**PIII-TAMU-1 WORKPLAN**

*Increasing utilization of cowpeas to promote health and food security in Africa*

**Lead U.S. Principal Investigator**
Joseph Awika, Texas A&M University

**Collaborating Scientists**
Susanne Talcott, Texas A&M University, USA  
Lloyd Rooney, Texas A&M University, USA  
Bir Bahadur Singh, Texas A&M University, USA  
Chitundu Kasase, University of Zambia, Zambia  
John Shindano, University of Zambia, Zambia  
Kalaluka Lwangamunyinda - University of Zambia, Zambia  
Kennedy Muimui, Zambia Agriculture Research Institute (ZARI), Zambia  
Abdul Faraj, Egerton University, Kenya  
Prisca Tuitoek - Egerton University, Kenya  
Amanda Minnaar, University of Pretoria, South Africa  
Gyebi Duodu, University of Pretoria, South Africa

**Project Problem Statement and Justification**

Many poor families in Sub Saharan Africa suffer high rates of malnutrition, especially among children, while diet-related chronic diseases have become a common phenomenon among urban African populations. Moreover, evidence indicates that childhood malnutrition may lead to increased risk of chronic diseases, e.g., cancer in adulthood. In fact nutrition-related chronic diseases are becoming increasingly common in Africa, especially in urban areas, thus putting a large strain on the limited health infrastructure and imposing economic burden among the poor.

In Africa, malnutrition is closely linked to food insecurity, and thus the most vulnerable groups are those in marginal rainfall rural areas, and the urban poor. Grain pulses are an important source of protein for these vulnerable groups. Cowpea is one of the most drought tolerant crops and has a big potential as a food security crop for many poor African subsistence farmers. A strong and broad demand for cowpea is needed for the small scale farmers in the marginal areas to realize economic benefits of cowpea production.

A limited number of studies have also demonstrated that cowpeas have high antioxidant capacity, cholesterol-lowering properties as well as chemo preventive potential. Cowpeas thus may produce additional health benefits commonly associated with fruits and vegetables. However, information on how cowpea and its constituents may provide directly impact human health is lacking. Additionally, how variations in cowpea genetics affect their composition of potentially beneficial compounds is unknown. This makes it difficult to promote cowpea as a healthy grain which dampens its demand and utilization. *Accurate and credible information on how cowpea may influence human health is important as a primary step in promoting wide consumption of cowpeas.* This will also allow for breeding of varieties with improved health properties that target specific applications or markets.

*Constraints to consumption cowpeas*
The image of cowpea as a healthy food lags behind other commodities. Part of this is due to lack of scientific data on health and nutritional benefits of cowpea. In many parts of East and Southern Africa, the common perception that beans, cowpeas, and other pulses are ‘poor man’s food’ has also been a major impediment to broader consumption of these grains. Thus most of cowpea use is still restricted to the low income population. This leads to weak demand and depressed economic value of the crop, which in turn leads to limited incentive to invest in cowpea production and utilization infrastructure. Thus even in higher use regions like West Africa, demand for cowpea is showing declining trends, especially in urban areas. In the USA, lack of nutritional benefit information limits incentive to promote cowpea use as a mainstream part of diet.

Project Rationale
Reliable scientific evidence is essential to make educated dietary recommendations on type of cowpea, level of consumption, and design of food processing strategies that maximize the beneficial effects. The evidence will also provide a basis for genetic and agronomic improvement aimed at optimizing composition of beneficial compounds. Sound scientific evidence is essential for consumer buy in. It is a first step in transforming cowpea into a primary food to address malnutrition in poor populations, and promoting cowpea as a mainstream part of healthy diet. This will lead to increased demand for cowpea and improvement in economic wellbeing of producers and overall health of consumers.

Planned Project Activities

Objective 1: Identify cowpea lines with high content of health enhancing compounds and their relationship to seed color and other seed traits.

Collaborators
Donna Winham, Arizona State University Polytechnic, USA
Jeff Ehlers, University of California, Riverside, USA
Philip A. Roberts, University of California, Riverside, USA
Boukar Ousmane, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
Ruth Oniang’o, Rural Outreach Program (ROP), Nairobi, Kenya
David Macharia, Kenya Agricultural Research Institute (KARI), Katumani, Kenya
Davies Lungu, University of Zambia, Lusaka, Zambia

Approaches and Methods: The goal is to determine genetic variability in cowpeas for the types and levels of key bioactive components [flavonoids, triterpenoids, phenolic acid esters, phytate, soluble and insoluble dietary fiber], as well as protein content and quality. Association between these traits and seed color and seed characteristics will be determined.

Gross phenolic composition. Based on screening tests from Year 1, selected lines will be crossed and their progeny screened for gross phenolic profiles. The following analyses will be used for the screening: gross phenol content, anthocyanin pigments, and tannins content, ground samples will be extracted in 0.12 mol/L HCl in methanol. Anthocyanin pigment content will be measured by pH differential method, which is based on
measuring absorbance in pH 1.0 and pH 4.5 buffers at $l_{\text{max}}$ using a scanning UV-Vis spectrophotometer. The Folin-Ciocalteu method will be used to estimate gross phenols content, by measuring reactant absorbance at 600 nm using gallic acid as the standard. The vanillin-\text{HCl} method will be used for condensed tannin assay; reactant absorbance (with blank subtraction to correct for non-tannin pigments) will be measured at 500 nm, catechin will be used as standard. (\textit{Egerton University, University of Zambia, Texas A&M}) Detailed characterization of specific compounds will be done using the following methods:

\textit{Flavonoids and terpenoids profiling}. Sample extracts obtained as described above will be washed through a C-18 column to remove sugars and other non-flavonoid constituents. Flavonoids will be eluted using 70\% acidified methanol, rotoevaporated and reconstituted in 10\% methanol containing 10 mL/L formic acid and filtered through 0.45 mm membrane before analysis. For terpenoids, n-butanol extracts fractions will be characterized. A reversed phase C-18 column will be used for separation; and an Agilent 1200 HPLC system will be used for characterization. MS analysis will performed using a Thermo-Finnigan TSQ7000 triple-quadrupole mass spectrometer equipped with an API2 source, and an Electrospray Ionization (ESI) interface. (Texas A&M)

\textit{Phenolic acid and phenolate esters}. Free phenolic acids will be measured in methanol extract whereas alkaline hydrolysis of residue will be used to measure esterified phenolic acids. Reversed phase HPLC separation, with appropriate standards, will be used to identify the compounds; LC-MS will be used for structural determination when needed. (University of Zambia, University of Pretoria, Texas A&M)

\textit{Protein content and quality}. These tests will be conducted on elite cultivars selected for crossing, and their selected progeny. To obtain relevant data from this procedure, samples will initially be cooked by boiling in water for 75 min, and then drying at 45 – 50 °C. Protein content will be measured using the combustion method (AOAC Method 990.03). Complete amino acid profile will be measured using the AOAC method 982.30, whereas available lysine will be measured using the OAC Method 975.44. In vitro protein digestibility will be determined by pepsin/pancreatin digestion method. (University of Zambia, Egerton, University of Pretoria).

*Bold denotes lead institution that will be primarily responsible for analysis and coordination of data for specific activity.

**Objective 2:**

**Collaborators**
Donna Winham, Arizona State University Polytechnic, USA  
Ruth Oniang’o, Rural Outreach Program (ROP), Nairobi, Kenya  
Fredie Mubanga, Nutrition, Food and Nutrition Commission (NFNC), Lusaka, Zambia

**Approaches and Methods**: This will establish how the phytochemical profiles affect the ability of cowpeas to influence metabolic, cardiovascular and chemoprotective health predictors \textit{in vitro}. The F1 and F2 progeny will be screened for predictors of bioactivity using the following methods:
**Hydroxyl/free radical scavenging properties:** protection against oxidative stress is an important component of chronic disease prevention. Both lipophilic (hexanes extract) and hydrophilic (aqueous acetone extract) antioxidant capacity of cowpeas and their fractions will be measured by two widely accepted methods that involve hydrogen atom transfer (HAT) and single electron transfer (SET) that have been shown to correlate with biological oxidative status measures. Oxygen radical absorbance capacity (ORAC), will be the HAT method. Ability of cowpea extract to protect fluorescein from free radical attack by AAPH will be monitored for 90 min at 37°C using a fluorescence spectrophotometer (excitation 485 nm, emission 528 nm). The Trolox Equivalent Antioxidant Capacity (TEAC) will be used for SET assay. Samples will be reacted with preformed ABTS° free radical, and ability of the sample to quench the free radical measured after 30 min by monitoring color at 734 nm. Trolox will be used as standard in both assays. (Texas A&M)

From these tests, representative crosses will be selected and tested using the following methods:

**Bile acid-binding assay:** Increased bile acid excretion by binding to food components is one of the most important mechanisms by which food components lower cholesterol. The bile acid binding assay as described by Ma and Xiong (2009) will be used to characterize cowpea for potential cholesterol-lowering properties. Freeze-dried cowpea extracts will be dissolved in pH 6.3 sodium phosphate buffer and incubated at 37°C for 2 h with bile acid solutions (2 mM) (Sigma, St Louis, MO). The bile acid binding assay kit (Kit 450, Trinity Biotech, Berkeley Heights, NJ) used to colorimetrically estimate bile binding (530 nm). (Egerton)

**Inhibition of low density lipoprotein (LDL) oxidation:** Oxidation of LDL leads to impairment in the regulation of cholesterol uptake. This potentially leads to development of atherosclerosis and cardiovascular disease. The ability of extracts from the cowpea/bean varieties to inhibit LD oxidation will be determined using the method described by Puhl et al. (1994) by monitoring formation of conjugated dienes at 234 nm. (University of Pretoria)

**Glycemic properties:** Procedures described by Goni et al (1997) will be used to measure rate of in vitro starch hydrolysis in selected cowpea lines. Hydrolysis index and estimated glycemic index will be calculated from area under curve (30 min intervals to 180 min digestion) as detailed by the authors, using fresh white bread as a control. (University of Zambia)

**Cell culture assays:** Two strategies will be used to assess how cowpea compounds can protect against cancer and also cardiovascular disease, two major chronic diseases: Anti-cancer effects:

- **Phase II detoxifying enzyme assay.** This method is based on the fact that enhanced activity of enzymes that detoxify potential carcinogens will lead to prevention of cancer initiation. We will employ the NAD(P)H:quinone oxidoreductase (NQO) inducer activity as previously described (Yang et al. 2009). Murine hepatoma (Hepa 1c1c7) cells will be incubated with various concentrations of cowpea extracts and
NQO enzyme activity as well as cytotoxicity measured as described by Prochaska, et al. (1992). Sulforaphane will be used as a positive control; this compound is a potent natural phase II enzyme inducer. (Texas A&M)

- **Anti-proliferation assays.** These methods will measure how the various cowpea extracts affect growth of pre-formed cancer cells. We will use the widely studied HT-29 and Caco-2 human colon carcinoma cells for this assay following the viable cell (MTT) and DNA (PicoGreen) procedures as recently modified (Awika et al. 2009). Various concentrations of the cowpea extracts will be incubated with the cells for 48 hr. after which the MTT assay kit (Sigma, St Louis, MO) will be used to measure viable cell population by established protocols. Double stranded DNA will be measured using the PicoGreen Quant-iT assay kit (Invitrogen Inc., Carlsbad, CA) as described by Ahn et al (1996). Genistein will be used a positive control in both assays. *Apoptosis* will be assessed by analyzing in cells by analyzing PARP-cleavage as previously described (Chintharlapalli, Papineni et al. 2009) (University of Pretoria/Texas A&M)

**Cardiovascular Disease:** In order to determine the in vitro effects of total polyphenolic extracts and fractions from cowpea on biomarkers for antioxidant properties and inflammation in vascular endothelial cells (HUVEC). We will measure:

- **Biomarkers for inflammation:** nuclear factor kappa B (NFkB), interleukins IL-6, IL-8, tumor necrosis factor TNF-a and NF-kB will be determined by ELISA assays obtained from E-bioscience, San Diego, CA and Life Diagnostics, West Chester, PA, as previously performed (76, 77). These biomarkers are typically used to assess inflammation and cowpea extract is expected to decrease LPS-induced inflammation these cells. (Texas A&M)

- **Antioxidant biomarkers:** As previously performed (71), cells will be treated with different extract concentrations and antioxidant effects will be determined after different incubation times with the ORAC assay as well as the generation of reactive oxygen species (ROS). Additionally, oxidative stress will be induced with hydrogen-peroxide and the mitigation of pro-oxidant potential by different concentrations of cowpea extract will be assessed. Oxidative DNA damage will be assessed in the same manner; after the induction of DNA-damage with H2O2, the alleviating effects of cowpea will be assessed with the ApoAlertTM DNA Fragmentation Assay (BD Biosciences) according to the manufacturer’s protocol. (University of Pretoria/Texas A&M)

*Bold denotes lead institution that will be primarily responsible for analysis and coordination of data for specific activity.

**Objective 3:** Elucidate the mode of inheritance (heritability) of selected bioactive traits in cowpea and genetic association between physical and bioactive traits.

**Collaborators**
Creighton Miller, Texas A&M University, USA  
Jeff Ehlers, University of California, Riverside, USA  
Philip A. Roberts, University of California, Riverside, USA  
Boukar Ousmane, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria  
Davies Lungu, University of Zambia, Lusaka, Zambia
**Approaches and Methods:** This objective will help determine the mode of inheritance and the extent of genetic associations of key bioactive traits in cowpea. This will open opportunities for genetic selection and improvement efforts as well as using modern molecular techniques to develop specific specialty cowpea lines for targeted health benefits.

We will continue genetic studies involving parents, F1s, F2s, and backcrosses. F3 seed will be obtained, F2 seeds will be backcrossed during this period. Crosses will also be made among popular national varieties in Zambia with lines identified to have high levels of phytochemicals and raise F1s. *(Texas A&M/University of Zambia)*

**Objective 4:** Establish strong linkages with HC policymakers and other stakeholders, and develop outreach strategies that will lead to long term increase in cowpea consumption for health and food security.

**Collaborators**
Gary J. Wingenbach, Texas A&M University, USA
Ruth Oniang’o, Rural Outreach Program (ROP), Nairobi, Kenya
Stephen Muliokela, Golden Valley Agricultural Research Trust (GART), Zambia
Paul Kapotwe, Program Against Malnutrition (PAM), Zambia
Fredie Mubanga, Nutrition, Food and Nutrition Commission (NFNC), Lusaka, Zambia

**Approaches and Methods:** Each HC PIs in collaboration with US PIs will organize a 1 day training workshop for key stakeholder representatives from the government ministries and research institutions, local NGOs and women organizations. The US PI will participate in the workshops. The aim of the workshops will be threefold; 1) Train the stakeholder representatives on how to interpret nutrition research findings and accurately convey the message to a lay audience, and also use it to influence policymakers, 2) Integrate preliminary findings into dissemination strategies developed in the first year, and 3) Establish networks among stakeholder and researchers that will lead to long term collaborations and development efforts. It is anticipated that each HC workshop will involve 15-20 stakeholder representatives. HC education and extension specialists will be engaged as consultants. The HC USAID country representatives will be invited to these workshops. From the workshops, a training manual and brochure for use by stakeholder representatives will be developed in Year 3. *(University of Zambia/Egerton)*

**Objective 5:** Strengthen cowpea nutrition research in Kenya and Zambia

**Collaborators**
Gary J. Wingenbach, Texas A&M University, USA
Donna Winham, Arizona State University Polytechnic, USA
Fredie Mubanga, Nutrition, Food and Nutrition Commission, Zambia

**Approaches and Methods:** Human capital development and strengthened host country research institutions are keystones to sustainable development and income generation. Elevating nutrition research profile through capacity building is especially critical to enable the nutritionists to influence policy and programs that will lead to development...
outcomes. Through Prof. A. Minnaar and her colleagues at the University of Pretoria (Dr. K.G. Duodu and Prof A. Oelofse), we will work closely with lead investigators in HC to provide training on cowpea health benefits and design public education material to promote cowpea as a healthy and nutritious food crop. University of Pretoria has exceptional facilities and is educating a large number of young scientists from Sub-Saharan Africa.

**Graduate training (long term)**

Each of the two HC graduate students will continue their training at University of Pretoria, South Africa. The two HC graduate trainees will spend 5 months at Texas A&M (July – November) for research and training using advanced instruments and techniques not readily available in Africa. They will conduct research on specific aspects of Objective 2 while at Texas A&M, while also learning other techniques, like purification and chemical characterization by LC-MS\(^n\) and NMR techniques. The students will also have an opportunity to establish relationships and networks with international researchers that will help them succeed in their careers. They will be expected to participate in a national scientific conference while at Texas A&M.

**Short term training**

Each HC PIs in collaboration with US PIs will organize a 1 day training workshop for key stakeholder representatives from the government ministries and research institutions, local NGOs and women organizations. The US PI will participate in the workshops. The aim of the workshops will be threefold; 1) Train the stakeholder representatives on how to interpret nutrition research findings and accurately convey the message to a lay audience, and also use it to influence policymakers, 2) Integrate preliminary findings into dissemination strategies developed in the first year, and 3) Establish networks among stakeholder and researchers that will lead to long term collaborations and development efforts. It is anticipated that each HC workshop will involve 15-20 *stakeholder representatives*. HC education and extension specialists will be engaged as consultants.

**Contribution of Project to Target USAID Performance Indicators**

Degree training – 3 PhD students (2 directly benefiting HC research capacity)
Short term training – 30-40 stakeholders training via workshops in HC
New nutrition research techniques will be available to the HC institutions
Host country partner institutions will directly benefit: University of Zambia, Zambia Agriculture Research Institute, Egerton University

**Target Outputs**

Associations between phenotype, chemical composition and bioactive properties determined.
Ability of elite cowpea cultivars to influence cancer initiation and growth determined.
Ability of elite cowpea lines to regulatory inflammation established.
Ability of elite cowpea lines to influence cardiovascular markers established.
F2 seeds obtained, filed crosses performed in Zambia and at TAMU.
F1 and F2 seeds characterized for heritability of key bioactive traits.
30-40 stakeholders from HC trained on nutritional/health benefits of cowpea and the role cowpea can play in ensuring food security and overall health.
Three graduate student trainees make progress towards graduation (2 from HC at University of Pretoria and 1 at Texas A&M).
Research findings published in scientific journals – at least 2.

Engagement of USAID Field Mission(s)
Both the US and HC PIs have communicated with the HC USAID Missions and they have expressed their support for the project. The US and corresponding HC PI plan to meet with the USAID Mission representatives in Zambia and Kenya during the US PI visits to the host country projects. We will discuss project goal and approaches in detail with the Mission representative and seek their input in fine tuning approaches if necessary to achieve maximum impact. We will also seek their input in leveraging other resources locally and internationally to improve overall project success and impact. Networking opportunities with key stakeholders will also be discussed with the country Mission Representatives.

Networking Activities with Stakeholders
In Zambia, we will meet with the head of Legume Program at Zambia Agriculture Research Institute (Dr. Kennedy Kanenga), along with Dr D. M. Lungu of University of Zambia to discuss progress and seek input on future efforts. We will also meet with representatives from the Food and Nutrition Commission (NFNC) in the Ministry of Health, and Program Against Malnutrition (PAM) (an NGO involved in community nutrition based interventions) to discuss the long term project goal and plan outreach strategies that would be locally suitable to influence policymakers and benefit vulnerable groups. The training workshop will also provide an opportunity to network with stakeholders.

In Kenya, we will continue to work Kenya Agricultural Research Institute (KARI) at Katumani to include local lines that meet nutritional quality criteria in local field testing. We will also meet with local government representatives from Ministries of Education, Public Health, and Agriculture, and discuss project progress and opportunities for future efforts, as well as strategies to disseminate findings. We will especially discuss with the stakeholders strategies to use the findings to develop nutrition-based interventions that can produce broad impact. We will continue involving local NGOs representatives, like Peter Mwangi of World Vision International, and Ruth Oniang’o of Rural Outreach Program. The training workshop will also provide an opportunity to network with stakeholders.

Leveraging of CRSP Resources
We plan to use data from this work to seek additional funding from NIH National Cancer Institute, American Institute for Cancer Research, and USDA-AFRI programs, as well as other international organizations like the Bill and Melinda Gates Foundation, and the McKnight Foundation.
Training/Capacity Building Workplan Format

Degree Training:

PhD Student 1:
First and Other Given Names: Twambo
Last Name: Hachibamba
Citizenship: Zambia
Gender: Female
Training Institution: University of Pretoria
Supervising CRSP PI: Amanda Minnaar, Gyebi Duodu
Degree Program for training: PhD
Program Areas or Discipline: Food Science and nutrition
If enrolled at a US university, will Trainee be a “Participant Trainee” as defined by USAID?
Host Country Institution to Benefit from Training: University of Zambia
Thesis Title/Research Area: TBD
Start Date: June 2010
Projected Completion Date: 2013*
Training status (Active, completed, pending, discontinued or delayed): Pending
Type of CRSP Support (full, partial or indirect) for training activity: Full

PhD Student 2:
First and Other Given Names: Alice
Last Name: Nderitu
Citizenship: Kenya
Gender: Female
Training Institution: University of Pretoria
Supervising CRSP PI: Amanda Minnaar, Gyebi, Duodu
Degree Program for training: PhD
Program Areas or Discipline: Food Science and nutrition
If enrolled at a US university, will Trainee be a “Participant Trainee” as defined by USAID?
Host Country Institution to Benefit from Training: Egerton University
Thesis Title/Research Area: TBD
Start Date: June 2010
Projected Completion Date: 2013*
Training status (Active, completed, pending, discontinued or delayed): Pending
Type of CRSP Support (full, partial or indirect) for training activity: Full

PhD Student 3:
First and Other Given Names: TBD
Last Name: TBD
Citizenship: TBD
Gender: TBD
Training Institution: Texas A&M University
Supervising CRSP PI: Joseph Awika, Susanne Talcott
Degree Program for training: PhD
Program Areas or Discipline: Nutrition and Food Science
If enrolled at a US university, will Trainee be a “Participant Trainee” as defined by USAID?
Host Country Institution to Benefit from Training: TBD
Thesis Title/Research Area: TBD
Start Date: August 2009
Projected Completion Date: Dec 2012
Training status (Active, completed, pending, discontinued or delayed): Pending
Type of CRSP Support (full, partial or indirect) for training activity: Partial
**Short-term Training:**
Type of training: Workshops
Description of training activity: Stakeholders will be invited to 2 day workshops on how to interpret nutrition research findings and accurately convey the message to a lay audience, and also use it to influence policymakers.
Location: University of Zambia; Egerton University, Kenya
Duration: 1 day
When will it occur: June-July 2011
Participants/Beneficiaries of Training Activity: Government representatives, NGO representatives, community leaders, research center representatives.
Anticipated numbers of Beneficiaries (male and female): 15 male, 15 female
PI/Collaborator responsible for this training activity: Chitundu Kasase, Abdul Faraj, Joseph Awika
List other funding sources that will be sought (if any): N/A
Training justification: 1) Train the stakeholder representatives on how to interpret nutrition research findings and accurately convey the message to a lay audience, and also use it to influence policymakers, 2) Integrate preliminary findings into dissemination strategies developed in the first year, and 3) Establish networks among stakeholder and researchers that will lead to long term collaborations and development efforts.
*Extension will likely be requested due to delayed project start date.*

**Equipment** (costing >$5,000): N/A
TMAC EVALUATIONS AND RECOMMENDATIONS
PLUS PI RESPONSES

Phase III Project: PIII-TAMU-1, *Increasing utilization of cowpeas to promote health and food security in Africa*

Lead U.S. PI: Dr. Joseph Awika, Texas A&M University

A. Comments regarding Project Performance
   1. TMAC views this as an important project for the Pulse CRSP.
   2. TMAC has some concerns about the genetic component of this project. The TMAC recommends that the project first obtain data on the nutritional parameters from the phenotyping of the parental cowpea lines, before extending out to breeding and extension. Phenotyping identifies what are the positive factors to be selected for in a breeding program.

   **PI Comment:** Based on phenotyping screening of available germplasm, parent lines with high and low levels of markers for bioactivity would be selected for the genetic and breeding activities.

   3. Generational means analysis has methodological flaws.

      **PI Comment:** Genetic populations comprising P1, P2, F1, BC1P1, PC1P2, and F2 will be generated and evaluated for the desired traits. The data will be analyzed using classical genetic method if the segregation is of qualitative nature and using generation mean analysis if the data show quantitative variations. The estimates of number of genes and heritability would be determined.

   4. The balance of expenses between expenditure lines suggests little support for the graduate research activities in terms of supplies and labor for the laboratory work.

B. TMAC Recommendations and PI Responses

The TMAC recommends the approval of the FY11 work plan and budget contingent upon satisfactory responses in writing to the following recommendations.

1. Recombinant inbred lines should be considered as an option for the breeding program.

   **Response:** Using the F2 populations, recombinant inbred lines would be developed through advancing the generations by single seed descent method and the RILs would be evaluated after F6-F8 generations and used in future breeding program if financial resources are secured.
2. Adequate resources for supplies and laboratory staff for graduate studies need to be identified, either within the project or from leveraged resources.

Response:
In the case of training the two PhD host country candidates, Ms. Alice Nderitu (from Egerton, University, Kenya) and Mrs. Twambo Hachibamba (from University of Zambia) at the University of Pretoria, South Africa, all costs (including tuition, stipends, medical insurance and actual research costs) are covered by the Degree Training component of the budget. Please refer to the table below for more details:

The funds requested per student per year therefore include tuition, accommodation, living allowance, and medical insurance as detailed below, as well as the actual costs for doing the research in the laboratories. The HC trainees will also spend 5 months at TAMU as explained under non-degree training component in FY011 in TAMU’s budget. During this time, the expenses of the students and their research will be covered by TAMU. The necessary allowances have been made in the budget.

<table>
<thead>
<tr>
<th>Cost per HC student at University of Pretoria</th>
<th>Y1 ($)</th>
<th>Y2 ($)</th>
<th>Y3 ($)</th>
<th>Total $</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuition and research fee*</td>
<td>6,930</td>
<td>5,123</td>
<td>4,571</td>
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<td>Medical Insurance</td>
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<td>1,184</td>
<td>1,302</td>
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<td>Living expenses</td>
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<td>8,634</td>
<td>10,971</td>
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<td>Total $</td>
<td>18,976</td>
<td>14,941</td>
<td>16,844</td>
<td>50,761</td>
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</table>

3. The PI needs to better explain, with relevant literature, why the in vitro assays are a clear indication of the compounds that would have positive health benefits. For example, could harmful compounds not also induce phase II detoxification enzymes?

Response:
Modulation of levels of biotransformation enzymes that facilitate the elimination of endogenous and environmental carcinogens is widely recognized as one of the successful strategies to retard or block carcinogenesis (Yu and Kensler, 2005). Induction of detoxification and antioxidant enzymes through the activation of intracellular signaling mediated by NF-E2-related factor 2 (Nrf2) is believed to be a central mechanism by which bioactive agents impart their chemopreventive activity (Lee and Surh 2005; Yu and Kensler 2005). Hence phase II enzyme activity induction is a good indicator of protection of animal cells against carcinogens and oxidant toxicity (Gao et al. 2006). Quinone reductase (QR) is a widely distributed enzyme that protects cells against toxicity of quinones and their metabolic precursors by promoting reduction of quinones to hydroquinones which are then susceptible to
conjugation; this enzyme is typically co-induced with other phase II electrophile-processing enzymes (Prochaska and Santamaria 1988). The unifying mechanism for chemoprotective effects of a variety of unrelated compounds in vivo has been shown to be induction of phase II enzymes (Wattenberg 1985).

Measuring QR activity in Hepa 1c1c7 murine hepatoma cell lines is a convenient, reliable and rapid index of phase II enzyme inducer activity (Zhang et al. 1992). An additional advantage of these cell lines is that bifunctional inducers (that induce both phase I and phase II activity) can be readily distinguished from monofunctional inducers (selectively induce phase II activity) using available aryl hydrocarbon (Ah) receptor defective mutant lines. This is important, since a balance between phase I (which can activate carcinogens) and the phase II enzyme activity is essential for regulating neoplasia (Prochaska et al. 1992).

References


Dry Grain Pulses CRSP
Research, Training and Outreach Workplans
(October 1, 2010 -- September 30, 2011)

FY 2011 PERFORMANCE INDICATORS
for Foreign Assistance Framework and the Initiative to End Hunger in Africa (IEHA)

Project Title: Increasing utilization of cowpeas to promote health and food security in Africa
Lead U.S. PI and University: Joseph Awika, Texas A&M University
Host Country(s): Zambia, Kenya

<table>
<thead>
<tr>
<th>Output Indicators</th>
<th>2011 Target</th>
<th>2011 Actual</th>
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<tbody>
<tr>
<td></td>
<td>(October 1, 2010-Sept 30, 2011)</td>
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<tr>
<td>Degree Training: Number of individuals enrolled in degree training</td>
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<tr>
<td>Number of women</td>
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<td>Number of men</td>
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<td></td>
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<tr>
<td>Short-term Training: Number of individuals who received short-term training</td>
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<td>Number of men</td>
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<td>Number of technologies and management practices made available for transfer</td>
<td>0</td>
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<tr>
<td>Number of policy studies undertaken</td>
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<tr>
<td>Beneficiaries:</td>
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<tr>
<td>Number of rural households benefiting directly</td>
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<tr>
<td>Number of agricultural firms/enterprises benefiting</td>
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</tr>
<tr>
<td>Number of producer and/or community-based organizations receiving technical assistance</td>
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<tr>
<td>Number of women organizations receiving technical assistance</td>
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<tr>
<td>Number of HC partner organizations/institutions benefiting</td>
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<tr>
<td>Developmental outcomes:</td>
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<tr>
<td>Number of additional hectares under improved technologies or management practices</td>
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## Dry Grain Pulses CRSP FY 2011 Workplans

### Dry Grain Pulses CRSP FY2011 Budget

**Increasing utilization of cowpeas to promote health and food security in Africa**

<table>
<thead>
<tr>
<th>Institution Name</th>
<th>U.S. Institution</th>
<th>U.S. for Host Country</th>
<th>HC or U.S. Institution (1)</th>
<th>HC or U.S. Institution (2)</th>
<th>HC or U.S. Institution (3)</th>
<th>HC or U.S. Institution (4)</th>
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<tbody>
<tr>
<td>Texas A&amp;M</td>
<td>Texas A&amp;M</td>
<td>ZAMBIA</td>
<td>KENYA</td>
<td>S. AFRICA</td>
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<tr>
<td>a. Personnel Cost</td>
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<tr>
<td>Salaries</td>
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<td>Fringe Benefits</td>
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<td>b. Travel</td>
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<td>$1,100.00</td>
<td>$500.00</td>
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<tr>
<td>c. Equipment ($5000 Plus)</td>
<td>$28,045.00</td>
<td>$17,300.00</td>
<td>$10,400.00</td>
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<td>d. Supplies</td>
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<td>e. Training</td>
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<td>Non-Degree</td>
<td>$3,900.00</td>
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<td>f. Other</td>
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<td>g. Total Direct Cost</td>
<td>$79,305.00</td>
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<td>$22,500.00</td>
<td>$12,790.00</td>
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<tr>
<td>h. Indirect Cost</td>
<td>$32,804.00</td>
<td>$12,555.00</td>
<td>$3,375.00</td>
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<td>i. Indirect Cost on Subcontracts (First $25000)</td>
<td>$6,153.00</td>
<td>$12,555.00</td>
<td>$3,375.00</td>
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<tr>
<td>j. Total Indirect Cost</td>
<td>$38,957.00</td>
<td>$12,555.00</td>
<td>$3,375.00</td>
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<td>Total</td>
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<td>$25,875.00</td>
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**Cost Share**

<table>
<thead>
<tr>
<th>Cost Share</th>
<th>U.S. Institution</th>
<th>U.S. for Host Country</th>
<th>HC or U.S. Institution (1)</th>
<th>HC or U.S. Institution (2)</th>
<th>HC or U.S. Institution (3)</th>
<th>HC or U.S. Institution (4)</th>
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<tr>
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<td>$ -</td>
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### Attribution to Capacity Building

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<thead>
<tr>
<th>Percentage of effort</th>
<th>U.S Institution (1)</th>
<th>U.S Institution (2)</th>
<th>U.S Institution (3)</th>
<th>U.S Institution (4)</th>
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<tr>
<td>100.00%</td>
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<td>$7,354.25</td>
<td>$29,045.19</td>
<td>$88,886.94</td>
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*U.S Institution PI: Joseph Awika*
### FY 2011 SEMI-ANNUAL INDICATORS OF PROGRESS BY INSTITUTIONS AND TIME PERIOD

**Project Title:**

Increasing utilization of cowpeas to promote health and food security in Africa

<table>
<thead>
<tr>
<th>Objective</th>
<th>Phytates</th>
<th>Protein content</th>
<th>Protein quality and digestibility</th>
<th>Screening backcrosses:</th>
<th>Phenolic tests</th>
<th>SET and HAT</th>
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<table>
<thead>
<tr>
<th>Objective 2</th>
<th>Bile acid binding assay</th>
<th>Inhibition of LDL oxidation</th>
<th>Glycemic Index</th>
<th>Phase II</th>
<th>Anti-proliferation</th>
<th>Biomarkers for inflammation</th>
<th>Antioxidant biomarkers</th>
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<tr>
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<table>
<thead>
<tr>
<th>Objective 3</th>
<th>Perform relevant crosses</th>
<th>Obtain F3 seed</th>
<th>Conduct field trials</th>
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<table>
<thead>
<tr>
<th>Objective 4</th>
<th>HC stakeholder workshops</th>
<th>Graduate training (PhD)</th>
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**Abbreviated name of institutions**

- TAMU
- UNZA
- EGER
- UP

<table>
<thead>
<tr>
<th>Identify Benchmark</th>
<th>TAMU</th>
<th>UNZA</th>
<th>EGER</th>
<th>UP</th>
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<tr>
<td>9/30/11</td>
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</tr>
</tbody>
</table>

**Name of the PI responsible for reporting on benchmarks**

| J Awika | C Kasase | A Faraj | A Minnaar |

**Signature/Initials:**

__________________________

**Date:**

__________________________