Research Proposal: Peanut Genomics

Description

Translational Genomics to Reduce Pre-harvest Aflatoxin Contamination of Peanut

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Geographical Locations

USA, India, Senegal, Niger

Project Duration Nov. 1, 2013 – Oct. 31, 2017

Executive Summary

Peanut/groundnut is a protein- and calorie-rich subsistence and cash crop in Africa serving as an excellent source of human nutrition as well as for soil enrichment due to its symbiotic nitrogen fixing capacity. Much of the crop is grown by small-holder farmers, frequently women. In the absence of severe disease pressure, haulms serve as livestock feed, thereby increasing the utility of the crop. Peanut yields are lower in Africa than in any other region of the world, and pod production is negatively impacted by many pests and diseases for which chemical control is not readily available. Apart from low yields, seed quality often declines under water deficit during maturation in part due to the incidence of aflatoxin contamination. Aflatoxin contamination of peanut is a global threat to human health that is largely controlled in developed countries by irrigation and post-harvest sorting. Small-holder farmers in developing and feed-the-future (FTF) countries lack water resources to reduce pre-harvest aflatoxin contamination (PAC) through irrigation and encounter significant crop loss with post-harvest sorting. Preharvest aflatoxin contamination contributes to the potential for contamination to proliferate during post-harvest storage under suboptimal conditions. While peanut is a highly nutritious addition to the diet, high levels of carcinogenic aflatoxin can have serious health consequences.

Objective

Selection for genetic resistance to PAC offers the most economical route to production of a quality peanut crop. PAC results from a complex interaction of plant host (peanut), fungal pathogen (*Aspergillus flavus* and *A. parasiticus*), and the environment. Genetic components for resistance to PAC are heterogeneous and quantitative in nature, and resistance mechanisms range from mechanical or biochemical impedance of pathogen invasion, biochemical interference with aflatoxin biosynthesis, to altered host stress responses that affect host vulnerability. Identifying and combining genetic components, underlying these and other mechanisms, that result in reduced PAC is the primary goal of this research. The advent of peanut genomics will enable genome-wide characterization of genetic differences among diverse germplasm that can contribute to reduced PAC while breeding for increased yield potential and resistance to other pests and diseases.

Approach

The research team assembled to explore genotype-phenotype associations for PAC includes leaders in the peanut genomics initiative and scientists with extensive experience in selection for resistance to PAC in peanut. Genotypic and phenotypic information will be generated by each group aligned with their respective expertise, and computational tools will be applied to identify genotype-trait associations. Well-established as well as advanced breeding tools and methods will be incorporated in the research plan including 1) structured segregating recombinant inbred line (RIL) populations, 2) association mapping panels, 3) multiparent advanced generation intercross

(MAGIC) populations, 4) interspecific introgression lines, and 5) genomic selection. Each population includes PAC resistant lines/parents identified after numerous years of testing. Extensive testing for PAC in germplasm selected for this project will be carried out.

Genomic information generated in the course of this project will be integral to the proposed goal of reduced pre-harvest aflatoxin contamination of peanut, but will have value that extends well beyond this specific goal. Translation of genome sequence to breeding application on the ground in Africa will be an ultimate objective that will be facilitated by Africa-based project scientists with capacity for marker-assisted breeding. All project scientists will be involved in some aspect of training (workshop, graduate students, or scientist exchange) to meet this goal.

Project Description

Goal

The overarching goal will be to associate molecular variation with resistance to pre-harvest aflatoxin contamination on a genome-wide scale. This goal will be enabled by the imminent release of a peanut genome sequence and affordability of re-sequencing methods for identifying diversity in peanut germplasm and populations.

Relevance and Justification

Peanut is a dietary staple and cash crop in sub-Saharan Africa, yet its production and quality are constrained by intermittent drought related to erratic rainfall patterns during maturation in the post- rainy season. Yield reduction by drought costs millions of dollars each year (Sharma and Lavanya 2002). Furthermore, peanut seed quality under water-limiting conditions also is affected by contamination with aflatoxin, a mycotoxin produced by the pervasive fungi Aspergillus flavus and A. parasiticus. Preharvest aflatoxin contamination (PAC) in peanut is an extremely variable characteristic that primarily presents under heat and drought stress (Holbrook et al. 2000a; 2009; Nigam et al. 2009). Although aflatoxin contamination of peanuts could be significantly reduced or eliminated by preventing drought stress through irrigation, irrigation is not available in areas that suffer the most from aflatoxin contamination. Dryland production predominates in developing countries and even continues to be widely practiced in the U.S. Therefore, breeding of varieties that are better adapted to these dryland conditions is part of the solution to aflatoxin contamination. While aflatoxin contamination of peanut also is a problem in industrialized countries such as the U.S., levels in food and feed are controlled to the recommended 0.5-20 ppb limits by extensive testing and segregation of peanuts and peanut products pre- and post- processing. Testing is costly, however, and often not implemented in the developing world, resulting in consumption of foods that far exceed the recommended limit. Chronic a group 1 carcinogen, exposure to aflatoxin, has serious health consequences, particularly in populations that also have a high incidence of hepatitis and immunosuppressive disease (Wild and Gong 2010). From an economic perspective, aflatoxin contamination leads to rejection of peanut lots from export markets. These factors result in significant negative societal and economic impact to the developing world.

Peanut breeding has been intensively conducted for the past half century, but as with most crops, breeders have preferentially utilized elite x elite crosses to make gains in disease resistance and yield. Large germplasm collections exist in multiple countries and are now being recognized as valuable sources of traits that are not present in elite lines. The cumulative peanut collections contain more than 30,000 germplasm entries, although, ironically the size of this resource makes it impossible to access in a direct, thorough, and systematic way. To enable the mining of useful traits from a manageable sample size, core, mini-core, and reference collections, representative subsamples of entire collections, have been established for peanut (Holbrook et al. 1993; Holbrook and Dong 2005; Upadhyaya et al. 2002, 2003; Hamidou et al. 2012). Preservation of genetic diversity in the U.S. core collection was demonstrated by identification of germplasm resistant to multiple diseases/pests such as tomato spotted wilt, nematode, and late leaf spot (Holbrook and Anderson 1995; Anderson et al. 1996; Holbrook et al. 2000b). Only in the last two decades have protocols that require extensive replication been developed for identification of field resistance to PAC. These protocols have resulted in selection of 19 and 21 resistant out of 831 and 2000 accessions screened from the U.S. core and ICRISAT collections, respectively (Holbrook et al. 2009; Nigam et al. 2009). However, incorporating unadapted germplasm into a breeding program was historically avoided by most breeders because of the negative consequences of linkage drag and the lack of understanding of the genetics of most traits of interest. More recently, breeder interest in accessing traits outside of elite and primary gene pools has expanded (Simpson 2001; http://www.ncfar.org/Germplasm_Presentation_062512.pdf)_and is being facilitated by the use of molecular markers (Fonceka et al. 2009).

Cultivated peanut is tetraploid (2n=4x=40; AABB genome) while most of its wild relatives are diploid. Cultivated peanut, Arachis hypogaea L., most likely evolved from a hybrid between two wild diploid progenitor species, A. ipaensis (BB genome) and A. duranensis (AA genome) upon spontaneous chromosome doubling (Kochert et al. 1996). The evolutionary bottleneck imposed by a suggested single tetraploidization event that led to domestication of peanut has minimized allelic diversity. Wild relatives of peanut, which are all native to South America, are a rich source of genetic diversity with new alleles that have been selected over millions of years under diverse biotic and abiotic stresses. For characteristics such as disease and pest resistance, the narrow genetic base of cultivated peanut presents clear and well documented limitations to crop improvement. Yet there is evidence that wild species can be used to broaden the genetic base of cultivated peanut for complex traits including disease resistance and drought tolerance (Leal-Bertioli et al. 2009, 2012; Fonceka et al. 2009, 2012a; Guimaraes et al. 2012). Molecular breeding tools are particularly beneficial monitoring of introgressions during breeding generations. for Ample polymorphism exists within AA and BB genome accessions which, along with the creation of a variety of synthetic tetraploids (Simpson 2001; Favero et al. 2006), contributes to the feasibility of marker-assisted introgression (Fonceka et al. 2009, 2012a,b).

Application of molecular markers in cultivated peanut has been slow to develop, primarily due to the low levels of polymorphism that have been

difficult to identify with the limited genome coverage of most marker types such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), and amplified fragment length polymorphisms (AFLPs). More recently, as genomic and transcriptomic sequence was generated, the hypervariable simple sequence repeat (SSR) marker type has predominated genetic mapping studies (Pandey et al. 2012). With the development of next-generation sequencing technologies, the is sequencing of peanut genome underway (<u>http://www.peanutbioscience.com/</u>) and genome re-sequencing has become affordable for identification of genome-wide single nucleotide polymorphism (SNP) diversity and implementation of genomic selection in breeding (Janila et al. 2013; Varshney et al. 2013).

Research Plan

Objective(s)

- Phenotype at least 800 inbred lines of peanut for preharvest aflatoxin contamination in replicated (time, location) field trials to obtain measures of absolute aflatoxin levels and variation in susceptibility to PAC under conditions of stress permissive for aflatoxin contamination.
- 2. Genotype all lines tested for PAC using genotyping-by-sequencing methods (currently being tested and refined with other genetic materials) and use computational methods to identify allelic diversity.
- 3. Apply statistical methods to test for significant associations between genomic regions and PAC and other traits for which genetic materials have been phenotyped during the execution of other projects.
- Develop breeding tools based on the above research results and disseminate information on implementation of discoveries for breeding to qualified programs in FTF countries through workshops and training opportunities.

Role of Each Scientist/Partner

Ozias-Akins, project PI, will be responsible for coordinating the project and reporting progress within the PMIL program.

U.S. scientists Ozias-Akins, Jackson, Bertioli and Holbrook will conduct genotyping and phenotyping of inbred panels, structured populations, and introgression lines available in the US and will genotype other materials for which only DNA may be available.

Dr. Rajeev K. Varshney, will be PI for ICRISAT and will co-ordinate the overall project activities together with Farid Waliyar, at ICRISAT, Hyderabad and ICRISAT, Mali, including making arrangements and directing the research, and coordinating the schedule of work to further meet the goals of the grant, and complete the project. At

ICRISAT, Rajeev K. Varshney and other staff (Farid Waliyar, Hari Upadhyaya, P. Janila and Pawan Khera) engaged in peanut projects will continue to contribute data relevant to this project.

ISRA scientists Fonceka and Faye will conduct phenotyping of CSSL populations available in Africa, and will be key contacts for educational

opportunities.

Annual Work Plan, Milestones and Timeline

Genetic materials

- 1. Aflatoxin core
 - a. In 2009, approximately 100 entries were selected and exchanged by Shyam Nigam/Farid Waliyar (ICRISAT) and Corley Holbrook (USDA-ARS), the majority of which represented lines with reproducibly less PAC in the tested environments along with high PAC controls. Each group has completed quarantine clearance and seed increase is in progress. These entries will be of highest priority within this project for genotyping and additional phenotyping since they already were identified as having potential to increase resistance to PAC. These entries will be tested both in Africa and in the U.S.
- 2. Recombinant inbred line (RIL) populations
 - a. One line in the aflatoxin core, C76-16, was bred and selected by Corley Holbrook for its drought tolerance and resistance to PAC in the southeastern U.S. (Dang et al. 2012). This line was used in the development of two RIL populations, each with a different female parent, either Tifrunner or Florida-07 (Table 1). Tifrunner is the genotype selected for the reference peanut genome sequence while Florida-07 is a high-yielding recent release from the University of Florida breeding program. These two populations are being used in independent studies on drought tolerance and transcriptional changes during differential response to drought and PAC. One of the two populations will be genotyped and phenotyped over multiple years in the field. The maximum population size is 361 lines.

CHECK								
Line ID	#	Relative	Relative	Maturity Class	Known traits			
	Tests	PAC*	Yield*					
Tifrunner	2	0.95	0.90	late (150 d)	TSWV resistance			
Florida-07	1	1.10	1.50	medium-late (140 d)	high oleic acid/low linoleic;			
					TSWV resistance			
C76-16	16	0.70	1.16	medium-late	drought tolerance; PAC			
					resistance			
A72	10	3.07	0.95	medium	PAC hyper-susceptible			

Table 1.	RIL	population	parents	and	susceptible
check					

* Values are relative to the test mean

3. ICRISAT reference collection

a. The ICRISAT reference collection is composed of 300 genotypes (Upadhyaya et al., 2010) encompassing the peanut mini-core (Upadhyaya et al. 2002). Its diversity recently has been assessed by a small set of SNPs (Khera et al. 2013) identified from transcriptional assemblies (Nagy et al. 2012; Guo et al. 2012). The reference set also has been extensively phenotyped for 50 traits including drought tolerance related traits and *Aspergillus* infestation (aflatoxin contamination).

- 4. MAGIC (multiparent advanced generation intercross) population
 - a. A total of eight highly diverse genotypes (Table 2) possessing variability for different components of drought tolerance along with several economically important traits such as disease resistance, dormancy, early maturity and oil quality etc., were selected as parents of the MAGIC population. One of the parents also has consistently demonstrated resistance to PAC.

Table 2. Genotypes for MAGIC population

		• •
S. No.	Genotype	Trait for which the parent was selected
1	ICGV 88145	Resistant to preharvest seed infection, colonization, and aflatoxin
		contamination and is used as check in ICRISAT aflatoxin trials
2	ICGV 00308	Short duration (90-95 days) and high yielding
3	ICGV 91114	Short duration (90-100 days), drought tolerant and high yielding
4	ICGV 06040	High Fe and Zn content (57 ppm Fe & 79 ppm Zn)
5	ICGV 00440	Confectionary type with high 100- seed weight (75 g) and low oil 45%;
6	ICGV 05155	High oil content (55 %)
7	GPBD 4	Foliar disease resistant (LLS score 4 and Rust 2 at 90 Days)
8	55-437	Drought tolerant and short duration
Crosso	among those gor	aturas have already been initiated

Crosses among these genotypes have already been initiated.

- 5. CSSL (chromosome segment substitution line) population
 - a. A collection of 122 BC_4F_3 CSSL lines were obtained from a cross between the synthetic amphidiploid (A. ipaensis KG30076 x A. duranensis V14167)4x and the cultivated variety Fleur 11. This collection of lines represents the entire wild genome in the form of overlapping chromosome segments averaging 39.2 cM in length, mapped and introgressed into the genome of the cultivated variety. Most of the lines contained a single wild chromosome segment in a homogeneous cultivated genetic background. Phenotypic comparison of each line to the cultivated parent makes it possible to analyze the effects of each segment of wild origin. A genetic study conducted during the construction process of the population showed the existence of wild alleles, which have a positive effect on the yield components and maturity under well-watered and water-limited conditions (Fonceka et al. 2012a). Following this work, a first characterisation of a subset of 80 CSSLs has confirmed the interest of the population for dissecting the genetic control of the morphological traits involved in the development of the plant (Fonceka et al. 2012b). Two years of phenotyping of the CSSL population (Fonceka et al., unpublished) showed that the lines segregate for several traits that are relevant to drought.

Screening for resistance to pre-harvest aflatoxin contamination

In the field, water extraction capacity based on larger root systems and assessed by visual stress ratings has been shown to correlate with a reduction in aflatoxin contamination (Holbrook et al. 2000). However, results from greenhouse screening did not strictly correlate with results from the field, although some genotypes were low in aflatoxin contamination under both environments. It is therefore imperative to evaluate materials in the field under natural or simulated post-rainy season water-limiting conditions during the developmental stage of pod filling. Genetic resistance to preharvest aflatoxin contamination has been examined in highly replicated *Aspergillus*-inoculated field trials under simulated drought conditions provided

by rainout shelters in the southeastern U.S. (Holbrook et al. 2009). A movable greenhouse system was developed to provide a screening site at Tifton, GA. Thirteen large (9.1 m wide x 25.5 m long) rainout shelters were constructed on skids. These structures can be moved in the field with tractors and are parked on the test plots for the 40 days immediately preceding harvest to provide the extended period of heat and drought stress necessary for consistent aflatoxin contamination of susceptible genotypes. Artificial inoculation following the technique of Will et al. (1994) uses a combination of A. flavus and A. parasiticus grown on sterilized cracked corn and applied to the test plots at mid bloom (60 DAP). Artificial inoculation helps to insure uniform testing conditions, which reduces the number of escapes and reduces variation in the data that could mask genetic differences. Drought simulation with rainout shelters results in an abrupt transition for plants from well watered to water-deficit conditions, that is affected by variations in daily high and low temperatures and often high relative humidity, but results in detection of significant quantitative differences in aflatoxin contamination with several years of data from replicated trials. This artificial imposition of stress is essential in the southeastern U.S. because of unpredictable rainfall that can occur at any time of the growing season. Semi-arid growing regions of Africa have erratic rainfall that can cause intermittent drought stress during the growing season along with a transition to the dry season during peanut pod maturation and pod filling. Testing of materials in different water deficit environments will allow common and unique genetic factors contributing to PAC resistance to be identified. Given the extreme variability frequently encountered with preharvest aflatoxin contamination, it is necessary to extensively replicate field trials. Putatively resistant genotypes will be tested at least 3 years with a minimum of 5 replications per year. The majority of susceptible lines will be eliminated from testing after as little as a single year of data. This progressive plan will allow us to accommodate multiple years of testing for putatively resistant lines.

Genotyping and genetic analysis of PAC resistance

PAC resistance in peanut has been shown to have low to moderate heritability in the few studies that have been conducted (Girdthai et al. 2010; Arunyanark et al. 2010). Mapping of quantitative trait loci (QTL) for PAC in maize (Warburton et al. 2011; Willcox et al. 2013) has demonstrated the potential of marker-assisted breeding for this trait, if genetic variation is available. The potential for "omics" tools to impact PAC has been recognized for some time (Bhatnagar et al. 2008), although the potential cannot be fully realized in peanut until considerable investment has been made to develop these tools, an investment that is underway (Varshney et al. 2013). While simple sequence repeat (SSR) markers currently are the preferred marker type for peanut (Pandey et al. 2012), the community soon will begin to transition to single nucleotide polymorphism (SNP) markers given the emerging genome sequence information and advancements in computational tools. As a result, a moderate number of SNPs has been identified from transcriptome sequencing of expressed sequence tags (ESTs) (Guo et al. 2012; Nagy et al. 2012; Chen et al. 2013b). In addition, two Illumina GoldenGate assays for genotyping 1536 SNPs and 768 SNPs have been developed at the University of Georgia and University of California-Davis (see

Pandey et al., 2012). However, GoldenGate assays are not cost-effective when there is a need for a varying number of samples that can be genotyped with a varying number of markers. Therefore, another SNP genotyping assay known as Kompetitive allele-specific PCR (KASP) assays have emerged for breeding applications in peanut (Khera et al. 2013). With the advent of next-generation sequencing (NGS) technologies, genotyping-by-sequencing (GBS) has become a robust approach (Elshire et al. 2011; Poland et al. 2012b). In GBS, large numbers of SNPs representing genome-wide molecular markers can be identified at a much lower cost as compared to whole-genome resequencing.

For the purpose of this project, genetic materials undergoing phenotyping also will be genotyped. Genotyping essentially will be conducted according to GBS protocols developed for other species that sample a fraction of the genome (reduced representation) based on restriction enzyme digestion, size selection and sequencing of barcoded DNA pools from multiple genotypes (Baird et al. 2008; Elshire et al. 2011; Poland et al. 2012a; Peterson et al. 2012). Sequences will be aligned with peanut reference genomes for tetraploid cultivated peanut (AABB, available 2014) and its AA and BB diploid progenitors (available 2013). The complexity of the peanut genome due to recent whole genome duplication and the high similarity between homoeologous sequences may require development of custom computational tools for reliable calling of SNPs (allelic differences between inbred lines) vs. inter-homoeolog polymorphisms, although progress has been made on this front for other polyploid crops (Chen et al. 2013a; Page et al. 2013). The genome-wide polymorphic SNP calls along with phenotypic scores will then be analyzed with more traditional as well as innovative methods to discover genotype-trait associations or calculate genomic-estimated breeding values (GEBV). For example, genetic maps will be constructed using JoinMap (Stam 1993), Mapmaker/QTL (Paterson et al. 1988; Lincoln et al. 1992), MSTMap (Wu et al. 2008), or other suitable software. Phenotype and SSR/SNP marker data will be combined for QTL mapping (Doerge 2002). Multiple methods for QTL detection will be tested including composite interval mapping (Zeng 1994) implemented in WinQTL Cartographer (Wang et al. 2011). Phenotypic coefficients of variation and heritabilities will be estimated. We also will evaluate the potential to use genomic selection (GS) (Meuwissen et al. 2001) in breeding for PAC resistance since certain sets of materials will be suitable as training populations and this approach will have the most value for complex traits such as PAC resistance. GS uses a 'training population' of individuals that have been both phenotyped and genotyped and basically attempts to capture the total additive genetic variance with genome-wide marker coverage and effect estimates (Jannink et al. 2010). Therefore, selection of an individual without phenotypic data can be performed based on this model (predicting the individual's breeding value, GEBV). To date, the publicly available results on large-scale GS performance are from animal breeding programs (Legarra et al. 2008; Hayes et al. 2009; Luan et al. 2009; VanRaden et al. 2009). However, most recently, GS has started to be used in some crops, e.g. maize, wheat, and soybean (Wong et al. 2008; Bernardo 2009, 2010; Heffner et al. 2009). Genetic diversity analyses in different sets of groundnut germplasm with genome-wide SSR markers have shown relatively large blocks (10-20 cM) of linkage disequilibrium (LD) in breeding lines. These results indicate a requirement of 600-1200 markers distributed on 20 linkage groups for generating genomewide marker profile data for use in GS. This marker density should be easily achievable using GBS.

Milestones

- 1. U.S. and ICRISAT locations will target testing of 200 entries with ample replication per year under stress conditions conducive for aflatoxin formation. One germplasm set, the "aflatoxin core", will be tested in both locations.
- 2. ISRA will grow an average of 80 entries (introgression lines) per year under stress conditions conducive for aflatoxin formation.
- 3. Aflatoxin data will be exchanged and evaluated at the end of each growing season, in particular to inform the selection of genotypes for evaluation in the following year.
- U.S. and ICRISAT scientists will genotype at least 1700 lines, most within the first two years of the project. Genotyping of MAGIC lines will be delayed until year 4 to allow time for the population to be developed (Fig. 1).
- 5. All genotype and phenotype data will be exchanged, and genotype-trait analyses will be conducted by each group with the populations most relevant to their environments and breeding programs. We expect to identify QTL and begin to pyramid these using marker- assisted backcrossing and marker-assisted recurrent selection. Genetic stocks with potential to contribute resistance to PAC will be made available to other groups conducting breeding activities.
- 6. A MAGIC population will be developed and testing of a population subset for PAC will be initiated late in the project.
- 7. The potential to apply GS to breeding for PAC resistance will be assessed late in the project.
- 8. A workshop for African breeders from FTF target countries will be organized in the third year of the project to inform them of recent developments in peanut breeding that can be directly applied to their own breeding programs or facilitated by interaction with other groups.

28 2-way crosses		A A E E E C C G D B A E C	8 8 8 0 G A G 8		G (C) R2012
14 4-way crosses (AB) (CD) (EF) (GI)	ACLEG BD FII AD FG BC EI	AE BF CG DH AF CH	BEVDG (AGVEN	CEXT ANY DE	G (F PR2012-13
7 8-way crosses (ABCD) (EFGH)	(ACEG BOFH) (ADFG BCEH)	(AEBF CGDH) (AFC	CH (BEDG) (AGBI	CEDF) (AHDE) BG	R2013
F ₁ s with genomic regions from all	(ACEGBDFH) (ADFGBCEH)	(AEBFCGDH) (A	FCHBEDG	BICEDF) AIDEBGCF	PR2013-14
eight parents	>1000 F ₂	plants from these abo	ve F ₁ s		R2014
	F ₃ plant pro	ogenies for phenotypin	g and selection		PR2014-15
	F ₄ plant pro	ogenies for phenotypin	ng and selection		R2015
	F _s plant pro	ogenies for phenotypin	ng and selection		PR2015-16
	F ₆ plant pro	ogenies for phenotypin	ng and selection		R2016
	Yield asses	sment through replicat	ted yield trial		PR2016-17

Figure 1 Diagrammatic outline for development of multi-parent advanced generation inter-cross (MAGIC) population in groundnut. Years/season are on right side.

Timeline

Activity/Yr	0-0.5	0.5-	1.0-	1.5-	2.0-	2.5-	3.0-	3.5-
		1.0	1.5	2.0	2.5	3.0	3.5	4.0
Teleconferencing semiannually for								
research planning and evaluation of								
progress	Х	Х	Х	Х	Х	Х	Х	Х
Planting and cultivation	Х		Х		Х		Х	
Harvest and aflatoxin quantification		Х		Х		Х		Х
Genotyping	Х	Х	Х	Х			Х	Х
QTL mapping					Х	Х	Х	Х
Genomic selection					Х	Х	Х	Х
MAGIC population development (Fig.1)	Х	Х	Х	Х	Х	Х		
MAGIC population subset testing							Х	Х
Training workshop						Х		

Gender research strategy

How will the research program yield benefits for both women and men farmers and processors in the target countries?

Translational genomics, which in the context of the proposed research means application of genomic information to peanut/groundnut crop improvement, is expected to impact all levels of the value chain where both men and women play significant roles. While the proposed project will be focused on reduction of pre-harvest aflatoxin contamination (PAC), the genomic information generated will have application beyond PAC resistance for crop improvement, specifically, phenotype scores for other traits (resistance to late leaf spot, groundnut rosette virus, insects, abiotic stress, etc.) from the germplasm sets to be genotyped can be easily integrated for QTL discovery and GEBV calculation to expand breeding applications. Improved varieties, when released, tested by national programs and adopted by small-holder farmers, largely women, will lead to increased yields, less disease, and less aflatoxin contamination. Reducing aflatoxin contamination will enhance the quality of the crop, making it more nutritious for local consumption and more marketable.

The direct beneficiaries of this project are households in Sub Saharan Africa, involving women who are actively involved in the cultivation, consumption, processing and marketing of peanut and peanut products (oil, peanut cake, peanut butter, etc.). Across all the FTF countries, women are mostly marketing peanut and peanut products and the crop serves as a key source of revenue for women. Implementing programs to reduce the levels of aflatoxin contamination is likely to generate social benefits. Boakye-Yiadom (2003) used an economic surplus model that incorporates trade, as well as domestic production and consumption, to assess the potential benefits from research into the aflatoxin- reducing program on high quality edible groundnut exports in Senegal. Various scenarios (from a 30% increased to a 60% increase in high-quality groundnut) of program-effectiveness were examined. The results support that, besides enhancing farmers' welfare, the adoption of the aflatoxin-reducing program is expected to yield an overall net-gain ranging between US\$0.56 million and US\$4.25 million. This study

does not account for benefits accruing from improved health, nutrition of women and children and livestock.

Public and private extension services including NGOs have largely invested in training farmers at production enhancing technologies but little progress has been made to tackle PAC in peanut. In addition, knowledge of farmers, processors, traders, policy makers and health authorities remain limited on the negative effects of aflatoxin contamination. Therefore, PAC resistance is needed to reduce the level of toxins in crops to guarantee a product with a better quality along the peanut value chain.

The peanut scientific community involving ICRISAT, USDA-ARS, U.S. Universities and National Africa Research Systems have recently developed new materials which have potential PAC resistance. Efforts now need to be initiated to ensure greater impacts by re-confirming PAC resistance and applying genomics tools to develop breeding lines and release new varieties in FTF countries. This project then will be linked to ongoing projects in the different FTF countries to promote adoption and diffusion of peanut varieties with PAC resistance by farmer organizations among which many are managed by women and related to women. Scientists will make an effort to balance genders involved in research and communication of results in order to maximize potential for achieving significant outcomes.

Outcomes and Impacts

Outcomes

Research

Assessment of genome-wide molecular variation at the single nucleotide level for at least 1700 genotypes for which phenotypes for preharvest aflatoxin contamination also will be measured for the majority of these.

Discovery of associations between genotype and phenotype and integration of this knowledge into breeding for reduced preharvest aflatoxin contamination.

Training

Student and/or scientist study visits will be incorporated into the project for technical and intellectual capacity building.

Education on advancements in breeding technologies will be conducted through a workshop targeting breeders from FTF countries.

Impacts

To be determined by Program Director

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