybrids (from either objective 1 or 2) will be propagated for use in introgression breeding back to both energycane (or miscanthus) and sorghum. Propagated hybrids will be grown in crossing greenhouses (either College Station or Weslaco) and induced to flower via the use of short day treatments (or winter planting). At anthesis, fertility of the hybrids will be assessed; a large amount of sterility is expected. To mitigate this issue, other clones of the same lines will be treated to induce chromosome doubling. This should enhance fertility and allow these lines to be backcrossed to either parent as appropriate. BC1 progeny will be for each will be recovered and grown for additional backcrossing until progeny are recovered that have stable chromosome levels and fertility. Once derived lines are obtained, these lines will be assessed using molecular and cytological testing for evidence of introgression. In addition, the derived progeny for each species will be evaluated in agronomic field trials similar to those described in Objective 2, except that the lines will be compared directly with the recurrent parents (ie, energycane vs. derived energycane).

Budget: $_____/year

$_____/year for breeding/cytological manipulations (Weslaco and/or College Station)

$_____/ year for evaluation – College Station

$_____/ year for evaluation – Weslaco

Objective 4: Use elite hybrids for introgression of desirable traits into both sorghum and energycane or miscanthus

Rationale: Seed based propagation of an energycane like crop would have world-wide impact due to the reduction in production and flexibility in production and management program. Seed based propagation costs are approximately 10% of vegetative propagation methods, resulting in savings ranging from $400 to 800/acre (depending on location) for planting annually. Our programs have confirmed that there are differences among energycanes in their effectiveness in pollinating [redacted].

Implementation Plan: Breeding programs to optimize hybridization will be developed for both sorghum (seed parent) and energycane derived hybrids (to be used as pollinators). The timeline on this objective is five years; at that time we expect to produce quantities of seed derived hybrids suitable for advanced testing and development.

a. Sorghum Seed Parental Line Development (iap) – improved sorghum seed parents are under development. These include iap sorghum with high stem sugar, enhance disease resistance, and improved drought tolerance. Both male fertile and male sterile versions are under development and this process is continuous throughout the project.
b. Energycane Pollinator Parent Line Screening – research in objective 1 will assess seed set of different energycane pollinators. This information will be used to develop energycane populations that will be screened for enhanced seed set on [redacted]. Selected hybrids from the best pollinators will be used to develop true breeding pollinators for hybrid seed production.
c. Cytological Manipulations of Pollinator Parent to Produce Uniform Commercial Hybrids. Because energycane is genetically heterogeneous, it will be necessary to stabilize the genetics of the pollinator to produce genetically uniform sorghum/energycane hybrids. The most logical approach to stabilize the pollinator parent and further enhance seed set is to create an amphidiploid of elite sorghum/energycane hybrids. These lines will be genetically stable (homozygous), and the presence of the sorghum genome will combine with the sorghum genome in the seed parent during hybrid seed production.

Budget: $_____/year

$_____/year for breeding sorghum (College Station)

$_____/year for breeding energycane (Weslaco)

$_____/cytological manipulations and crossing trials (College Station)

Objective 5: Molecular Characterization of iap and other important genes that influence intergeneric hybridization and recombination.

Rationale: Identifying the gene responsible for the iap phenotype will facilitate (1) marker-assisted breeding to enhance the breeding program, (2) assess in the identification of additional genes involved in enhancing wide hybridization not only in sorghum but in other crop species as well.

Implementation Plan: Initial mapping of a small subset of progeny has located the iap location to linkage group __ on chromosome __. To fine map the iap locus, a large (300 individual) BC_1F_1 population has been created and is ready to be phenotyped.

Budget: $_____/year

$_____/year for phenotyping mapping population (College Station)

$_____/year for genetic analysis (Weslaco)