Appendix 2. Additional methods for Objectives 1, 2, 3, and 4.

Objective 1. Methods continued:
Germination testing was initiated in 2002 at the Griffin location, and to date at least one inventory has been tested for over 81% of all accessions. Standard germination tests will be conducted on the entire range of crop and wild relative accessions in the germplasm collection with emphasis on completing germination testing on existing accessions of sorghum, warm-season grasses, and Vigna spp. Germination testing will follow standard procedures developed by the Association of Official Seed Analysts (AOSA, 2011) for major crop species and/or the Handbook of Seed Technology for Genebanks - Volume II: Compendium of Specific Germination Information and Test Recommendations (Ellis et al., 1985). When standardized germination protocols do not exist for a particular species, the crop curator, cooperators at NCGRP, S-009 scientists, and curators or crop experts for other minor crops and crop wild relatives will be consulted for alternative germination techniques. The germination assays provide seed viability information to curators for establishing regeneration priorities. Germination tests will be conducted using 100 seeds for accessions with adequate seed numbers and follow a sliding scale decreasing to 10 seeds on accessions with minimal seed numbers. Test results will be entered into GRIN-Global.

Accessions are evaluated for trueness to type at various steps in the genebank conservation process. Prior to incorporation into the collection, seeds from completed regenerations are compared to original samples during seed processing. Seed mixtures, incorrect taxonomic identifications, or other concerns are noted during seed cleaning, germination testing, characterizations, or regenerations. Cooperators or users may report that a particular accession is incorrectly identified during their research. When inventories or accessions with questions on trueness to type are identified during these processes, they are referred to the appropriate curator for growouts or other evaluations to determine the correct taxon or designation for the sample.

Plant pathogen testing will include evaluating 25 to 100 peanut quarantine accessions per year for seed-borne virus infection, field or greenhouse diagnoses, screening plants or seed lots for diseases, and verification of plant or seed health as possible when required by other countries for satisfying their import permits. A seed assay for potato spindle tuber viroid will be developed due to requests for screening on pepper, sweetpotato, and other vegetable accessions prior to distribution to other countries. In cooperation with North Carolina State University scientists, 1,600 Citrullus accessions will be screened for downy mildew (Pseudoperonospora cubensis) resistance to identify new sources of resistance in this germplasm collection. Disease resistance data will be entered into GRIN-Global.

Sweetpotato, perennial wild peanuts which produce few seeds, bamboo, Chinese water chestnuts, bermudagrass, and other warm-season grass accessions are maintained as clones rather than seeds. The sweetpotato clonal collection consists of over 750 accessions maintained in tissue culture. Sweetpotato accessions will be maintained in vitro in a pathogen-free state on semi-solid Murashige and Skoog (MS) media in test tubes on a 10 hr photoperiod at ~20 C and are recultured every 6 to 12 months to ensure viability and availability. Clonal accessions of 321 warm-season grass, 175 wild peanut, and 6 water chestnut accessions are currently maintained as live plants in the greenhouse. An additional 38 warm-season grass (Pennisetum purpureum)
accessions are maintained in the field at Griffin. Clonal bamboo accessions are maintained in separate 5 x 6 m plots at the USDA, ARS, Byron, GA, location.

More than 95% of crop seed accessions and approximately 89% of the sweetpotato in vitro culture collection are conserved at a second location at NCGRP, Ft. Collins, CO. Backups will be conducted for accessions not already conserved at another location including newly acquired accessions and other accessions. Over time, accessions in the collection will be sent to a third site at Svalbard, Norway, as an additional backup. Cryopreservation of warm-season grass and sweetpotato accessions is conducted at NCGRP with 132 sweetpotato and 20 clonal warm-season grass accessions presently preserved. Ten warm-season grasses and 12 to 25 sweetpotato in vitro cultures will be sent to NCGRP per year or as requested by NCGRP.

**Objective 2 Methods continued:**
The entire sweetpotato (*Ipomoea batatas*) collection will be grown in the field in Charleston, SC, in collaboration with ARS cooperators. Standard descriptor data will be collected on all accessions and entered into GRIN-Global. DNA will also be extracted from leaf tissue of all accessions for long-term storage and use in parallel studies involving the utilization of molecular markers (microsatellites) as a germplasm management tool. The seashore paspalum and zoysiagrass clonal collections will be screened for salt tolerance (Lee et al., 2004). Plants will be grown under greenhouse conditions and flood irrigated twice daily with different salt levels (0, 15, 30 and 45 dS-m). Based on the results, an overall salt rating will be assigned to each accession and the ratings will be entered into GRIN-Global.

Germplasm accessions can be genetically characterized with a set of non-specific DNA markers (not necessarily associated with a specific trait) or candidate-gene markers (probably associated with a specific trait). The characterization choice depends on the species and/or traits of interest. Non-specific DNA (SSR or SNP) markers are now readily available for most major and minor agronomic crops. Candidate-gene markers can be developed from existing databases or newly-generated genomic resources (such as RNA-Seq) from the lab. Using previously published castor SSR markers (Qiu et al., 2010), a set of the most polymorphic SSR markers will be selected for initial testing of castor accessions. Seed tissue from about 669 accessions will be collected and extracted DNA used to genotype the accessions. Morphological descriptor data from GRIN-Global will be combined with the genotyping results to select 103 accessions to construct the U.S. castor core collection. Polymorphic SSR or SNP markers will also be used for genotyping 150 *Cucurbita argyrosperma*, 750 hexaploid sweetpotato, 350 chili pepper (*Capsicum annum*), and 750 eggplant accessions and the entire seashore paspalum and watermelon (*Citrullus lanatus*) collections using procedures described by Shirasawa et al. (2012), Roullier et al. (2011), Sandlin et al. (2012), Gong et al. (2008), and Barchi et al. (2011). SSR data will be used to identify potential redundancies in the collections, provide a fingerprint of clonal accessions (sweetpotato and seashore paspalum) as a tool to maintain genetically pure clones in tissue culture and greenhouse, and evaluate the diversity of these collections for association mapping. The obtained genetic characterization data will be entered into GRIN-Global or other databases (such as GenBank) for other scientists to use.

Genotyping by sequencing (GBS) can be used as an alternative method for genetically characterizing germplasm accessions using SNP markers. A total of 300 accessions will be
selected from 1,000 sorghum accessions which have been genotyped by sequencing and grown in Manhattan, Hay, and Colby, KS, for morphological observation and biomass chemical composition analysis. Using genetic and chemical analyses along with field observation, desirable accessions will be recommended to breeders or researchers for developing new cultivars or hybrids as bioenergy feedstocks. Based on fatty acid profiles generated by biochemical analysis, high and low oleic sesame accessions will be utilized to determine the association of oleic acid content to variability found in the sesame fatty acid desaturase (FAD2) gene (Kim et al., 2006). If polymorphic sites are linked with the level of oleic acid, a functional DNA marker can be developed for marker-assisted selection of oleic acid in sesame breeding. Similar to the procedure utilized for the FAD2 genes, functional DNA markers will be developed using real-time PCR or allele-specific PCR (AS-PCR) for tannin in sorghum. The developed functional DNA markers can be used in marker-assisted selection in sorghum breeding programs. RNA-Seq is a new approach to transcriptome profiling by using deep-sequencing technologies (Wang, Z. et al., 2009). A subset of peanut accessions that are empirically determined to have high and low oil content will be characterized by RNA-Seq in order to reveal the transcriptome profile and information on gene regulation of oil metabolism. Functional DNA markers will be developed from the target sequence identified from RNA-Seq.

Objective 3 Methods continued:

One aspect of improving the efficiency and usefulness of the collection as documented in GRIN-Global is the identification of duplicate (identical) or redundant (very similar) accessions as compared to other genetic resources already in the active collection. To identify duplicate or redundant accessions, analysis of data will be conducted by comparing taxonomy, plant names (i.e. cultivar, collector number, local name), the origin of the accession, other genebank identifiers, and donor information. Potential new genetic resources will be assessed for duplication or redundancy prior to incorporation into the collection. For additional comparison, the GRIN-Global database information will be compared to international databases (i.e. International Crops Research Institute for the Semi-Arid Tropics, GENESYS) for crops including sorghum, peanuts, Vigna spp., peppers, and various millets. Other resources for identification of duplicates include original field notes, files and notes of retired and current scientists, publications, and other historical documents. Annual clover duplication will be checked using historical documents from the Norman L. Taylor, Lexington, Kentucky Clover Collection, and tropical legume duplication will be checked using passport data from the Albert Kretschmer, Jr., University of Florida collection (Kretschmer and Wilson, 1996). Identification and confirmation of duplicate or redundant accessions will also be determined by genotyping suspected duplicates using a reference set of DNA markers. Evaluating all these resources, not only identifies duplicate or redundant accessions, but also provides additional passport and other data that can be put into GRIN-Global to provide better information for requestors. When any data is added or updated in GRIN-Global, the source of the information will be cited. For duplicates, a plant identifier is added to the duplicate accession record which says, “Duplicate of accession id” and the identifier type is set to “Duplicate”. The duplicate record is set to “Inactive” and the corresponding inventories are relinked to the primary accession. Accession action records are added to show when the accessions were checked for duplication, when the duplicate accession was set to inactive, and who checked the records for duplication. Every effort is made to document each addition or update made in GRIN-Global. By this procedure, duplicate
accessions are clearly identified in GRIN-Global and further regeneration will not be conducted on these duplicates.

**Objective 4 Methods continued:**
Sweetpotato clonal materials will be shipped to the user as small plantlets or in vitro cultures. One to three in vitro cultures of sweetpotato are typically provided per request depending on the needs of the user. In vitro cultures (semi-solid media) are packaged in boxed cardboard sleeves (25 x 150 mm culture tubes) or styrofoam packing material (liquid media). Vegetative material of other Ipomoea spp., perennial wild peanuts, and warm-season grass accessions which do not produce seed will be shipped as bare-rooted cuttings; wrapped in moist paper towels, foam root cubes, or moss; placed in plastic bags; and shipped in packing material within a cardboard box. Constant communication is maintained with the user so that if damage occurs in transit, arrangements for the replacement of damaged materials can be arranged quickly. Nursery certificates issued by the Georgia Department of Agriculture will be kept current to allow for legal domestic distribution of live plant material. Routine inspections of greenhouse facilities and bamboo field plots will be conducted twice a year by the Georgia Department of Agriculture to ensure compliance with the Imported Fire Ant Quarantine, Nematode Quarantine, Federal Japanese Beetle Quarantine, and North Carolina Tropical Spiderwort Quarantine. In addition, seed storage personnel will query Department of Agriculture websites for the destination state prior to shipment of material to determine if specific state import requirements limit the shipment of live plant material. When it is determined that live plant material cannot be distributed due to state-imposed import restrictions, the requestor will be contacted by the curator. Bamboo rhizomes will be distributed once a year in February to all requesters from the bamboo plots at the USDA, ARS, Byron, GA, location. Proper documentation (e.g. official fire ant stamp) showing legal compliance will be sent with each live plant shipment.