USDA's Foreign Agricultural Service (FAS) is seeking to identify U.S. universities willing to host English speaking agricultural scientists from Malawi, Mozambique, South Africa and Zambia under the Norman E. Borlaug International Agricultural Science and Technology Fellowship Program (Borlaug Fellowship Program). These Fellows have been selected competitively based on research priorities, academic and work accomplishments, commitment to Borlaug Fellowship goals and leadership qualities. It is recommended that the program begin in September 2011, however, priority should be given to a time that is appropriate for the Fellow's proposed topic of research. The program should last for a period of 8-12 weeks.

Each Fellow has specific research topic interests. Please find below a brief description of the Fellows' research/fellowship interest, educational and research background, and program goals. Click on the hyperlink for more details regarding each Fellow's expressed interest as indicated in his/her proposed action plan.

- 1. <u>Fellow #1</u> (male-Malawi)
 - Background: Master of Science in Horticulture. Principal Research Scientist at the Department of Agricultural Research Services.
 - Main Objective: receive training in the molecular characterization of cashew nut trees; investigating the genetic diversity using AFLPs.
- 2. <u>Fellow #2</u> (female-South Africa)
 - Background: Master of Science in Genetics. Currently working as a Researcher at the Agricultural Research Council.
 - Main Objective: detect quantitative traits loci that have effect on tick resistance, growth rate, post weaning growth, carcass composition, meat quality in beef cattle using SNPs marker in an F2 design experiment.
- 3. <u>Fellow #3</u> (male-Zambia)
 - Background: Master of Science in Agronomy. Senior Seeds Officer at Ministry of Agriculture working on plant variety registration and testing.
 - Main Objective: assess the genetic variability of maize populations and elite germplasm.
- 4. <u>Fellow #4</u> (male-Mozambique)
 - Background: MSc in Plant Protection. Head of Plant of Quarantine department at Ministry of Agriculture.
 - Main Objective: obtain skills and knowledge and ensure the implementation of SPS/WTO agreement on trade.
- 5. <u>Fellow #5</u> (female-Zambia)
 - Background: Ph.D. in Horticulture and Master of Science in Environmental Science. Lecturer at University of Zambia teaching soil microbiology, soil physics and soil conservation.

• Main Objective: enhance phosphorus solubilization by use of soil microbiota to improve crop productivity.

Institutions may submit proposals to host more than one Fellow. Institutions interested in hosting one or more Fellows should submit a proposal following the guidelines below:

- Indicate the name of the institution and mentor applying to host the Fellow(s);
- Indicate the country, research interests and reference number of each Fellow;
- Provide a tentative action plan based on the Fellow's research proposal and action plan, including topics covered, field visits and other activities;
- Provide a summary of relevant institutional capabilities for hosting international scientists and policymakers in the proposed field;
- Briefly describe the research expertise and international experience of the mentor in the Fellow's field of interest;
- A 1-2 page curriculum vitae should be provided for mentors and other collaborating researchers involved in the proposed program. This is not included in the page count provided maximum noted below.
- Identify the expected skills or knowledge to be acquired by the Fellow at the end of the program;
- Complete a budget based on the attached template with budget notes justifying the budget. If attendance at the World Food Prize in Des Moines, Iowa in October, 2011 is feasible, the budget should include time and funding for the Fellow to attend;
- Complete the following checklist on university administrative policies;
- Include all components of the proposal in a single PDF document, and;
- Proposal, excluding the budget, should not exceed 3-4 pages. If more than one Fellow is requested, an additional two pages per fellow is permitted.

Please submit the proposal, university administrative checklist and estimated budget via email to: Natasha Acheampong at <u>Natasha.Acheampong@fas.usda.gov</u> or Karen Uetrecht at <u>Karen.Uetrecht@fas.usda.gov</u>. FAS would like to receive all expressions of interest by June 6, 2011.

Funding support will be provided through USDA as part of the Borlaug Fellowship Program. For more information on the Borlaug Program, please visit our website at: http://www.fas.usda.gov/icd/borlaug/Borlaug.asp.

The Norman E. Borlaug International Agricultural Science and Technology Fellowship Program aims to promote food security and economic growth by increasing scientific knowledge and collaborative research to improve agricultural productivity. This program targets promising young scientists and policymakers from developing or middle income countries. The Fellows spend 8-12 weeks in the United States and work one-on-one with a U.S. scientist in their field. The mentor coordinates the Fellow's training and in order to continue collaborative efforts, visits the Fellow's host country for 7-10 days within 6-12 months after completion of the training in the U.S.

During the program, the Fellows learn new research techniques, gain exposure to the latest scientific developments in various fields of agriculture, access fully-equipped laboratories and libraries, and learn about unique public-private partnerships that help fund agricultural research and science. Equally important, this program provides international scientists and policymakers with opportunities to establish long-term contacts with U.S. scientists and apply newly gained knowledge from U.S. institutions to their country's research and development programs.

The World Food Prize is awarded annually during the Norman E. Borlaug International Symposium in Des Moines, Iowa. This year the World Food Prize is scheduled for October 12-14, 2011. The USDA Borlaug Fellowship Program organizes a side-event each year which includes activities for Borlaug Fellows that provide important networking opportunities for Fellows and international agricultural researchers, policy makers and the non-profit sector. The following link provides more information about the World Food Prize Borlaug Dialogue: <u>http://www.worldfoodprize.org/index.cfm?nodeID=25286</u>.

Host University Administrative Checklist

Please fill out the following checklist concerning the university's policies on the administrative aspects of hosting a fellowship.

| Host University Policies | YES | NO |
|--|-----|----|
| Will all mentors listed in the proposal be present for the majority of the | | |
| fellowship? | | |
| Will the university be able to provide per diem within the first week of the | | |
| Fellow's arrival? | | |
| Will the university be able to provide fully furnished lodging with kitchen | | |
| facilities? | | |
| Does the university tax participants' per diem and housing (if so, please | | |
| include in the budget)? | | |



Fellow #1 (male-Malawi)

MOLECULAR CHARACTERIZATION OF SELECTED CASHEW NUT TREES IN THE FIVE POPULATIONS IN MALAWI

The cashew nut (*Anacardium occidentale*) belongs to the Anacardiaceae family of plants, which also include the mango, the pistachio and the poison ivy. The tree is native to the coastal parts of north eastern Brazil but has spread to the other parts of tropical south central America, Mexico, West Indies and introduced to Africa and India by the Portuguese traders (Azam-Ali and Judge, 2000). Cashew provides food, employment, income, wood which is used for carpentry, firewood, and shade. The tree has other uses which include reforestation and buffering roadside. The world production of edible nuts is around 2,082,101 metric tones produced in 32 countries with insignificant contribution by Malawi (MCEI, 2005). The Malawi cashew industry dates back to 50 years. The majority of cashew trees in the orchards (about 3, 000ha) are of seedling origin and hence low yields (Kachule *et al.*, 1998). Cashew is freely marketed in the country and the biggest consumer is Air Malawi and retail shops and the price per kilogram of processed kernels is above the prices of other export crops.

A reliable method for plant DNA isolation is required for polymerase chain reaction analysis in plant breeding, plant gene mapping and plant genetic diversity. Cashews have high amount of secondary metabolites, orthohydroxyphenols and polysaccharides (Rout *et al.*, 2003), which are powerful fuloxidising agents to interfere with genomic DNA. Some varieties are recalcitrant to inhibit the Polymerase Chain Reaction (PCR) amplification.

The AFLP Assay

Amplified Fragment Length Polymorphism (AFLPs) are generated by complete restriction endonuclease digestion of total genomic DNA, followed by selective PCR amplification and electrophoresis of a subset of the fragments, resulting in a unique, reproducible fingerprint for each individual (Meudt and Clarke, 2007). The markers that make up the fingerprint, although often concentrated in centromeric regions, are widely distributed throughout the genome.

AFLP analysis has several advantages over other molecular techniques, one of which is high multiplex ratio (Meudt and Clarke, 2007). Fluorescent labeling and automated fragment detection method of AFLP offers significant improvements over the radiolabeling method by improving the scoring accuracy and safety. The large numbers of AFLP fragments generated translate that AFLPs can out-perform microsatellites for discriminating taxa and populations.

DNA Markers

Scientists (Karp *et al.*, 1996; Kumar, 1999) have demonstrated that DNA markers were until now the most promising technique used to differentiate among genotypes at species and subspecies level. DNA genome provides a significantly more powerful source of genetic polymorphism (Beckmann and Soller, 1986). They allow direct comparison of genetic diversity to be made at the DNA level, have the potential to identify a large number of polymorphic loci with an excellent coverage of an entire genome, are phenotypically neutral, allow scoring of plants at any developmental stage and are not modified by environment and management practices (Messmer *et al.*, 1993). In this

experiment ninety cashew trees will be investigated for their genetic similarity using AFLP markers.

Problem Statement and Justification

Information on cashew nuts genetic diversity is lacking in Malawi. Cashew industry in Malawi lack high yielding planting materials that will produce nuts of high quality. Verification of cashew morphological characterization results by Chipojola *et al.* (2009) is paramount.

Objective

To evaluate genetic diversity of cashew nut populations using AFLPs (molecular markers)

Null Hypothesis

There are no genetic differences in the populations of cashew nuts in Malawi.

Materials and Methods

DNA Isolation

The week prior to departure semi-mature healthy leaves from ninty accessions idntified by Chipojola *et al.* (2009) will be collected, preserved and transported to USA. The five populations of cashew will include Karonga in addition to the othe four used by Chipojola *et al.* (2009). DNA extraction as described by Vos *et al.* (1995) will be conducted in a laboratory in USA. Restriction digestion of genomic DNA; ligation of adapters; pre-amplification using primers and selective amplification will be executed using primers pairs. The gel will be visualized and photographed under UV light.

Data Evaluation

Consistently reproducible amplified DNA fragments will be transformed into binary character matrix (1=presence, 0=absence). Genetic variation within and among populations will be analysed on the basis of banding profile using polymorphism, diversity among populations, fixation index, and genetic distance using Nei Li index (Nei, 1978). The Dendogram will be constructed by statistical program NTSYS using the UPGMA (Un –weighted pair group means average0 cluster analysis).

Fellow #2 (female-South Africa)

The goal of the study is to identify quantitative traits loci (QTL) that affect tick resistance, post-weaning growth rate and feed efficiency, carcass and meat quality traits using an AngusxNguni F2 design in beef cattle

Objectives

The primary objective of the current study is to: • detect QTLs that affect tick resistance, post-weaning growth rate and feed efficiency, carcass and meat quality traits using SNPs markers

The secondary objectives of the study are to:

 evaluate growth performance, carcass yield, stress adaptability and meat quality traits of F2 progeny finished under intensive feedlot conditions

 determine colour and shelf life characteristics of meat produced from AngusxNguni F2 progeny Experimental design and breeds involved

An F2 design method has implemented in the current study in creating a resource population for QTL detection. The F2 design was chosen to allow for the estimation of both the additive and the dominance effects. The Angus and Nguni cattle were used as parental breeds. The main objective of the current study is to search for QTL for resistance to ticks and therefore the Nguni was an obvious choice. The other trait group considered in the current is carcass traits and thus the Angus was found to be a suitable breed since it is known for good carcass quality. The Angus was also preferred since it is known to be susceptible to ticks. Two Angus bulls and 2570 Nguni cows was bred and will also be bred again in 2011 for two consecutive years and their F1 progeny will in turn be bred with the hope of producing 250-300 F2 progenies.

Statistical analyses

Least squares method will be used for QTL analysis (Haley et al., 1994). The least squares analysis allows performing data permutation to determine genome-wide significance thresholds. The standard errors and confidence intervals will be obtained via bootstrap sampling technique.

Outcomes of the research

Successful completion this project will give a good opportunity to enhance the methodology of animal breeding and improve livestock production via South African indigenous breeds for economic important traits.

Issues

This fellowship will be used to pave a way by attending training on SNPS markers, gene expression, QTL statistical methods, and QTL design methods

Work plan:

First week will includes introduction course focus on the background of Single Nucleotide polymorphism (SNPs) in livestock production

Second and third week will include training in SNPs applications, design, methodologies

Forth to eight week will includes training in SNPs analysis in QTL experimental design

Nine to Ten week will be data interpretation and analysis

Eleven to Twelve week : general analysis on real time PCR looking at expression level of fatty acid genes in beef cattle

Fellow #3 (male- Zambia)

BACKGROUND

Zambia has a challenge of developing maize genotypes that are tolerance to abiotic and biotic stresses. Low nitrogen and drought has been cited as one of the major constraints of maize production among the smallholder farmers. In Zambia, low pH and aluminium toxicity are also known to limit maize production in the high rainfall areas. Therefore, developing maize genotypes tolerant to these stresses would be key to sustain maize production in challenging environmental conditions of Zambia.

Hence, the availability of genetic diversity will be essential to achieve significant genetic progress for generating high yielding maize genotypes adapted to marginal areas. Genetic resources have been linked to efficient cultivar development and thus they should be utilised efficiently. One of the ways of exploiting the genetic resources is by assessing the genetic variability of germplasm using molecular markers. Knowledge on the genetic variability of germplasm will strengthen the link between applied breeding programs and efforts of germplasm conservation thereby leading to its improvement and utilization.

Problem statement

Recent studies have indicated that the genetic base of maize has been narrowing due to the breeding methods employed. Germplasm with a narrow genetic base is vulnerable to pests and disease, apart from reduced genetic progress. Several causes have been attributed to this of which recycling of inbred lines is one of the key causes. In developing countries like Zambia, where funding for agricultural research and development has been reducing, the situation can be worse. It is for this reason that genetic evaluation studies are being done to identify genetically diverse germplasm that are trafts specific for stress tolerance. This information enhances plant breeding and maintains genetic diversity. In Zambia, there is little information available on the diversity of germplasm as well as inbredies.

Therefore, the purpose of the proposal will be to assess the genetic variability of local germplasm and elite germplasm. The major objective of the proposal will be to contribute to the breeding of genetically enhanced maize hybrids by generating baseline information on local germplasm. The specific objectives are:

(i) Study the molecular diversity within and among maize germplasm

(ii) To investigate the amount of genetic diversity captured by maize inbrediines combared to that in the populations.

(iii) To determine the distribution of genetic variation within and among agro-ecological regions of Zambia
 (iv) Identify mini cores and cores for germplasm conservation and population development
 (v) Compare 55R and SNP in genetic diversity studies of populations and inbredlines

MATERIALS AND METHODS

Malze genotypes kept by the national and SADC regional gene bank will be used. In addition, all elite germplasm and populations commonly used by breeders will be included. Bulk DNA obtained from a minimum of 5 plants will be used for genotyping. SSR markers, selected to cover wide maize genome, will be used in conjunction with selected SNPs. Depending on resources available, all maize accessions in the national gene bank will be used or else selected accessions will be used. In the laboratory, the genotypes will be assessed for tolerance to aluminium toxicity and pH.

EXPECTED OUTPUTS

(a) Genetic potential of germplasm determined. This will result in using maize breeding strategies aimed at maintaining the genetic base as well as increasing breeding progress.

(b) Enhance plant variety protection as elite germplasm would have been genotyped

(c) Identification of core and mini core collections that are traits specific for stress tolerance and are genetically diverse, to enhance the use of germplasm in breeding and development of broad based breeding populations

USDA Request for Proposals- 2011 Borlaug Fellowship Program (Southern Africa)

WEEK 1

ACTIVITIES

· Soft skills in good laboratory practice and molecular data analysis

desk study on understanding recent advances in molecular techniques related to diversity study and
molecular breeding

Importation of maize genotypes for study

EXPECTED OUTPUTS/VERIFIABLE INDICATORS

Fine tuning of the research proposal methodology

· Procurement of reagents, chemicals and other laboratory accessories

WEEK 2

ACTIVITIES

· Identification and optimization of the laboratory protocol

Actual research activities: growing of maize seedlings for DNA extraction.

EXPECTED OUTPUTS/VERIFIABLE INDICATORS

Protocol optimized

Records of reagents and chemicals stocked

WEEK 3

ACTIVITIES

DNA extraction, Data collection and analysis

Draft report writing

EXPECTED OUTPUTS/VERIFIABLE INDICATORS

Draft report

DNA results

· Records of reagents and chemicals used

WEEK 4

ACTIVITIES

- · Finalisation of proposal report and drafting of manuscript for journal publication
- Drawing of plant of action for Zambian activities inclusive of supervisory visits by the USA supervisor
 EXPECTED OUTPUTS/VERIFIABLE INDICATORS
- Completion report
- Plan of action
- Journal manuscript

Fellow #4 (male- Mozambique)

Armando Marcos Wane Come is an agronomist – Plant Protection Subject with 22 years of work experience in Agriculture. His is well trained in agriculture, crop protection and pest management. Although he has not experienced work on

research however could be area of interest in this present fellowship applicant.

Fellow #5 (female- Zambia)

Through this fellowship I wish to study the soil microbiota associated with phosphorus solubilization and the possibility of developing a methodolgy to develop inoculum from appropriate fungal and / or bacterial cells that can be used to Improve crop productivity. Additionally, in order to further improve the capability of soil microbiota to synthesize the metabolites involved with phosphorus solubilization, I intend to study the genes involved in the biosynthesis of these metabolites, to establish the factors affecting their expression levels as and the relationship between the expression levels and the accumulation of the metabolites. This information could be useful in developing microbial genotypes that are capable of over expressing the trait for metabolite accumulation and further enhance P solubilization and consequently, crop productivity.

Phosphorus is an essential nutrient element and is classified based on its abundance in plants. In agriculture, P has a direct influence on yield as it is involved with the metabolic and physiological processes in the life of plants. In the soil P, is involved in major chemical reactions and also has an influence on the physical properties of the soils.

Phosphorus (P) is the second most limiting nutrient next to nitrogen in Zambia. Zambian soils are typical of many tropical soils which have been generally characterized as being very deficient in P and this is related to the degree of weathering. In addition many of the soils have capacity to fix fertilizer P. As a result a constant supply of P to the soil is needed to replenish the depleted P. For this purpose, mostly water soluble commercia P fertilizers are used by Zambian farmers. Water soluble fertilizers are expensive and therefore their use is limiting to small scale farmers. Alternative sources of P have been looked into. Zambia has phosphate deposites in Mubwa, Chilembwe, Kaluwe and Nkombwa. So far acidulated phosphate rocks have yielded some promising results. The direct use of rock phosphate as a fertilizer will require an effective means for solubilization.

Microorganisms are an integral part of the soil phosphorus cycle and are important for the transfer of P between different pools of soil P. Soil microorganisms play an important role in the mobilization of soil P and that detailed understanding of their contribution to the cycling of P in soil-plant systems is required for the development of sustainable agriculture. Already, soil bacteria has been shown to increase P nutrition of plants, through increased calcium-phosphate. Prospects still remain for enhancing phosphorus mobilization by soil microorganisms. An opportunity exists for genetic manipulation of soil microorganisms and also the use of fungal and bacterial inoculum on soils low in plant available P also still exists as an area of intervention.

Through this fellowship, it is believed that a contribution will be made to the productivity of crops in Zambia through the introduction of a technique to produce fungal and bacterial inoculum. Additionally, preliminary information on the microbial genetic influences on the accumulation of P solubilizing metabolites will be obtained, with the purpose of genetically manipulating the microbes in future. This too, will contribute to the productivity of crops through enhanced P solubilization and availability to the plants.

Week1: Objective: To isolate the soil microbiota from the soil Activities: Collect soil samples from field

Set up appropriate plate cultures for the isolation

Use a combination of methods to isolate the microbiota

Requirements: Petri dishes, common non-differential medium, selective medium, shakers, incubators Outcome: Specific microbes will be isolated from a mixture of soil microbes

Week 2-3: Objective: Culture the microbiota in appropriate media and extract the exudates in the culture media Activities: Develop protocols for extracting metabolites from culture media

Extract metabolites

Optimize HPLC conditions for analyzing metabolites

Use HPLC to perform qualitative and quantitative analyses

Requirements: HPLC machine, organic acid extracton solvents, HPLC liquid phase solvents.

Outcome: Different types of amino acids, and other products exuded into medium will be identified

USDA Request for Proposals- 2011 Borlaug Fellowship Program (Southern Africa)

Week 5: Objective: To test P solubilizing capability of extracted metabolites

Activities: Set up cultures with rock P

Determine rate of solubilization

Determine efficacy of metabolite for p solubilization: dose-response experiment

Requirements: Rock phosphate, HPLC machine

Outcome: P solubilizing metabolites will be identified and linked to microbes producing them

Week 5-7: Objective: To determine the expression of some of genes involved in metabolite biosynthesis Activities: Design primers for some genes in metabolite biosynthesis

Extract RNA from microbial cells in culture

Prepare cDNA from RNA extracts

Preform polymerase chain reaction experiments

Requirements: Primers for cDNA synthesis, RNA extraction reagents, gene primers, PCR machine, Outcome: Relationship between gene expression levels and metabolite characteristics established Suggest points of genetic modification to enhance P solubilation

Week 8-11: Objective: To develop microbial inoculum

Activities: Identify microbes most effective in solubilizing P through production of metabolites Develop and test different encapsulation procedures

Requirements:

Outcome: Methods of developing inoculum suggested, atleast.

Weeek 12: Objective: Wrap up and report writing