Description of document:	Note and Budget, Identification of genes conferring resistance to black pod disease in cacao (Theobroma cacao L.) and their linked markers (FULBRIGHT-ARS: 2015-2016, DS-2019: N 0016391104, Program: G-1-00005) Project Title: Identification of genes conferring resistance to black pod disease in cacao (Theobroma cacao L.) and their linked markers
Requested date:	16-December-2018
Release date:	10-December-2019
Posted date:	11-February-2020
Source of document:	FOIA Request Department of Agriculture Departmental FOIA Officer 1400 Independence Avenue, SW South Building Room 4104 Washington, DC 20250-0706 Email: USDAFOIA@ocio.usda.gov

United States
Department of
Agriculture

Office of the General Counsel
1400 Independence Ave. SW
Washington, DC 20250-1400

November 10, 2019

Delivered via Electronic Mail

RE: Final Response for Freedom of Information Act (FOIA) Request
FOIA Request No. 2019-FAS-01538-F

This is the Departmental FOIA Office's (DFO) final response to the above-referenced FOIA request, on behalf of the Foreign Agricultural Service (FAS). As of October 1, 2019, FOIA requests sent to the FAS will be processed by the DFO. You requested a copy of the Statement of Work, and a copy of the final report/presentation provided to FAS under the contract by Pennsylvania State University, for contract number AG3151P160184 (Cacao Research Project Transcriptome Sequencing).

This request has been processed under the FOIA, 5 U.S.C. § 552.

A search for responsive records was conducted by the FAS. The search identified one (1) agency record, titled 'Concept Note,' that is responsive to your request. The record is being released to you in its entirety, with no FOIA exemptions applied.

By way of explanation, the responsive 'Concept Note' is being provided to you in place of the requested 'Statement of Work.' The FAS located and provided the 'Concept Note' in response to your request because it was submitted by Penn State in association with the referenced contract, and was determined by FAS to be equivalent to (in lieu of) the Scope of Work. The FAS did not locate a 'final report;' however, a research paper resulting from the contract work is publicly available at the link below: https://doi.org/10.1007/s11103-019-00832-y.

You may appeal this response by email at USDAFOIA@usda.gov, or by mail to Inga Bumbary-Langston, Deputy General Counsel, Room 101-W, Jamie L. Whitten Federal Building, U.S. Department of Agriculture, 1400 Independence Avenue, S.W., Washington, D.C. 20250-0103. Your appeal must be in writing, and it must be received no later than 90 calendar days form the date of this letter. The OGC will not consider appeals received after the 90 calendar-day limit. Appeals received after 5:00 p.m. EST will be considered received the next business day. The appeal letter should include the FOIA tracking number listed above, a copy of the original request, the DFO's response to your original request, and a statement explaining the basis of your appeal. For quickest possible handling, the subject line of your email, the appeal letter, and its envelope, if applicable, should be marked "Freedom of Information Act Appeal." You should also reference FOIA No. 2019-FAS-01538-F.

You may seek dispute resolution services from the DFO's FOIA Public Liaison, Ms. Camille Aponte. Ms. Aponte may be contacted by telephone at 202-690-5260, or electronically at Camille.Aponte@usda.gov or USDAFOIA@usda.gov.

You also have the option to seek assistance from the Office of Government Information Services (OGIS). Please visit https://ogis.archives.gov/mediation-program/request-assistance.htm for information about how to request OGIS assistance in relation to a FOIA request.

If you have any questions regarding the processing of this request, please contact Mr. Jeff Crile by telephone at 202-720-7732, or electronically at Jeffrey.crile@usda.gov or USDAFOIA@usda.gov.

For additional information regarding USDA FOIA regulations and processes, please refer to the information available online at www.dm.usda.gov/foia.

The DFO appreciates the opportunity to assist you,

Sincerely,

Alexis R. Graves

Alexis R. Graves
Departmental FOIA Officer
Office of the General Counsel

Enclosures: Responsive Records (7 pages)

CONCEPT NOTE and BUDGET

FULBRIGHT – ARS: 2015-2016

DS-2019: N 0016391104

Program: G-1-00005

Project Title

Identification of genes conferring resistance to black pod disease in cacao (Theobroma cacao L.) and their linked markers.

Grantee

POKOU N'DA Désiré, Cacao geneticist, CNRA, Côte-d'Ivoire,

Faculty Associate

Mark GUILTINAN, Professor, Penn state University

I- Background

Cacao beans obtained from *Theobroma cacao* L. are an income generating commodity in Africa. More than 70 percent of the world's cocoa comes from West and Central African countries including Cote d'Ivoire, Ghana, Nigeria and Cameroon. Export of dried cocoa beans to chocolate companies makes the largest agricultural commodity contribution to foreign exchange earnings, gross domestic product, and development of producing countries. Cultivation of cocoa is one of the predominant income producing enterprises in rural areas of Côte-d'Ivoire, the largest producing country, where more than 95% of cacao is produced by smallholder farmers.

Unfortunately, cocoa cultivation is severely hampered by a diverse array of pests and diseases. It is estimated that over 40% of all cacao production in the world is lost annually to just five diseases (e.g Black pod, Cacao Swollen Shoot Virus disease, Frosty pod, Vascular Streak Dieback and Witches' Broom) with additional losses inflicted by insects and rodents. Globally, the most widespread disease is *Phytophthora* pod rot, also called black pod, which is caused by four species of fungus, all beloning to the Phytophthora genus.The most common worldwide is *Phytophthora palmivora*. The more aggressive, *Phytophthora megakarya*, is an African species that predominates in Cameroon, Nigeria, Ghana, and Côte-d'Ivoire. Losses due to *P. megakarya* reach 80% of farm production if no control measures are taken. Chemical control of black pod is possible but expensive in relation to the low average productivity obtained by thousands of the smallholder cocoa growers in Africa.

Continuing the development and cultivation of improved planting material with both increased productivity and improved pathogen resistance, is critical to increasing the efficiency of cacao production and farmer's income. Genetic resistance to *Phytophthora* pathogens has been a target of cacao breeders for over eighty years, and genotypes with partial resistance have been the subject of QTL mapping. However, applying markers from QTLs identified with different populations and other regions of the world might not be effective to combine into single varieties different sources of resistance. Therefore a larger, focused evaluation of germplasm and conditions in West Africa is essential.

In Côte-d'Ivoire, the cacao breeding program follow a reciprocal recurrent selection scheme. The goal is to improve the resistant to diseases and simultaneously increase the yield. The program started in 1990 with resistance to *Phytophthora* as the main criteria. Significant variation in genetic resistance has been observed in germplasm collections and breeding trials.

Identification of genes conferring this resistance and their linked markers will help breeders speed up the development of new varieties.

New genomic and transcriptomic experimental strategies offer a reliable method enhance to speed of breeding in many crops. Thus, we have submitted this proposal to Fulbright in order to identify genes conferring the resistance to *Phytophthora* through transcriptome sequencing and to develop genetic markers linked to these genes to improve the breeding for resistance to *Phytophthora*. Professor Mark Guiltinan of Penn State University, who has an experience in cacao transcriptome analysis through different projects conducted in his lab, has accepted to host this work.

II- Objective

The main objectives of this project is to develop markers for genes conferring resistance to *P. megakarya*. To be specific, this project will analyze differentiation of the expressed genes against *P. megakarya* through transcriptome sequencing. Then, we will used the transient assay to validate the high priority candidates and sequence those genes in plant material from diverse genetic groups to develop markers for tracking these genes.

III- Plant material

1- For gene expression analysis, the plant material is composed of two genotypes, SCA6 and NA32. The performance of these genotypes has been observed at CNRA in Côte-d'Ivoire using the pod rot rate in the field over several years and the detached pods and detached leaf test in the lab. SCA6 is known to be highly resistance to *Phytophthora* while NA32 is highly susceptible. Each genotype has six replicates, made up of a set of three plants to be tested at different times including T0, T6 hours, T24 hours, T48 hours and T72 hours. Infected plants will be compared to a mock-inoculated (water-treated) control.

2- The development of genes markers will be done using the following 40 genotypes representing the diversity of the current breeding populations in Côte d'Ivoire (*Pokou et al*, 2009). These genotypes have been evaluated using detached leave test and showed a range of resistance to susceptibility (*Lachenaud et al*, 2001).

Resistant	Moderately susceptible	susceptible
ICS 95, IFC 371, T 60/887, ICS 84, PA 150, P 7, T79/501, MO 98, P 19 A, IMC 57, SCA 6	ICS 46, NA 58, IFC 1, W 41, ICS 39, IFC 29, ACU 85, WA 40, MAT 1-9, PA 4, MO81, CC 10, IFC 6,	GS 29, ICS 89, NA 32, IFC 5, UF 676, IFC 304, UPA 134, IFC 303 SNK12, IMC 6, ICS 6, UPA401, UPA413, IMC67, IFC 14, N38, UPA 409

IV- Methodology

Activity 1-Transcriptome sequencing.

We grew 3 month old plants from the SCA6 and NA32 genotypes in greenhouse at CNRA, Côte-d'Ivoire. Each genotype has six replicates of three plants for the treatment and the control. Plants were selected for treatment in a complete randomized design. The two treatments applied are: spraying *P. megakarya* inoculum suspension at a zoospores density of 3 x 10^5 per ml using manual vaporization, and the second, spraying distilled water as control. A blank control without treatment is added to the analysis. Sampling is performed at several time points. To prevent any loss of replicate, two sampling is performed at each time point. The sampling is consist of taking one young leaf per plant at the same vegetative stage. At the starting point (T0), only the blank control will be subject of sampling. Then, the two treatments will be applied on the other plants (zoospores and distilled water). Six hours later, a set of 18 plants is randomly selected for each treatment to compose the sampling point with a pool of three of plants per replicate. The same sampling strategy is repeated 24, 48 and 72 hours after treatments. All the replicates collected will be used for RNA extraction and then for reverse transcription to obtain cDNA. Then, the qRT-PCR using primers for several genes already known to be induced during infection with *Phytophthora* will allow us to select the replicates that have been successfully inoculated. After checking this reaction to the pathogen using qRT-PCR, a subset of 24 samples per genotype will be used for transcriptome sequencing.

Activity 2- Validation of Candidate genes

To facilitate the study of gene function in cacao, *Fister et al.* (2016) developed a rapid *Agrobacterium*-mediated transient genetic transformation protocol at Penn State University. This protocol will be used to assess effects of over-expression of individual candidate genes on detached leaves. The plant material for this test will be collected from the Penn State

greenhouses. Priority will be given to genes located in known consensus QTLs for resistance to *Phytophthora*. Genes which were up-regulated and genes which were down-regulated at the different post-inoculation times will be identified as candidate genes.

Activity 3- Development of gene markers

A set of 40 genotypes known to be the founders of the genotypes currently used in breeding in Côte-d'Ivoire have been selected base on their genetic variation and their range of resistance to *Phytophthora*. All the validated genes from the transient assay will be sequence for each of the above genotypes as well as their surrounding area (promoter). Comparative analysis of genes sequence will allow to identify SNP markers inside the gene or in the promotor.

V- Expected results

We are expecting the following result from our work:

1- All the genes of cacao up regulated and down regulated after inoculation by *P. megakarya* are known.

2- Genes from known QTLs that have truly effect on the genetic resistance to *Phytophthora* are validated and the relevel of effect is determined.

3- At least one SNP marker linked to each of the validated genes has been developed.

4- Two manuscripts are published.

4.1. "Transcriptome sequencing and comparative expressed genes in cacao (*Theobroma cacao* L.) after inoculation by *Phytophthora megakarya*.

4.2. Development of Single Nucleotide Polymorphism (SNP) markers of genes conferring resistance to *Phytophthora* in cacao (*Theobroma cacao* L.)

Reference

Fister S. Andrew, Zi Shi, Yufan Zhang, Emily E. Helliwell4, Siela N. Maximova and Mark J. Guiltinan. 2016. *Plant Method. 12-19*

Lachenaud Ph., A. Eskes, J. A. K. N'Goran, D. Clément, I. Kébé, M. Tahi et C.Cilas. 2001. Premier cycle de sélection récurrente en côte d'ivoire et choix des géniteurs du second cycle. *13th International Conference of Cocoa Research. Kota Kinabalu, Malaisie, 9-14.*

Pokou N. D., J. A. K. N'_Goran , Ph. Lachenaud , A. B. Eskes , J. C. Motamayor, , R. Schnell, M. Kolesnikova-Allen, D. Clement and A. Sangare. 2009. Recurrent selection of cocoa populations in Côte d'Ivoire: comparative genetic diversity between the first and second cycles. *Plant Breeding 128, 514-520*

Appendix

I- Budget Summary per quarter

Task	**Cost in (US $)**			**Total cost (US $)**
	Q1	**Q2**	**Q3**	
RNA extraction (84 samples)	2,000.00			2,000.00
DNA extraction (40 samples)	1,000.00			1,000.00
qPCR reaction for gene expression checking (84 samples)	2,000.00			2,000.00
Transcriptome sequencing (48 samples)		27,000.00		27,000.00
Transient assay		5,000.00		5,000.00
Sanger sequencing of DNA			5,000.00	5,000.00
Publication			2,000.00	2,000.00
TOTAL COSTS PER QUARTER	5,000.00	32,000.00	7,000.00	**44,000.00**

II- Gantt chart

Task Name	Q1	Q2	Q3
RNA extraction			
DNA extraction			
cDNA construction			
Check for gene expression (qPCR)			
Transcriptome sequencing			
Sequence analysis			
Transient assay			
Sanger sequencing of DNA			
Publication			

Note: Q1: March- April May

Q2: June July August

Q3: September-October-November