A. **Program Description**

**Program Overview, Objectives, and Priorities**

The Borlaug International Agricultural Science and Technology Fellowship Program (Borlaug Fellowship Program) advances USDA’s agricultural research goals of promoting collaborative programs among agricultural professionals of eligible countries, agricultural professionals of the United States, the international agricultural research system, and United States entities conducting research by providing fellowships to individuals from eligible countries who specialize or have experience in agricultural education, research, extension, or other related fields. Fellowships promote food security and economic growth in eligible countries by educating a new generation of agricultural scientists, increasing scientific knowledge and collaborative research to improve agricultural productivity, and extending that knowledge to users and intermediaries in the marketplace. The collaborative nature of the training and research programs not only benefits the Fellow, his or her home institution, and partner country; the U.S. host institution, its professors, researchers, and students; and the global agricultural sector by improving agricultural productivity, systems, and processes in partnering nations through the transfer of new science and agricultural technologies.

USDA will identify Borlaug Fellows based on country-specific topics of importance to international, agricultural trade. USDA then places Fellows with U.S. research institutions for 10-12 week, intensive programs. These programs are expected to contribute to the strategic goals and objectives of the fellow and those institutions through a hands-on experience in a “real-world” agricultural research scenario, providing opportunity for application of research agendas where they can have a direct impact on food security and economic growth in an emerging economy. It is hoped that host institutions will share the knowledge gained through the program in their classroom and extension work with their faculty, students, extension officers, and constituents; and that they will continue to maintain professional contacts with the fellows after their departure from the United States.

Borlaug fellows may be identified in any of the topics listed below:

(A) Animal health, production, and well-being  
(B) Plant health and production  
(C) Animal and plant germ plasm collection and preservation  
(D) Aquaculture  
(E) Food safety  
(F) Soil, water, and related resource conservation and improvement  
(G) Forestry, horticulture, and range management  
(H) Nutritional sciences and promotion
(I) Expansion of domestic and international markets for agricultural commodities and products, including agricultural trade barrier identification and analysis

(J) Information management and technology transfer

(K) Biotechnology

(L) The processing, distributing, marketing, and utilization of food and agricultural products

PLACE OF PERFORMANCE

• The applicant is expected to host fellows at a research facility on their campus in the United States.
• The mentor is expected to make a reciprocal visit of up to two weeks to the fellow’s home institution, which may be in a developing country.

EXPECTATIONS:

(1) Assignment of a Principal Investigator (Training Coordinator)
The host institution will designate a contact person as the Principal Investigator (PI) responsible for coordinating all administrative and programmatic arrangements.

(2) Assignment of a Mentor
A key component of the program is matching the Fellow with a mentor. The host institution will select an appropriate mentor for one-on-one work with the Fellow for the duration of the program.

- The mentor will establish a professional relationship, providing guidance and training in the Fellow’s research and studies.
- The mentor will work with the Fellow before arrival to discuss appropriate work plan, site visits, and other arrangements. A work plan should be agreed upon and finalized no later than 2 weeks after the program start date.
- The mentor will provide draft of work plan through the PI to USDA/FAS for consultation and approval approximately 2 weeks before the commencement of the program.
- The mentor agrees to commit a significant amount of time each week for one-on-one work with the Fellow during the program.
- The mentor will continue communicating with the Fellow beyond the end of the program in the U.S. through the mentor visit.
- Mentor will submit quarterly progress reports that indicate all program activities conducted (form SF-PPR).
- The mentor may assign other faculty members to assist with Fellow’s training and research activities.
- Mentor may not be assigned to multiple Fellows during the same time frame.

(3) Mentor Follow-up Visit

- The mentor visit is a required component of the Borlaug Fellowship Program.
- The mentor will work with the Fellow to plan a follow-up visit to the Fellow’s home country. The trip should occur within 6 months to 1 year after the program ends.
• The PI should provide USDA/FAS with an agenda for mentor’s travel, including goals and objectives. The mentor’s travel information must be provided for emergency contact purposes and country clearance (if required by the cognizant FAS Overseas Office).
• The mentor will provide a trip report highlighting the trip’s activities and results through the PI to USDA/FAS within 30 days after the visit.
• The mentor should plan to meet with the USDA/FAS Attaché or staff from the U.S. Embassy while they are traveling, if feasible. USDA/FAS can assist with coordination prior to the trip.

(4) Visa
• USDA/FAS will provide a DS-2019 for the Fellow to request and obtain a J-1 Visa. USDA/FAS will provide instructions to the Fellow regarding the application process, the amount of lead-time needed, and any paperwork required. The visa start and end date will be coordinated with the host institution who will be responsible for purchasing round trip plane tickets for the fellow to come to the U.S. for his or her program.
• Fellows, including those already in possession of another valid U.S. visa, must still obtain a J-1 visa to participate in the program. Fellows will be refused entry if they arrive in the United States without the appropriate category of visa.

(5) Travel and Transportation
• The host institution must comply with the Federal Travel Regulations (41 CFR 300 et seq.).
• The host institution will provide round trip, economy class, international airfare from the Fellow’s home to the university.
• The host institution is responsible for arranging and purchasing all domestic travel related to the Fellow’s training program.
• The host institution will provide housing for the Fellow for the duration of the training program, taking into account gender and cultural norms.
• The host institution will pay lodging fees directly. The host institution will not require the Fellow to pay for his or her lodging expenses, whether through reimbursement or advance payment.
• Lodging will include a private bedroom, private or shared bathroom, access to a laundry room, and access to a kitchen with pots, pans, and utensils.
• Basic necessities, such as sheets, towels, and cleaning supplies (if not already provided), will be provided for Fellow’s use. The Fellow should not have to pay for these items.
• Lodging will be within walking distance to the campus/training location or easily accessible by public transportation.
• If public transportation is required to access campus/training location, the host institution will provide the Fellow with a bus pass or proper allowance for transportation expenses.
• When planning lodging options, the host institution should check with the Fellow and account for any special dietary restrictions or preferences.

(6) Meals and Incidentals (M&IE)
• The host institution will provide each Fellow with meal and living allowances for the duration of stay.
• Daily M&IE allowance may not exceed current GSA per diem rates.
• The host institution can determine the frequency of per diem allotments, but the Fellow must receive per diem within the first week of the Fellowship. The PI must inform the Fellow and USDA/FAS immediately if this cannot be accommodated.

(7) Emergency Health Insurance
• The host institution will purchase emergency health insurance for the Fellow for the duration of stay, as required for all J-1 Visa holders (22 CFR 62.14).
• The Fellow will not be required to purchase his or her health insurance and then be reimbursed.
• The host institution will educate the Fellow as to what is covered under health insurance policy, especially highlighting that pre-existing medical conditions are not covered.
• The host institution will alert USDA/FAS staff if any health/medical conditions arise during the Fellowship.

(8) Communication
• The host institution will initiate contact with the Fellow as soon as possible.
• The host institution will develop the training program in consultation with USDA/FAS and the Fellow.
• The host institution will keep USDA/FAS informed regarding any logistical or program planning.
• The host institution will notify USDA/FAS immediately upon Fellow’s physical arrival and departure from the U.S. to comply with U.S. Department of Homeland Security requirements.
• The host institution will provide USDA/FAS with the Fellow’s temporary U.S. address and phone number, and emergency contact numbers for the PI, mentor, or other appropriate institution personnel. This information is required so that Fellow can be reached in the event of an emergency.

(9) Fellowship Program
• The host institution will provide educational materials and supplies to each Fellow necessary for their full participation in the fellowship.
• The host institution will pay for all fees related to the Fellow’s training program, such as (but not limited to) technology fees, administrative fees, laboratory fees, etc.
• The host institution will arrange relevant field visits as applicable to the Fellow’s training program.
• The host institution will ensure the Fellow submits an interim and final report (2-3 pages each) to USDA/FAS before the Fellow leaves the United States.

(10) Orientation
The PI/Training Coordinator will communicate directly with the Fellow at least 4-8 weeks before his or her arrival in the U.S. to ensure that all pertinent information is provided, including:

- Name and contact information of PI/Training Coordinator
- Name and contact information of mentor
- Institution information, weather information, and clothing needs
- Housing and M&IE allowance
- Program plan and anticipated site visits
- Professional development expectations
- Reminder to bring any necessary prescription medications
- Explain what is and is not covered under emergency health insurance policy (e.g. no pre-existing conditions, no dental, etc.)

Institution will provide an orientation upon the Fellow’s arrival to acquaint them with campus and community resources, such as:

- Explanation and demonstration of local bus/transportation options
- Explanation of cultural and legal expectations

USDA will provide a welcome and orientation packet for mentors

Issued By
Foreign Agricultural Service, Office of Capacity Building & Development, Trade & Scientific Exchanges Division, Scientific Exchanges Branch

Catalog of Federal Domestic Assistance (CFDA) Number and Title
10.777
Norman E. Borlaug International Science and Technology Fellowship Program

Notice of Funding Opportunity Title
Borlaug Fellowship Program

NOFO Number
USDA-FAS-10777-0700-10.-18-0001; Fellow 1 Bangladesh
USDA-FAS-10777-0700-10.-18-00032; Fellow 2 Cambodia
USDA-FAS-10777-0700-10.-18-0009; Fellow 4 India
USDA-FAS-10777-0700-10.-18-00012; Fellow 5 India
USDA-FAS-10777-0700-10.-18-00013; Fellow 22 Peru
USDA-FAS-10777-0700-10.-18-00014; Fellow 7 Indonesia
USDA-FAS-10777-0700-10.-18-00015; Fellow 8 Malaysia
USDA-FAS-10777-0700-10.-18-00016; Fellow 9 Malaysia
USDA-FAS-10777-0700-10.-18-00017; Fellow 10 Mongolia
USDA-FAS-10777-0700-10.-18-00018; Fellow 11 Myanmar
USDA-FAS-10777-0700-10.-18-00020; Fellow 13 Sri Lanka
USDA-FAS-10777-0700-10.-18-00021; Fellow 14 Thailand
USDA-FAS-10777-0700-10.-18-00027; Fellow 15 Vietnam
USDA-FAS-10777-0700-10.-18-00028; Fellow 16 Vietnam
USDA-FAS-10777-0700-10.-18-00022; Fellow 17 Colombia
Authorizing Authority for Program
The legislative authority for the Borlaug Fellowship Program is provided in Sec. 7139 of
the Food, Conservation, and Energy Act of 2008 (PL 110-234), as incorporated in to the
National Agricultural Research, Extension, and Teaching Policy Act of 1977, as
amended.

Appropriation Authority for Program
Consolidated Appropriations Act, 2017 (PL 115-31)

Program Type
New

B. Federal Award Information
Award Amounts, Important Dates, and Extensions

Available Funding for the NOFO: Each award (for one fellow) is up to $50,000.

Projected number of Awards: 19 per fiscal year (in total)

Number of Project Budget Periods: 1

Projected First Budget Period: N/A

Projected Period of Performance Start Date(s): Subject to the availability of
implementer and Fellows.

Projected Period of Performance End Date(s): 18 months after the start date

Extensions are allowable, please see Section H. Additional Information to see how to requests
one should the need arise.

Pre-Award costs: Not Allowable

Cost Share or Match requirements: A cost match or cost share is not required.

Funding Instrument
USDA will enter into a cost reimbursable agreement under 7 USC § 3319a with selected
universities.
C. Eligibility Information

Eligible Applicants
Proposals may be received from U.S. State Cooperative Institutions or other colleges and universities, including minority serving institutions (MSIs).

A single mentor may not host two fellows simultaneously. Both the PI and mentor must hold positions at an eligible U.S. institution.

Eligibility Criteria
All applicants must have an active registration in the SAM database at www.sam.gov – pending or expired registrants are not eligible. This requirement must be met by the closing date of the announcement and will not be waived. Please contact the program officer listed if you have questions about this requirement.

In addition to obtaining a DUNS number and registering in SAM, you must also obtain Level 2 eAuthentication to apply for this funding opportunity in ezFedGrants (eFG). You must submit an online form requesting access. Normally you will receive an email within 24 hours of your submission, if your request is approved. After this occurs, you will need to schedule an appointment with an LRA. Once you meet with the LRA, your Level 2 eAuthentication should be granted within 2 to 3 days after that meeting. See Section D of this NOFO for detailed information.

Maintenance of Effort (MOE)
MOE is not allowable.

D. Application and Submission Information

Key Dates and Times

Application Start Date: 05/21/2018

ezFedGrants Posting Date: 05/21/2018

Application Submission Deadline: 06/18/2018 at 11:59PM EST

Anticipated Funding Selection Date: Approximately 2-3 weeks after the submission deadline, subject to the availability of funding

Anticipated Award Date: Approximately 2-3 weeks after selection, subject to the availability of funding

Address to Request Application Package
This NOFO represents the full application information.

Applications will be processed through the ezFedGrants portal at https://grants.fms.usda.gov – prospective applicants are encouraged to register for this portal. Applicants that are unable to access the ezFedGrants portal should contact the program manager for alternative submission instructions. Note that if selected, registration is a requirement of performance.

Content and Form of Application Submission
Institutions must be able to host multiple groups over the period of performance and should submit a proposal following the guidelines below:

- Required forms and certifications, including:
  - SF-424 version 2.1, with an OMB Expiration Date of 10/31/2019
  - SF-424A version 1.0, revised July 1997. This should be accompanied by a detailed budget worksheet and a detailed budget narrative (NOTE: A budget narrative must be provided). All line items should be described in sufficient detail that would enable FAS to determine that the costs are reasonable and allowable for the project per federal regulations. An example budget narrative is included in the appendix, but is not required.
  - AD-3030, revised February 2016
  - AD-3031, revised February 2016
- Indicate the name of the institution applying to host the Fellows.
- Indicate the country, research interest, and reference number.
- Identify a Primary Investigator.
- Identify a Mentor. A Mentor may not be assigned to multiple Fellows who are in the U.S. at the same time.
- Provide a tentative research plan based on the Fellow’s research proposal and action plan, including topics covered, field visits, and other activities.
- Include a narrative description of the proposed fellowship, how it will be administered, and the role of the university faculty and support staff.
- 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.
- Provide a one to two page curriculum vitae for the mentor and other collaborating researchers involved in the proposed program.
- Identify the expected skills or knowledge to be acquired by the Fellow at the end of the program
- If attending the World Food Prize, the budget should include time and funding for the Fellow and Mentor to attend. An adjustment to the Fellow’s M&IE must be made for the time spent in Iowa.

The SF-424 and SF-424 A can be completed within the ezFedGrants platform. However, the other required forms must be downloaded from the Forms sections on Grants.gov. The
Certification regarding Lobbying and the Grants and Agreement Coversheet will be sent to you along with this NOFO.

**Unique Entity Identifier and System for Award Management (SAM)**

The link below provides information on 2 CFR §25.110. Please read.

[https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=7a45f973880240465cd255471f1380ef&ty=HTML&h=L&mc=true&n=pt2.1.25&r=PART](https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=7a45f973880240465cd255471f1380ef&ty=HTML&h=L&mc=true&n=pt2.1.25&r=PART)

FAS is using ezFedGrants to post NOFO’s and issue agreements, which is an electronic grants management system. Applicant(s) with electronic access are to submit their applications electronically through:

[https://grants.fms.usda.gov](https://grants.fms.usda.gov)

Before you can apply, you must have a DUNS number, be registered in SAM, and have access to the ezFedGrants website.

**Applicants are encouraged to register early. Due to recent changes in the SAM platform, the registration process can take 6-8 weeks to be completed.** Therefore, registration should be done in sufficient time to ensure it does not impact your ability to meet required submission deadlines.

**DUNS number.** Instructions for obtaining a DUNS number can be found at the following website: [http://www.dnb.com/duns-number.html](http://www.dnb.com/duns-number.html)

The DUNS number must be included in the data entry field labeled "Organizational DUNS" on the Standard Forms (SF)-424 forms submitted as part of this application.

**System for Award Management.** In addition to having a DUNS number, applicants applying electronically through ezFedGrants must register with SAM. Step-by-step instructions for registering with SAM can be found here:

[www.sam.gov](http://www.sam.gov)

Failure to register with SAM will result in your application being rejected during the submissions process.

**ezFedGrants System Access and Electronic Signature**

**Level 2 eAuthentication.** The next step in the registration process is to obtain a Level 2 eAuthentication account that will allow access to the ezFedGrants system. Instructions for getting a Level 2 eAuthentication account can be obtained by emailing ezFedGrants@cfo.usda.gov.
You may also request Level 2 eAuthentication online at:
https://www.eauth.usda.gov/MainPages/index.aspx

If you experience any issues with self-registration or have eAuthentication-related questions, please contact the eAuthenticationHelpDesk for assistance:
By email to eAuthHelpDesk@ftc.usda.gov

**Requesting a role in ezFedGrants.**
After obtaining eAuthentication, users will need a role in the system. Descriptions of the roles available and instructions on how to request a role can be obtained by emailing ezFedGrants@cfo.usda.gov

You may also go into the link below for instructions on requesting eFG access. The document is called “External Portal Access Request Submission”.

https://www2.nfc.usda.gov/FSS/Training/Online/ezFedGrants/access_user_roles.php

**Electronic Signature.** Applications submitted through ezFedGrants constitute a submission as electronically signed applications. When you submit the application through ezFedGrants, the name of your Signatory Official on file will be inserted into the signature line of the application.

If you experience difficulties accessing information or have any questions please email the Helpdesk at ezFedGrants@cfo.usda.gov.

The Federal awarding agency may not make a Federal award to an applicant until the applicant has complied with all applicable DUNS and SAM requirements and, if an applicant has not fully complied with the requirements by the time the Federal awarding agency is ready to make a Federal award, the Federal awarding agency may determine that the applicant is not qualified to receive a Federal award and use that determination as a basis for making a Federal award to another applicant.

**Intergovernmental Review**
This program is not subject to E.O. 12372.

**Funding Restrictions**
This will be a cost reimbursable agreement issued under 7 USC § 3319a. University indirect costs for cost reimbursable agreements are limited to 10% of modified total direct costs (MTDC).

**Allowable Costs:**

1. Salaries and Fringe Benefits:
Requested funds may be allocated toward salaries, fringe benefits, or the combination thereof.
No more than 20% of the requested funds may be allocated toward salaries, consultant fees,
fringe benefits, or the combination thereof. Only individuals that hold positions at eligible U.S. institutions should be listed in this category.

2. Travel:
For domestic travel, provide the purpose of the travel and information used in calculating the estimated cost, such as the destination, number of travelers, and estimated cost per trip. There are several restrictions associated with traveling on federal funds. In most cases, airfare must be purchased in economy class from a U.S. carrier. Travelers must also adhere to federally mandated domestic per diem guidelines. Additional information may be found in the circulars listed in the “Legislative Authority” section of this announcement.

3. Supplies:
All personal property excluding equipment, intangible property, and debt instruments as defined in this section.

4. Other Direct Costs:
Other Direct Costs are those anticipated charges not included in other budget categories, including materials and supplies, lab fees, publication costs, reasonable consultant fees, computer services, sub-awards (the level of detail required for the sub-award budget is the same as the recipient organization), equipment rental, facility rental, conferences and meetings, speaker fees, honorariums.

5. Indirect Costs:
Indirect Costs may not exceed 10% of direct costs (7 USC 3319a).

6. Tax Withholding:
Borlaug Fellows (as trainees, not students) are considered EXEMPT INDIVIDUALS under the IRS Substantial Presence Test for tax purposes. The exemption falls under one or both of the following categories: either the Foreign Government-Related Individuals standard or the Closer Connection Exception. Tax treaties might also exist between the U.S. and the Fellow’s home country. The only requirement is to complete IRS Form 8843 (Sections 1 and 2). No taxes should be withheld from Borlaug Fellows since they are exempt.

Unallowable Costs:

General purpose equipment (no particular scientific, technical, or programmatic purpose) and scientific equipment exceeding $5,000 or more; entertainment; any stipend or remuneration for the fellow, other than ordinary allowances for meals and supplies; capital improvements; thank you gifts, and other expenses not directly related to the project are not allowed. “Please note, Borlaug Fellows (as trainees, not students) are considered EXEMPT INDIVIDUALS under the IRS Substantial Presence Test for tax purposes. The exemption falls under one or both of the following categories: either the Foreign Government-Related Individuals standard or the Closer Connection Exception. The only requirement is to complete IRS Form 8843 (Sections 1 and 2). These funds are for federal financial assistance; as such no taxes should be withheld from Borlaug Fellows since they are exempt.”
Management and Administration (M&A) Costs:
M&A costs are not allowable.

Indirect Facilities & Administrative (F&A) Costs.
By statute, indirect costs for cost reimbursable agreements cannot exceed 10% of direct costs.

Other Submission Requirements
All applications must be submitted electronically as indicated above.

E. Application Review Information
Application Evaluation Criteria

Prior to making a Federal award, the Federal awarding agency is required by 31 U.S.C. 3321 and 41 U.S.C. 2313 to review information available through any OMB-designated repositories of government-wide eligibility qualification or financial integrity information. Therefore application evaluation criteria may include the following risk based considerations of the applicant: (1) financial stability; (2) quality of management systems and ability to meet management standards; (3) history of performance in managing federal award; (4) reports and findings from audits; and (5) ability to effectively implement statutory, regulatory, or other requirements.

Technical Expertise and Experience (40 points)
Mentor must have appropriate technical background to provide the desired, advanced training. If necessary, other appropriate collaborating scientists should be identified to meet any of the objectives which the mentor cannot address. Mentor’s experience and knowledge of relevant agricultural conditions within the Fellow’s country or a similar location will be considered as appropriate. The trainer’s experience with international training and adult-education will also be considered.

Overall Program (35 points)
The overall program plan and design should be relevant to the Fellow’s objectives background. The program plan should be thorough, and it should help achieve the desired post-program deliverables and the Fellow’s research goals and objectives. Relevant agricultural practices within the region of the university will be considered as appropriate. Relevant university resources should be identified. Additional resources/organizations should be identified as appropriate. Site visits and meetings should be meaningful to the content of the program, if included.

Budget (25 points)
The proposed budget should be appropriate for the number of Fellows and length of the program. The budget should include appropriate cost savings where available and narrative should accompany each line item. Host is strongly encouraged to use the Budget Worksheet provided in this NOFO.
Review and Selection Process
In all cases, the Program Manager will ensure application is submitted on time as specified in this announcement. Also, the Program Manager will ensure the organization is capable of delivering the program/activities as described in the announcement based on the applicant’s project narrative.

Qualified applications will be referred to a panel of 2-3 program staff and/or technical experts, and adjudicated among the criteria described above. In general, the highest-rated proposal will be selected, however, FAS may occasionally select out of score order for policy reasons, such as geographic distribution, incorporation of minority-serving institutions, past experience, etc.

Confidentiality and Conflict of Interest
Technical and cost proposals submitted under this funding opportunity will be protected from unauthorized disclosure in accordance with applicable laws and regulations. FAS may use one or more support contractors in the logistical processing of proposals. However, funding recommendations and final award decisions are solely the responsibility of FAS personnel.

FAS screens all technical reviewers for potential conflicts of interest. To determine possible conflicts of interest, FAS requires potential reviewers to complete and sign conflicts of interest and nondisclosure forms. FAS will keep the names of submitting institutions and individuals as well as the substance of the applications confidential except to reviewers and FAS staff involved in the award process. FAS will destroy any unsuccessful applications after three years following the funding decision.

F. Federal Award Administration Information

Notice of Award
Notice of award will be given to the institution via email. This email is not an authorization to begin performance. The notice of Federal award signed by the grants officer (or equivalent) is the authorizing document through electronic means. It should also indicate if there are any pass-through obligations that successful applicants are required to meet upon receiving award funds, including specific timeline requirements.

Administrative and National Policy Requirements
All successful applicants for all grant and cooperative agreements are required to comply with Standard Administrative Terms and Conditions for Overseas Federal Assistance Awards, which can be found on the FAS website:

https://www.fas.usda.gov/grants/general_terms_and_conditions/default.asp

The applicable Standard Administrative Terms and Conditions will be for the last year specified at that URL, unless the application is to continue an award first awarded in an earlier year. In that event, the terms and conditions that apply will be those in effect for the year in which the award was originally made.
Before accepting the award the Recipient should carefully read the award package for instructions on administering the grant award and the terms and conditions associated with responsibilities under Federal Awards. Recipients must accept all conditions in this NOFO as well as any Special Terms and Conditions in the Notice of Award to receive an award under this program.

Reporting

Federal Financial Reporting Requirements. The Federal Financial Reporting Form (FFR), as known as the SF-425, must be submitted semi-annually (the reporting period ending every 6 months after the start date of the agreement) within 30 days of the end of the reporting period, with the final FFR submitted within 90 days of the end of the agreement. The required form is available online at:

https://www.grants.gov/web/grants/forms/post-award-reporting-forms.html#sortby=1

At the top of the website select FORMS, and from the drop down box select POST AWARD REPORTING FORMS.

Program Performance Reporting Requirements.
Performance Progress Reporting must be submitted semi-annually (the reporting period ending every 6 months after the start date of the agreement) within 30 days of the end of the reporting period, with the final PPR submitted within 90 days of the end of the agreement, and should include details the activities undertaken and progress made during the reporting period.

Program Performance Requirements.
• Ensure that each Fellow completes the Borlaug Fellowship Program Evaluation.
• A brief Fellow final report before the fellow departs the U.S. (Template will be provided).
• The Principal Investigator or Mentor will submit a final report to USDA/FAS within 30 days after the Mentor visit. (Template will be provided).
• The Principal Investigator or Mentor will submit semi-annual progress reports.
• Reports should include the following:
  o Summary of activities, accomplishments, and any problems encountered or overcome
  o Photographs, when possible
  o Completed program evaluations and action plan
• An invoice/claim cannot be paid if a progress report is past due, and will not be paid until the required report has been received.

Close Out Reporting Requirements.
Within 90 days after the end of the period of performance, or after an amendment has been issued to close out a grant, whichever comes first, recipients must submit a final
FFR and final progress report detailing all accomplishments and a qualitative summary of the impact of those accomplishments throughout the period of performance.

After these reports have been reviewed and approved by OCBD, a close-out notice will be completed to close out the grant. The notice will indicate the period of performance as closed, list any remaining funds that will be de-obligated, and address the requirement of maintaining the grant records for three years from the date of the final FFR.

The recipient is responsible for returning any funds that have been drawn down but remain as unliquidated on recipient financial records.

G. Awarding Agency Contact Information

Contact and Resource Information

For all general questions, contact:
Tim Sheehan, Branch Chief
Hours of operation: 9:00 AM – 4:30 PM Eastern Standard Time
Telephone: (202) 690-1940
E-mail address: BorlaugProposals@fas.usda.gov
1400 Independence Ave, SW #3226-South
Washington, DC 20250-1031

H. Additional Information

1. Extensions

Extensions to this program are allowed.

Applicants may request a no-cost extension in order to complete all project activities. The request must be submitted 60 days prior to the expiration of the performance period. Requests for extensions are subject to approval by FAS.

2. Prior Approval

The Recipient shall not, without the prior written approval of the FAS Program Manager, request reimbursement, incur costs or obligate funds for any purpose pertaining to the operation of the project, program, or activities prior to the approved Budget Period/Performance Period.

3. Budget Revisions

a. Transfers of funds between direct cost categories in the approved budget when such cumulative transfers among those direct cost categories exceed ten percent of the total budget approved in this Award require prior written approval by the FAS Program Manager.

b. The Recipient shall obtain prior written approval from the FAS Program Manager for any budget revision that would result in the need for additional resources/funds.

c. The Recipient is not authorized at any time to transfer amounts budgeted for direct costs to the indirect costs line item or vice versa, without prior written approval of the FAS Program Manager.
## Appendix A

### Borlaug Fellowship Program for Asia and Latin America

#### Index of Fellowships

<table>
<thead>
<tr>
<th>Fellow Reference Number</th>
<th>Country</th>
<th>Gender</th>
<th>Fellowship Length (weeks)</th>
<th>Research Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bangladesh</td>
<td>Male</td>
<td>12</td>
<td>Identification of wheat blast resistant lines under natural and inoculated condition and association mapping for blast resistance.</td>
</tr>
<tr>
<td>2</td>
<td>Cambodia</td>
<td>Male</td>
<td>12</td>
<td>Identification of Invitro Antimicrobial Activity Determination of Plant Derived-Products from Cambodia</td>
</tr>
<tr>
<td>4</td>
<td>India</td>
<td>Male</td>
<td>12</td>
<td>Quantitative Microbiological Risk Assessment for Ensuring Food Safety</td>
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<tr>
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Individual Proposals and Action Plans

Fellow #1, Bangladesh, Male/ NOFO: USDA-FAS-10777-0700-10.-18-0001

Proposal
1. The goal of my research is to identify the sources of resistance to wheat blast
2. Objectives:
   a) Screening of wheat lines and varieties under natural and inoculation
   b) Characterization of resistant lines based on 2NS translocation
   c) Selection of promising lines and association study
3. Background information: Wheat blast, a fearsome fungal disease caused by Magnaporthe oryzae (syn. Pyricularia oryzae) pathotype Triticum was first discovered in Parana State of Brazil in 1985. Since then, it has spread to central and southern Brazil, Santa Cruz region of Bolivia, south and south-eastern Paraguay, and north-eastern Argentina. The pathotype Triticum is genetically distinct from rice-infecting pathotype of M. oryzae. However, the blast pathogen is very diverse and exhibits many pathotypes that can mutate and may cross-infect different hosts leading to genetic recombination and evolving new strains with increased virulence. The typical symptom of wheat blast is characterized by rachis infection resulting in partial or complete bleaching of spikes with no grain formation or shrivelled and light-weight deformed grains. Leaf symptom appears as eye-shaped grayish to tan necrotic lesions surrounded by dark margins. Infected seed, airborne spores, infected crop residues and some alternate grass hosts are known to serve as the sources of inoculum for wheat blast. The disease has increasingly become a serious biotic constraint to wheat production in the warmer areas of the Southern Cone region of South America affecting up to 3 million hectares with yield losses ranged from 10 to 100% depending on year, genotype, sowing time, rainfall and disease severity.

In February 2016, the first incidence of wheat blast beyond South America was recorded in several southwestern and southern districts of Bangladesh. Morphobiometrical and molecular marker analysis established that the wheat blast observed in Bangladesh was caused by M. oryzae pathotype Triticum. The incidence was significantly widespread accounting for approximately 15% of Bangladesh’s total wheat area and affecting about 15,000 hectares with yield losses reaching up to 100%. This large scale detection of wheat blast is very alarming and significant given the levels of crop losses incurred by small farmers relying on wheat as the second most important cereal crop. Most importantly, this emerging disease represents a serious threat not only for Bangladesh wheat production but also for regional food security in South Asia, where the people consume over 100 million tons of wheat per annum. Before wheat blast incursion in Bangladesh this region received low attention. There is not enough information available about blast pathogen biology and epidemiology. This triggers urgent need to develop integrated disease management solutions through multiple interventions to mitigate the threat of wheat blast in Bangladesh and its potential spread to South Asia and other wheat growing regions with similar climates.
4. During the fellowship, I want to accomplish the following tasks:
• Techniques of isolation, multiplication and preservation of blast pathogen
• Inoculation of wheat lines with blast isolates
• Scoring wheat lines for blast reactions
• Identify elite wheat lines showing blast resistance
• Characterizing the lines based on 2NS translocation
• Association analysis for blast resistant

At present I am working with the project entitled "Identifying sources of resistance to wheat blast and their deployment in wheat varieties in wheat varieties adapted to Bangladesh". I have a total of 14 years of experience in wheat breeding. I had a comprehensive training on QTL mapping for the traits associated with heat tolerance in wheat during my PhD research. My previous experience in phenotyping of large population, DNA and marker techniques gives my adequate confidence of carrying out the proposed work. Most of the wheat lines used in Wheat research Centre, Bangladesh are CIMMYT based lines. CIMMYT is going to establish a precision phenotyping platforms (PPP) in Bangladesh. CIMMYT germplasms that will be grown in ppp are already genotyped through USAID funded project led by KSU, USA. My proposed program can be carried out at USDA-ARS, Fort Detrick or KSU, Manhattan with the help of CIMMYT. I believe, USDA fellowship could play an important role in scientific exchange and our capacity building so that we can continue varietal development program efficiently.

5. Bangladesh experienced wheat blast for last two consecutive years. In the first year (2016) this disease was only confined to the 8 southwestern and southern districts of Bangladesh. In 2016 an estimated loss owing to blast disease was about 230 million US$. If this disease spread to other parts especially the northern wheat growing zones, there will be a huge economic impact. Wheat blast could be a potential thread not only for Bangladesh but also for the neighboring countries where rainfall and temperature patterns are similar to Bangladesh. Deployment of blast resistance to the adapted Bangladeshi wheat varieties will be a safeguard for wheat productivity and national food security.

Action Plan
1 University and laboratory orientations
2 Isolation of wheat blast pathogen from symptomatic plant parts. Culture the pathogen and observation under microscope. Use of diagnostic markers for confirmation of MoT.
3 Multiplication and preservation of blast pathogen
4 Multiplication and preservation of blast pathogen
5 Blast disease scoring techniques in green house.
6 Identification of elite wheat lines showing blast resistance
7 Identification of elite wheat lines showing blast resistance
8 DNA extractions and characterizing the lines based on the presence or absence of 2NS translocation
9 DNA extractions and characterizing the lines based on the presence or absence of 2NS translocation 10 Association analysis for blast resistant based
11 Association analyses for blast resistant and identification of new gens or genomic regions
12 Report writing, presentation and closing
Fellow #2, Cambodia, Male/NOFO: USDA-FAS-10777-0700-10.-18-0032

Proposal

(1) Goal: The main aim of this collaborative research is the identification of invitro antimicrobial active plant extracts and substances derived from Cambodian traditional medicinal plants.

(2) Research Objectives
2. Specific objectives are i) Fractions separation of plant extracts by prepHPLC; ii) Identification of main present secondary metabolites in plant extracts by HPLC with diode array detector and/or by GC/MS, NMR; iii) Determination of antimicrobial activity of plant extracts and compounds against important human and animal pathogens with special focus on inhibitory activity against antibiotic resistant bacteria and biofilms; and iv) Determination of antimicrobial combinatory effect of plant extracts and compounds against antibiotic resistant pathogens. The research will be focused not only on intensive antimicrobial activity screening and direct antimicrobial effect, but mainly on advanced testing of antimicrobial activity e.g. antimicrobial combinatory effect evaluation, experiments carried out with antibiotic resistant bacteria and biofilms. For extracts exhibiting a strong inhibitory effect HPLC, GC/MS and NMR analysis will be used to determine main present secondary metabolites and these will be further tested for its antimicrobial activity.

3. Plants offer an infinite number of compounds supposing to exert direct and synergistic activity and many of them have already been reported to have such ability. However, most of these compounds, direct effect and especially synergistic activity have been tested just in combination with several antimicrobials or with particular antimicrobial groups, whereas interactions with other preparations were not scrutinized. We therefore suppose systematic search through plant secondary metabolites can lead to discovery of antimicrobials active against microorganisms, esp. resistant strains and biofilms which act as resistance-modifying or otherwise potentiating agents. Based on literature review, selected based on phytochemical and ethnobotanical data, were identified edible and medicinal plant species traditionally used by indigenous people in Cambodia, 20 plant species will be used. Other plants will possibly be included, according to results of screening tests. Some of selected extracts and substances are currently available in our laboratories collected during ethnobotanical field visits in 2014-2016 in Cambodia, whereas the remaining will be performed during the research. Extracts will be prepared by maceration in a solvent (ethanol or methanol for 24 hours, filtration, and evaporation under vacuum), water distillation using Clavenger apparatus or by supercritical fluid extraction (SFE). Standards, antibiotics, solvents, growth media and other chemicals will be purchased from commercial sources (e.g. Sigma-Aldrich, Oxoid). Extracts will be first separated into several fractions by prepHPLC. These fractions will be tested for their antimicrobial activity and active fractions will be separated again. Analyses of extracts as well as identification of isolated compounds or their synthesized analogs will be performed using conventional analytical methods such as HPLC coupled with diode array detector and/or MS, GC-MS, NMR. Test samples will be evaluated for their inhibitory effect against both aerobic and anaerobic microorganisms such as enteropathogenic bacteria, oral pathogens and bacteria responsible for respiratory infections, which considered as the most common pathogens and whose inhibition is associated with a variety of problems, such as antibiotic resistance or formation of biofilms.
Standard strains (Oxoid) and clinical isolates, in its antibiotic susceptible and resistant forms will be used. Antimicrobial activity will be evaluated in vitro based on minimum inhibitory concentrations (MIC) using broth microdilution method according to CLSI (2009) modified following Cos et al. (2006) for effective testing of antimicrobial potential of substances. Based on its MIC selected samples will be tested for its combinatory antimicrobial effect with antibiotics by checkerboard method (Odds, 2003). Antibiotics will be selected based on resistance of individual microorganisms (oxacillin, tetracycline).

4. My research interests and scientific background are linked to this proposed collaborative research. I am now working on food and nutrition quality and safety and studies on medicinal plants and test their biological activity on growth inhibition of bacteria and yeast causing human and veterinary diseases or food poisoning and spoilage. The United State of America has numerous Universities and Research Centers internationally recognized with long standing experience in teaching and agricultural research, e.g. Michigan State University and University of California, Davis have a rich tradition in research on tropical and subtropical plants in fields of phytochemistry and pharmacology as well as food safety and quality assurance, of which are essential for my fellowship in terms of advisory and technical support. My main goal is to extend research and collaboration with U.S. Universities and Research Centers to work on improving food safety and antimicrobial resistance in Cambodia public health concern. Integration of my knowledge on studies of plants derived antimicrobials from Cambodia and microbial food safety integration will be made of good use. For these reasons, I would like to spend my fellowship weeks through Borlaug Fellowship Program of next winter semester (Oct-Dec 2018) for my research stay at the State under supervision of U.S. mentor. By participating in the fellowship, I will be able to upgrade my capacity and skills to become more competence to educational and economically contribute towards of local and regional development as well as establishment of scientific networking and collaboration between my home university and U. S. institutions.

5. Systematic research focused on direct and combinatory antimicrobial activity evaluation of plant extracts and their secondary metabolites may lead to identification of new effective compounds with potential use in various sectors of food, cosmetic and pharmaceutical industries. Expected results can be used in development of new dietary supplements and animal feed, cosmetics, disinfectants, or herbal drugs, with special focus on infectious diseases and antibiotic-resistant organisms. Effective combinations can significantly improve efficacy of antimicrobials against both resistant and sensitive pathogens. This would also enable to reduce dosage of drugs and thus contribute to reduction of overall antibiotic consumption. Moreover, lower doses would help to minimize risk of undesirable side effects associated with antibiotic treatment. Positive combination can also increase efficacy and extend activity spectrum of antimicrobials used in food industry. Successful food safety improvements through reduction of microbial pathogens, esp. resistant strains will strengthen food security through improving the health and nutrient uptake of food insecure populations, as well as supporting improved productivity and livelihoods.

**Action Plan**

Research will be mainly carried out in Laboratory of Microbiology, University of Battambang, Cambodia and partially some experiments will be conducted at U.S University or Research Center during the fellowship, which are equipped by variety of facilities and equipment needed
for activities of the team in this research that enable a complex analysis of plant extracts and derived products – from bioactivity screening, separation of active fractions and compounds, to chemical characterization and structure identification. Prior to the fellowship stay in the State, plant raw materials for tests of bioactivities and phytochemical analyses will be already obtained from wild collections in Cambodia and crude plant extracts will be ready prepared for delivery to the State, and subsequently tested in order to evaluate their antimicrobial effect using various types in vitro methodology studies as described earlier.

Monthly research plan during the fellowship

- First month (Week 1-4): Plant extract will be distilled or extracted from plant materials. First experiments to screen and determine the antimicrobial activity of extracts in vitro will be performed. Active extracts will be separated to individual fractions using prepHPLC, whereas fractions will be further tested for antimicrobial effect.

- Second month (Week 5-8): In active extract fractions will be identified the main secondary metabolites using advanced chromatographic methods. The standards of these metabolites will be tested in vitro for their direct as well as combinatory antimicrobial activity. The initial screening and evaluation of antimicrobial activity from plants extracts as well as fractions identification of active extracts using prepHPLC will be performed and the detailed testing in vitro for their direct and combinatory antimicrobial activity will continue throughout the third month.

- Third month (Week 9-12): In the third month the detailed in vitro assays focused on direct as well as selected combinations against expanded spectrum of bacteria will be performed and the identification of substances using advanced chromatographic methods will intensively continue. This is the ideal arrangement of research stay during the fellowship. However, realistically, the performance of particular research phases can extend between the years. Especially the fractions identification is planned to be performed partially simultaneously with the detailed antimicrobial testing, or immediately after the detailed testing of particular active extracts or group of compounds is finished. Considering the time and labor demands of these experiments, it is difficult to predict if the detailed testing will be finished for all the perspective combinations during two years. This depends mainly on the number of positive interactions obtained from the screening tests.

Results obtained from screening studies of antimicrobial activities and their active compounds will be presented at international symposiums and published in scientific journals (e.g. Planta Medica, Journal of Antimicrobial Chemotherapy, Journal of Ethnopharmacology, Fitoterapia, Pharmaceutical Biology, or Phytomedicine) and in international symposiums or conferences (e.g. Phytochemical Society or International Union of Microbiological Societies, Society for Medicinal Plant Research, International Society for Ethnopharmacology, etc.). In other words, this collaboration will promote cooperation and solidarity among scientists and scholars between the partner institutions and beyond. In the area of human resources, it contributes to the enhancement of academic and professional staff expertise, production and transfer scientific and scholarly knowledge and information on research and development activities.
Fellow #4, India, Male/NOFO: USDA-FAS-10777-0700-10.-18-0009

Proposal

(1) Goal: The goal of my research is to carry out Quantitative Microbiological Risk Assessment of Water Supply in Dairy and Food Industry.

(2) Research Objectives

(i) Defining the Problem Statement with respect to microbiological concerns on dairy water supplies.

(ii) To carry out Microbiological Risk Assessment through a structured approach of hazard identification and characterization followed by exposure assessment and risk characterization.

(3) Background Information

Quantitative microbial risk assessment (QMRA) is a framework and approach that brings information and data together with mathematical models to address the spread of microbial agents through environmental exposures and to characterize the nature of the adverse outcomes. While most microbes are harmless or beneficial, some are extremely dangerous – these are called Biological Agents of Concern (BAC). All BAC can cause serious and often fatal illness, but they differ greatly in their physical characteristics, movement in the environment, and process of infection. Ultimately the goal in assessing risks is to develop and implement strategies that can monitor and control the risks (or safety) and allows one to respond to emerging diseases, outbreaks and emergencies that impact the safety of water or food.

Risk assessment takes place within a risk management context, to aid decision-making on managing a microbiological hazard, and considers knowledge on the nature of the hazard and the likelihood of exposure to that hazard.

The Codex document lists steps in the risk assessment process: statement of purpose, hazard identification, hazard characterization, exposure assessment, risk characterization,

Hazard Identification

Hazard identification is the first step in risk assessment. It is a qualitative process and, in addition to selecting an organism (or organisms) of concern, serves to document the important information known about the pathogen, food product and host interface. Hazards can be identified from publically available information such as published literature, epidemiological studies, foodborne disease reports, etc. In the description of the hazard, the hazard identification step will usually also summarize other aspects, such as the types of disease caused (e.g. acute or chronic) and the susceptible populations; and the mode with which the organism effects the host (e.g. through the action of toxins or through infectious mechanisms).

Hazard Characterization

Hazard characterization is defined as “The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents a dose–response assessment should be performed. For biological or physical agents a dose–response assessment should be performed if the data are obtainable”.

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Exposure Assessment

Exposure assessment is defined as “The qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposures from other sources if relevant”.

Risk characterization

Risk characterization is defined as “The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment”. Risk characterization brings together all of the qualitative or quantitative information of the previous steps to provide a soundly based estimate of risk for a given population.

(4) Describe what you hope to accomplish during your fellowship. How do your research interest and scientific background relate to the goals of your proposal? How will working with a mentor in the US help to achieve your research goals?

Microbiological hazards are the most common cause of food borne diseases worldwide. These pathogenic microorganisms gain entry in the food systems through various routes and pose great challenge to manufacturers and regulatory agencies for providing safe food for consumers. The water used for unit operations in dairy processing plants can be a potent source of pathogens and other spoilage organisms. Thus quality of water for dairy and food industry needs to be monitored in order to ensure food safety. In case of my selection for this prestigious fellowship, I plan to prepare a scientific framework in the paradigm of Microbiological Risk Assessment (MRA) for dairy water supply. This MRA shall be helpful to make guided decisions for the overall Risk Management for any food operations.

My research and teaching experience in the field of dairy microbiology complemented by my past industry stint as production officer in one of leading dairy industry in India, has prompted me to propose this initiative. Microbiological hazards can cause life-threatening consequences for consumers and in turn lead to economic and legal troubles for the manufacturers. All the stakeholders involved in food safety such as government agencies, the food business operators, testing laboratories and scientific community thus need a structured framework for assessment and management of microbial threats.

The Food Safety Modernization Act (2011) of the United States has mandated the requirement of a comprehensive science based preventive control across the food supply. Thus the proposed work falls in line with the already in-process initiatives of the US govt. and thus working with a mentor in US will help define and address the fine details of the study.

(5) How will a Borlaug Fellowship contribute to enhanced agricultural productivity, economic development, and/or food security in your country?

India is the largest producer of milk in the world but the processing and exports have been largely limited mostly owing to quality and safety issues. Though, the Food Safety and Standards Authority of India (FSSAI) was established in 2011 as a single window system for all issues related to food safety regulations in the country, many regulatory and scientific homework is still underway for effective implementation of food safety principles. The country wise priority area defined by Borlaug Fellowship for 2018 has highlighted food safety as one of the key areas for
consideration. The proposed project work seamlessly fits with the set priority area as well as with the contemporary issues of food safety concerns in India.

**Action Plan**
- **Week-1:** Orientation and Staff introduction
  Orientation program to understand the workings and know the personnel of the university and lab.
- **Week-2:** Defining the problem statement
  The statement of problem will be elaborated based on the existing infrastructure, sample area, target microorganisms and other related factors. The statement shall be important in terms of understanding the depth and extent of work.
- **Week-3-7:** Hazard Identification & Characterization
  The hazard identification will require collection of incoming water samples from dairy and food industries.
  These collected samples will be tested for total coliform count and fecal coliform count using Most Probable Number (MPN) technique. Hazard characterization will involve qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the microbiological hazard.
- **Week 8-9:** Carrying out Exposure Assessment / Dose response
  Exposure assessment will be carried out to determine the dose of the microbial hazard that an individual ingests or comes in contact with.
- **Week 10-11:** Risk Characterization
  Risk characterization will be carried out by integration of information on how much dose was received (from the Exposure Assessment) with how much risk is associated with different doses (from the Dose Response Assessment) to estimate a probability of harm (that is, risk).
- **Week 12:** Preparation and submission of project report:
  The findings of the study will be compiled in form of a project report under the guidance of the mentor and shall be submitted to the USDA and with the host university/department.
Fellow #5, India, Male/NOFO: USDA-FAS-10777-0700-10.-18-0012:

Proposal

1. Goal: Wheat blast has become a serious biotic constraint to wheat (Triticum aestivum L.) production in parts of the warmer wheat growing areas of the Southern Cone region of South America, causing yield losses of 10 to 100% in recent years depending upon the environmental conditions and genotype of the host. The disease was first reported in Parana state of Brazil in 1985 (Igarashi et al., 1986) but the first large-scale epidemic was reported from Bolivia in 1996 (Barea and Toledo, 1996) and then in Paraguay and Argentina in 2002 and 2007, respectively causing 70–80% wheat production loss (Alberione et al., 2008; Viedma and Morel, 2002). There are still some regions in South America where wheat is not cultivated because of the potential threat of this disease (Callaway, 2016). The pathogen causing the disease has been identified as Magnaporthe oryzae B.C. Couch (syn. Pyricularia oryzae Cavara). M. oryzae is composed of a range of morphologically identical but genetically different host-specific pathotypes that are specialized for infecting rice (Oryza pathotype), wheat (Triticum pathotype - MoT), perennial and annual ryegrass (Lolium pathotype), foxtail millet (Setaria pathotype), and many other graminaceous hosts. Isolates from different hosts are genetically distinct, although cross infection occurs to some extent (Duveller et al. 2016).

Wheat blast was observed for the first time outside of South America during the 2015-16 cropping season in the districts of Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, Bhola, and several other districts in the south of Bangladesh (Malaker et al, 2016) which poses a serious threat to the neighboring countries of India and Nepal. In India, the nearest state bordering Bangladesh is West Bengal which has an area of 0.3 million ha. under wheat cultivation with an average production of 0.9 mton. Although this state has a limited area devoted to wheat production, the concern is that the pathogen will spread to the major wheat producing districts of Murshidabad, Malda, and Nadia that are situated very nearer to the location of Bangladesh where the disease infestation has occurred. The pathogen has the potential to spread to the adjoining major wheat growing states of Bihar, Jharkhand, Uttar Pradesh, Haryana, and Punjab if appropriate steps are not taken to contain the disease. Developing blast resistant varieties is one of the best strategies to control this disease. This approach may be difficult as most of the cultivated wheat varieties are very susceptible to this disease (CIMMYT, 2016). However, a recent study described the possibility of breeding blast resistant wheat cultivar through introducing a short chromosomal segment called “2NS” from Aegilops ventricosa to the wheat (Cruz et al., 2016). According to another report, cultivars derived from the CIMMYT line Milan appear to contain high levels of resistance under field conditions (Kohli et al., 2011).

In this present situation, as a wheat breeder with 12 years’ experience, I would like to work on DNA marker development for wheat blast resistance by screening wheat genotypes against artificial inoculation of the disease and making primer design by DNA sequencing of resistant cultivar having 2NS background.

The goal of my research is to make DNA profiling of 2NS translocation segment of wheat (T. aestivum) and finding its relationship with wheat blast resistance.

2. Specific research objectives:

1. Screening of wheat genotypes with and without 2NS translocation segment by artificial inoculation.
2. Genomic DNA isolation and development BAC library from resistant source having 2NS background.
3. Primer designing and synthesis from sequenced database (BAC library).
4. Screening of resistant and susceptible cultivars by synthesized primers for identification of polymorphic and robust primers.

BGRI fellowship in this regard will be extremely useful to gain knowledge and skills for screening of wheat genotypes against wheat blast. With this fellowship I may be able to obtain hands on experience on culturing the pathogens, conducting inoculations, evaluating and scoring for resistance, molecular detection of pathogens, etc. at the Foreign Disease-Weed Science Research Unit (FDWSRU), USDA at Ft. Detrick, Marryland, USA. Facilities like biosafety-level-3 (BSL-3) laboratory for artificial inoculation under containment, BAC library development could be accessed to carry research programme.

**Action Plan**

1st week Visit to the laboratory, acquaintance with staff and facilities
2nd week Preparation of culture medium for MoT pathogen
3rd week Preparation of disease inoculum with spores from MoT culture and inoculation of plants with the inoculums
4th week Disease development under greenhouse condition at 28 ± 2°C (14-h day) and 23 ± 2°C (10-h night) with at least 150 μmol/m/s light intensity
5th week 1. Disease evaluation and disease scoring
2. gDNA isolation and sequencing by BAC (outsourcing) from already resistant cultivar having 2NS translocation segment
6th week Disease evaluation and disease scoring and confirmation of disease resistant and susceptible cultivar
7th week 1. Disease evaluation and disease scoring and confirmation of disease resistant and susceptible cultivar
2. Primer design and synthesis (outsourcing) from the sequenced database
8th week gDNA extraction from resistant and susceptible cultivar
9th week Screening of resistant and susceptible cultivars by synthesized primers for identification of polymorphic and robust primers
10th week PCR reaction with polymorphic primers; gel electrophoresis and subsequent analysis
11th week PCR reaction with polymorphic primers; gel electrophoresis and subsequent analysis
12th week Phenotypic and Genotypic data interpretation and report submission.
Fellow #7, Indonesia, Male/NOFO: USDA-FAS-10777-0700-10.-18-0014:

Proposal
1. The goal of my research is to find a more objective and modern method of calculating the harvesting area, productivity, production of rice, corn, and soybeans.
2. By studying the applications of remote sensing and/or Geographic Information System (GIS) Agricultural, it is expected to know how to calculate the area of harvest, productivity, production more accurately, faster, and cheaper to be applied in Indonesia.
3. There are several ways to calculate the area of harvest by applying remote sensing and GIS, some of which are analysis of vegetation index of remote sensing result and also sampling area. Based on trials conducted in 2017, calculations by area sampling provide more accurate results. But the resources and costs used to calculate by sampling area are much larger than the remote sensing analysis. It is expected that in this course, can be studied in more depth about both of these methods and synergized to get the most suitable method to be implemented in Indonesia. So far, the development of remote sensing and GIS applications in Indonesia is still limited to the analysis of the rice crop harvested area.
4. By following this fellowship, is expected to be obtained a better way to analyze harvested area and also productivity. And it would be better if corn and soybeans can also be analyzed. Working with a mentor in the US is an excellent opportunity to understand everything about applications of remote sensing and/or Geographic Information System (GIS) Agricultural. In addition, because there is no expert in Indonesia who has mastered remote sensing and can make in-depth analysis on increasing production, economic growth, and food security, then learning it with a mentor in the US would be a very valuable experience.
5. Rice, corn, and soybeans are important staple foods in Indonesia. Therefore, production data of these plants have an important role. Indonesia has long been collecting production data in a very simple way. The harvested area is calculated based on the administrative records of agricultural officers. The calculations are made indirectly based on information on the use of seeds, irrigation blocks, eye estimates, and farmer reports. Record based on eye estimation is the most commonly used method. Productivity is calculated in a more scientific way through crop cutting surveys. Production calculated in this way creates inaccurate production data and tends to overestimate. As a result, the government has become unable to make accurate food policy. The Borlaug Fellowship can contribute by providing assistance to BPS, the National Statistic Office of Indonesia, to make a better estimation of harvested area, yield, and production. This is will have a good impact on food security, economic stability, and the credibility of the government itself.

Action Plan
Week 1: Orientation and Introduction
Week 2: Learn how to estimate harvested area using remote sensing and GIS
Week 3: Learn how to estimate results and production using remote sensing and GIS
Week 4: Learn about the possibility of calculating corn and soybeans
Week 5: Practice using Indonesia data
Week 6: analysis of plant monitoring and food security
Week 7-8: Learn about the development of sampling area estimates
Week 9: compare between remote sensing analysis and sampling area estimation
Week 10: learn the scripting language so that the analysis / estimation process is faster and easier.
Week 11: evaluation
Week 12: discuss further plans
Fellow #8, Malaysia, Female/NOFO: USDA-FAS-10777-0700-10.-18-0015:

Proposal
1) The goal of my research is to develop a reliable molecular toolbox for simultaneous analysis of multiple functional genes in rice
2) The principal objectives are to identify and validate the publicly available functional markers associated with rice grain quality characteristics and disease resistance, especially blast resistance, and to develop a simple yet reliable multiplex PCR assay to analyze multiple functional genes in rice simultaneously.
3) Rice is a staple for nearly half of the world’s seven billion population, including more than fifty million undernourished people living in Asia. To increase the level and stability of rice production, we need varieties with higher yield potential, superior grain quality, and durable resistance to diseases and pests. Rice breeders these days often aim to develop new high-yielding varieties which exhibit various desirable traits hence, it is crucial to develop a rapid and reliable screening tool which can enable the breeders to screen and select multiple traits within a breeding program. It is also important to note that the tool should not be too costly, as most of the rice-growing countries are either developing or underdeveloped, putting majority of the breeders between low to medium income groups. Molecular approaches are becoming increasingly important to accelerate rice breeding programs, particularly on quality and agronomic traits that are inherited in a complicated way and they are difficult to select through conventional breeding. The last two decades have seen a growing number of genes controlling various key traits in rice discovered, and the underlying polymorphisms of some of these genes have been converted into functional markers. In the currently proposed research, previously reported genes with developed functional markers would be detected and analyzed using a multiplex PCR assay. These include, among others, the GS3 gene for rice grain shape, and Xa5, Xa13, and Xa21 genes for blast resistance. The development of this molecular toolbox will greatly benefit the current breeding programs in many rice-growing countries, especially those with limited resources.
4) As a researcher who is making a living in a developing country, I am familiar with the despair and frustration endemic to other similar nations struggling with sufficient food production. My short-term goals include advancing my knowledge in the current state-of-the-art sequencing technologies for plant genomics research, and understanding the mechanics of creating major impacts in the field of agriculture. This could be achieved through the Borlaug Fellowship. I am attracted to this professional fellowship because it offers the kind of collaborative experience which suits my personal needs and professional goals. I believe that the assigned Mentor in the U.S. will be able to guide me in my specific practice area. In the long run, my aim is to make use of all the knowledge and experience that I have acquired to help cultivate awareness among society, institutions and governments, in view of improving food and nutrition security, particularly in countries that are underdeveloped or in the developing stage. I am determined to leave a valuable imprint in the efforts to raise agricultural productivity, and therein accomplish my life's mission to contribute in the perseverence and sustainability of the world's food demands.
5) "Agriculture Biotechnology - to conduct research using marker assist breeding to identifying fungal pathogens and viral infection in developing a new rice variety that is resistant to diseases and infections" - This targeted research area for the Malaysian applicants is in line with one of our national policies (i.e., National Agro-Food Policy 2011-2020) that emphasizes on expanding
food production to ensure that food supplies are sufficient, especially for rice which is the staple of the country. My past research on rice was focused mainly on improving the quality traits of our local elite varieties, and my recent publication was on developing a local fragrant variety through marker-assisted backcrossing (MABC) [Cheng, A. et al. (2017). Rapid and targeted introgression of fgr gene through marker-assisted backcrossing in rice (Oryza sativa L.), Genome, DOI: 10.1139/gen-2017-0100]. My current research goal is to develop a reliable molecular toolbox that can screen and select multiple traits, including disease resistance traits, simultaneously within a breeding program. I am certain that the Borlaug Fellowship is a stepping stone for me to achieve my current goal. Ultimately, the Fellowship will be beneficial to the breeding programs in Malaysia and also other rice-growing countries that are often constrained in resources and high-end technology.

**Action Plan**

**Week 1:** Activities - Laboratory orientations and staff introductions; review research activities with the assigned Mentor

Planned outcomes - Completed laboratory orientations and introductions; research activities evaluated and finalized by the Mentor

**Week 2 and Week 3:** Activities - Plant rice seeds in containers or pots; exploitation of the Rice SNP-Seek database and other relevant databases to mine and select suitable markers for multiple key traits in rice, including rice grain size, plant height, aroma, amylose content, brown planthopper resistance and blast resistance.

Planned outcomes - Plant materials sown in shade house; Rice SNP-Seek database and other relevant databases exploited and suitable markers selected for further analysis.

**Week 4 and Week 5:** Activities - Extraction of genomic DNA from plant leaves; analysis of publicly available functional markers by performing singleplex PCR. Previously reported genes with developed functional markers, such as GS3 gene for rice grain shape, fgr gene for aroma, Waxy gene for amylose content, and Xa5, Xa13, and Xa21 genes for blast resistance will be used and analyzed.

Planned outcomes - Genomic DNA extracted; analysis of publicly available functional markers completed

**Week 6 and Week 7:** Activities: Development of functional markers for a particular trait in the event where previously reported markers may not be effective

Planned outcome - Functional markers for the targeted trait developed using an array-based SNP detection platform or next-generation sequencing

**Week 8:** Activities: Analysis of functional markers obtained from the current research by performing singleplex PCR

Planned outcome - Analysis of developed functional markers completed

**Week 9 and Week 10:** Activities - Development and optimization of multiplex PCR assay(s) for three or more important traits in rice

Planned outcomes - Multiplex PCR assay(s) developed and optimized

**Week 11:** Activities - Validation of the developed multiplex PCR assay(s) using at least twenty different rice genotypes with a wide range of grain size, quality and different levels of resistance

Planned outcomes - Multiplex PCR assay(s) validated for use

**Week 12:** Activities - Data reporting and discussions with Mentor on the best way forward

Planned outcome - Results reported to the research team and evaluated by the Mentor.
Proposal

1. The goal of my research is to devise a strategy to develop a N-enriched biochar-based fertiliser that nutrients released patterns are dependent on plant activity. Carbon sequestration are a process of removing carbon dioxide from the atmosphere by storing it in the soil. Implementing a global scale of biochar program could potentially offset 12% of the current anthropogenic CO2-C equivalent emissions. In agronomic perspective, the benefits of biochar are reducing leaching of inorganic-N, N2O emissions and ammonia volatilisation. Soil amended with biochar has been shown to increase CEC and water retention capacity and thus reducing the leaching of NO3 and NH4+ respectively.

2. The majority of Nitrogen in soil exists in organic forms that need to be ammonified (NH4+) and then nitrified (NO3−) before it can be incorporated by the plant. Biochar could be used to control the rates of Nitrogen cycling in the following ways: (i) enhancing the soil content of NH4+ by adsorption of the biochar. Oxygen-functional groups of biochars could help to increase the CEC of soil. (ii) enhancing the soil content of NO3− by adsorption of the biochar. Water retention capacity of biochars could help to increase the AEC of soil. (iii) large surface area could provide ideal habitat for microorganisms which could enhanced the nutrients transformation more efficiently. Thus, biochar is a alternative tool in reducing the emission of pollutants while improving soil quality by effective sequestration and bioremediation.

3. The Nitrogen leached to lakes and reservoirs are contribute greatly to the pollution and the eutrophication. Plants Only recovered 30 – 40% of Nitrogen applied as Urea. Leaching is the most critical problem caused by the use of fertilizer. It could cause problems associated as: soil acidification, increase in fertilizer costs, reduce of crop yields and affects the quality of surface and groundwater. Eutrophication is a problem due to the high concentration of nutrients especially phosphates and nitrates in the water. The abundance of these nutrients encourages the growth of algae. Decomposition of dead algae depleted the available oxygen in the water that could lead to the death of fish and other aquatic species. The contamination of ground water with Nitrates will caused Methemoglobinemiae. When large amounts of nitrates are ingested, the nitrate then reacts with oxyhemoglobin (the oxygen-carrying blood protein) to form methemoglobin, which cannot carry oxygen. That caused body tissues may be deprived of oxygen, causing possibly digestive and respiratory problems. Soil amendments by biochar addition has been proven to increase soil pH, water holding capacity, increased the cation exchange capacity (CEC) and improve mycorrhizal response in soils. Thus, biochar addition to soil could contribute to soil fertility with long lasting effects. Biochar with high minerals content (Calcium and Magnesium) could increase AEC thus possibly increased Ammonium and Phosphate adsorption capacity.

4. The proposal will focus on cocoa seedlings with the intentions of reducing fertilizer doses to achieve considerable savings of input cost. This will address of leaching studies on inland due to heavy rainfall or are made unavailable (such as P) to the plant due to the high nutrient fixation properties of these soils. Hopefully by working with the mentor, the benefits of different types of biochars could be tailored according to the farmer’s needs.

5. The Nitrogen and Phosphate losses through leaching contribute greatly to the pollution and the eutrophication of lakes and reservoirs. Hopefully by introduction of biochar usage to the farmers it will help farmers of saving foreign exchange and the huge cost of conventional fertilizers.
Action Plan
(Week 1).
Introduction to university staff and laboratory orientation.

(Week 2 - 4).
In this study we focussed new promising low temperature MicroWave pyrolysis technology and we compared the solid products with those produced by slow pyrolysis/torrefaction in the same temperature range on low temperature thermochemical conversion (up to 350 °C). This study will presents results from our experimental investigation of the impact of production conditions, i.e. pyrolysis temperature and heating method on the biochar product, its properties and stability.

(Week 5 - 8).
In this study we focussed on increasing the CEC by increasing the oxygen-functional groups found on biochar surfaces include hydroxyl, carbonyl, carboxylate, hydrogen (H), and ether that largely responsible for the CEC of such biochar. Thus, biochar ultimately increases in CEC as it ages and will contribute to reduced leaching of cations from the soil.

(Week 9 - 12).
Leaching loss of NO-3 and phosphate from agricultural soils is largely attributed to lack of anion exchange capacity (AEC). These anions leached out due to the lack of positively charged exchange sites in the soil. Coulombic interaction with positive surface charges NO-3 and phosphate will retain anions is strictly due on the AEC of soil.
Fellow #10, Mongolia, Female/NOFO: USDA-FAS-10777-0700-10.-18-0017:

Proposal
1. The goal of my research is to introduce new, cost and time efficient technology (NIRS) for implementing a concept “Healthy feed-Healthy food – healthy people” of nomadic Mongolia.
2. To achieve this goal, I am intending to adopt Near Infra Red Spectroscope (NIRS) technology in both agriculture and food sector research of Mongolia. Having the NIRS adopted in Mongolia will enable us to improve food safety issue through better analysis of animal feed and human food contamination at all levels. Adopting technology is only a start of the complex research which will require more follow up references, recommendations and methodologies to be translated into Mongolian circumstances. The following objectives are developed in consultation with the Mongolian University of Life Sciences (MULS) in order to adopt NIRS technology in Mongolia:
   - To determine sample types depending on the research object and prepare the samples for scanning;
   - To select and test appropriate methods and techniques appropriate for the research;
   - To analyze preliminary results and workout calibration formula for each type of samples;
   - Verification of results; and
   - Reporting the results in scientific articles.
3. Mongolia, with about 3 million people living on 1.5 million square kilometers, is the world’s most sparsely populated country. Mongolian food consumption is dominated by local animal products from the livestock grazed on pastureland with no proper management. Meat industry plays an important role in the food sector and is considered to be one of the most potential export sectors for future development of the country. Mongolia annually produces 200-250 thousand tons of meat from 8.2 million livestock and self-sufficient for its domestic meat consumption (MoIA, 2013). Mongolia also exports meat and meat products to Kazakhstan, Japan, Ukraine, Iran, Vietnam and China.
   As per statistics of 2007, only 6.4% of the consumed meat and 2.2% of milk were industrially processed, whereas other animal food products were not processed and had inadequate veterinary and hygiene control. Raw animal food materials and products sold in the markets were from uncertain origins and had no quality assurance and hygiene certificates.
   Grasslands and arid grazing cover 1 210 000 km2 (80% of the land area) and livestock is fully dependent on it with no monitoring of its’ substance.
   Mongolian meat and its sub products might be considered ecologically clean due to its’ free range nature of animal growth. However, pasture and food quality can be affected by several factors, such as i) pasture degradation, increase of weed plants, bare ground and decrease of hay preparation; ii) uncontrolled animal drug utilization and lack of monitoring of drug used animal slaughtering and its’ production; iii) growth in number of illegal artisanal miners (illegal use of mercury and cyanide contaminates the water and soil) and etc.
   Mongolia has professional organizations that analyses and controls animal health and food safety. However, these organizations have no constructive technology that monitors all levels of animal food production including soil; pastureland; animal and animal production stages.
   There is a need to develop a rapid, non-destructive and inexpensive technology /NIRS/ to conduct research for reliable quality of pasture and animal feed to produce safe food to the population. Adopting NIRS in Mongolia will avail reliable data source to monitor feed and food quality and security through improved information supply.
For many years, NIRS has been used by the feed and food chain as the main technique to identify chemical composition and contaminants in developed countries. In forages, in feces, in raw agricultural materials including food, NIRS methods are used to determine dry matter, ash, protein, ammonia, fat, pH, lactic acid and volatile fatty acid and heavy metals, antibiotic content as well as in vitro and in vivo digestibility (e.g., NDF, ADF) parameters.

4. I hope to learn how to use NIRS machine (both stand and portable type) at selected university or institution and process and analyze machine obtained data. I have experience in evaluating chemical composition of animal feed and the effect of protein fraction of animal feed on rumen degradability and whole tract digestion (by in situ, in vitro and in vivo method) during my PhD study at Chungbuk National University of South Korea. Since my PhD completed, I have been working as a lecturer at MULS. The University has 6 animal science research laboratories that analyze feed proximate on samples of hay and fodder concentrates; evaluate chemical composition on meat and dairy products by traditional method. There is a need to upgrade my study/research by NIRS assessment for feed and food safety. We have portable NIRS-512 machine that is sponsored by Texas Agricultural and Mechanical University project which is currently used for only animal nutritional trail. However, we are lacking in analyzing the trail data produced by NIRS – 512 due to lack of knowledge on calibration method. Upon my learning of using NIRS, I would like to extend usage of the machine for feed, fecal and food analyzes through Norman E. Borlaug Fellowship program.

5. Nowadays NIRS technology is a new approach to assess both feed quality and food safety. So I would like to have technical training on usage of NIRS machines and its’ result analysis and evaluations through Borlaug Fellowship program at the appropriate institute, university and research laboratory.

If my application is successful, the usage of NIRS technology will enable me to contribute to the following achievements in my country with my extended knowledge and experience of using NIRS technology and evaluating the results:

1. Contribute to pasture quality map development providing appropriate information on pastureland quality through NIRS analysis;
2. Contribute to develop pastureland management plan and relevant guidelines to local administrative units based on different status of pasture quality patterns and its’ safety measures to graze livestock for healthy products;
3. Consult to determine contamination free zones for meat and dairy production;
4. Establish network with peer researchers and improve technology transfer between American and Mongolian research institutes especially in agricultural sector; and
5. Provide appropriate information and informed recommendations to policy makers.

Action Plan
I have developed my study plan with the expectation to achieve my objectives identified in research proposal as part of my application.

Week 1: Travel; settle in; University and laboratory orientations; staff introductions
Tentative study plan
Period Study area
1 week Travel, settle in, University and laboratory orientations and staff introductions.
2 week Finalize the research proposal in consultation with the identified institute and mentor and learn to operate NIRS machine and related devices. Infrared spectroscopy has attained a primary position in monitoring the composition of feed and food products before, during, and following
processing. It has a wide range of feed and food applications and has proven successful in the laboratory, at-line, and on-line.
Infrared spectrometers must be calibrated for each analyte to be measured and the analyte must be uniformly distributed in the sample.
3 week Learn and practice feed and food sample preparation method for NIRS analysis:
4 week Analyze feed chemical composition by NIRS. Chemical compositions such are dry matter, ash and etc.
5 week Spectra and reference analyze by software application and calibration method Learn to analyze chemical compositions of feed by the NIRS test and evaluate how to make the test results useful in Mongolian meat production chain
6 week Analyze of feed contamination by NIRS The soil, water and pastureland are contaminated by heavy metals, which directly effects agricultural production.
7 week Spectra reference analyze by software application and calibration method
8 week Analyze of meat chemical composition by NIRS method Consumable heavy metals from contaminated agricultural crops on a long-term basis can accumulate in plant crops, and therefore in animals which consume the crops. Once accumulated in animals or crops, to decontaminate or excrete can be difficult, even though the immobilizer-assisted management skill is suggested for removing metal-contaminated agricultural soils for safer food production (Kim KR and Kim JG, 2009).
In meat-producing (growing) animals deposition of toxic substances in edible products is much harder to establish and adequate sampling and analysis of the excreta for the toxic substance studied is often not feasible (Cornelis Adriaan Kan, 2009).
9 week Spectra reference analyze by software application and calibration method
10 week Analyze of food contamination by NIRS
The food chain that starts with farmers and ends with consumers can be complex, involving multiple stages of production and distribution (planting, harvesting, breeding, transporting, storing, importing, processing, packaging, distributing to retail markets, and shelf storing. Various study methods can be employed at each stage in food chain.
Each of this stage is not controlled regularly because of special nomadic culture in Mongolia.
The primary advantage of NIR spectroscopy is that once the instrument has been calibrated, several constituents in a sample can be measured rapidly (from 30 s to 2min), simultaneously and easy. This is desirable advantage for Mongolian sparsely populated nation.
11 week Spectra reference analyze by software application and calibration method
12 week- Produce and submit final report of study
NIRS method for feed and food quality can play a major role in Agricultural sector in Mongolia nowadays because possibility of contamination pastureland is maybe higher in some areas where rapid growth in number of illegal artisanal miners. This will enable us to closely monitor where to produce meat and its sub products to establish healthy food chain which is a key factor to have healthy population.
In addition, being personally exposed to the institution with similar research area and interest, it will expand my network to my peers in American research institutes and it will develop more collaborative research.
Fellow #11, Myanmar, Female/NOFO: USDA-FAS-10777-0700-10.-18-0018:

Proposal
1. The goal of my research is to get skills and confidence to complete Pest Risk Analysis for new fruits and vegetables.
2. The specific research objective(s) that will achieve my goal are,
   a. To study the overall process of Pest Risk Analysis (PRA) of the United State Department of Agriculture's (USDA) to be familiar with the structure and function of the PRA section
   b. To get hands-on experience by conducting a number of trial PRAs
   c. To develop the skills to complete PRAs within the context of the IPPC.
3. Background information of Research
Myanmar is situated in Southeast Asia and is bordered on the north and north-east by China, on the east and south-east by Laos and Thailand, on the south by the Andaman Sea and the Bay of Bengal and on the west by Bangladesh and India. It is located between latitudes 09 32'N and 28 31'N and longitudes 92 10'E and 101 11'E.
The country covers an area of 677,000 square kilometers (261,228 square miles) ranging 936 kilometers (581 miles) from the east to west and 2051 kilometers (1275 miles) from north to south. It is a land of hills and valleys and is rimmed in the north, east and west by mountain ranges. Enclosed within the mountain barriers are the flat lands of Ayeyarwady, Chindwin and Sittaung River valleys where most of the country's agricultural land and population are concentrated. The climate of Myanmar is roughly divided into three seasons: Summer, Rainy Season, and Winter Season. The country as a whole can be divided into five physiographic regions—the northern mountains, the western ranges, the eastern plateau, the central basin and lowlands, and the coastal plains which generate a diversity of climatic conditions. This is why different kinds of fruit and vegetables such as tropical fruits as well as temperate fruits are available all-year round.
Myanmar is a member of World Trade Organization since 1995 and one of the contracting parties of IPPC.
Myanmar has abruptly increasing import partners such as 126 in 2012 to 167 in 2016 together with same trend of imported number which is increased from 3272.00 in 2012 to 3607.00 products in 2016. About 16 categories of products have been imported to Myanmar including various fresh fruits and vegetables as shown in following figure. Among them, about 66 countries including United States (US) import vegetables to Myanmar and US is the 9th largest import partner by contributing the import value of 10768.05 (us $ thousand) with import product share 4.97% in 2016 (https://wits.worldbank.org).
With increasing the importation of different kind and quantity of fresh fruits and vegetables and other agricultural commodities to Myanmar from the various parts of the world, the risk of introduction of new dangerous pests together with them is extremely high. WTO stated that every country has its own right to protect his nation by using The WTO Agreement on the Application of Sanitary and Phytosanitary Measures (WTO-SPS Agreement) which is how governments can apply food safety and animal and plant health measures while facilitating trade. With regard to plant health, the WTO-SPS Agreement allows countries to set their own standards to protect their economy or environment from damage due to the entry, establishment or spread of pests of plants. At the same time, it encourages them to use international standards, guidelines and recommendations, where they exist, when developing their sanitary and phytosanitary measures (Article 3 of the WTO-SPS Agreement).
The WTO-SPS Agreement encourages its members to harmonize their sanitary and phytosanitary measures on the basis of international standards. The WTO recognizes the IPPC as the relevant international standard setting body for plant health. The IPPC is an international treaty, binding to contracting parties. Contracting parties have the right to use phytosanitary measures to regulate imports, but have an obligation to do so only where necessary and technically justified. Conducting pest risk analyses. Like the IPPC, the WTO-SPS Agreement states that measures must be science-based and not used for the purpose of trade protection. It requires that phytosanitary measures be based on an assessment of the risk to human, animal or plant health, taking into account risk assessment techniques developed by the relevant international organizations, and that they should be technically justified.

Pest risk analysis (PRA) is a form of risk analysis conducted by regulatory plant health authorities to identify the appropriate phytosanitary measures required to protect plant resources against new or emerging pests and regulated pests of plants or plant products.

National Plant Protection of Myanmar never conducted the PRA for imported agricultural commodities before. Now it is considered that PRA for every imported agricultural commodity should be conducted to protect nation’s plant, animal and environment’s health and to provide the list of quarantine pest and their phyto-sanitary risk management to importing partners. However, recent working staff in our NPPO has not got the international hand-on trainings on PRA and do not have enough skill and confidence to complete the PRA process. Therefore, it is urgently needed to study on Pest Risk Analysis for fruits, vegetables at prestige NPPO of the United State of America.

4. During my fellowship, I hope that I will be familiar with the structure and function of a PRA unit and documents. In addition to this, I have conducted a number of trial PRAs as well as seen and discussed examples of many more. I should have the self-confidence to complete PRAs and will know where to look for information and where to seek help when required. To accomplish my objectives, I will work closely together with the mentor in the U.S and they will guide me how to conduct Pest Risk Analysis (Pest Risk Assessment and Risk Management) based on commodity as well as pest.

5. A Borlaug Fellowship will take responsible to conduct PRA for imported fruits and vegetable, surveillance activities for quarantine pests and conducting training for the pest management of quarantine pests for the government staff, farm workers and private entrepreneurs. In addition to this, she will participate to develop the proper pre shipment phytosanitary treatments for exporting of fruits and fruiting vegetables. By doing so, her efforts will be very valuable to facilitate the import and export of fruits and vegetable as well as agricultural commodities which lead to improve the economic development and food security of the country. By sharing her expertise on the quarantine important pests and their management, the various stakeholders will improve the knowledge on the area of quarantine pests, their management and important of food safety issue for export market and produce better quality and safe products which will enhance the agricultural productivity of the country.
## Action Plan

<table>
<thead>
<tr>
<th>Week</th>
<th>Activities</th>
<th>Outcomes</th>
<th>Special Material requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lectures on -Plant Quarantine in USA - Structure, Duties and Functions of Pest Risk Analysis and Market Access Information Unit in USDA</td>
<td>Have got knowledge on -the structure, duties and function of Plant Quarantine System in USA; -PRA and Market Access Information Department/Unit in USDA</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Lectures: - Pest Risk Analysis and Related ISPMs</td>
<td>Have got the knowledge on why and how to conduct PRA based on commodity/pest by USDA</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Doing Arthropod Pest Risk Analysis</td>
<td>Improve skill and confidence to complete PRA</td>
<td>A laptop if possible</td>
</tr>
<tr>
<td>4</td>
<td>A climatic prediction analysis NAPPFAST, CLIMEX analysis, Ecoclimatic index</td>
<td>Have got technology and skill for climatic prediction analysis for pest establishment and distribution to new area to evaluate the impacts of the pest</td>
<td>A laptop if possible</td>
</tr>
<tr>
<td>5</td>
<td>A climatic prediction analysis NAPPFAST, CLIMEX analysis, Ecoclimatic index</td>
<td>Have got technology and skill for climatic prediction analysis for pest establishment and distribution to new area to evaluate the impacts of the pest</td>
<td>A laptop if possible</td>
</tr>
<tr>
<td>6</td>
<td>Doing Arthropod Pest Risk Analysis</td>
<td>Improve skill and confidence to complete PRA</td>
<td>A laptop if possible</td>
</tr>
<tr>
<td>7</td>
<td>-Field visit to Pest Free Area of Fruit fly - Doing Arthropod Pest Risk Analysis</td>
<td>-Have got knowledge and experiences on the implementation of pest free area or low pest -prevalence area</td>
<td>A laptop if possible</td>
</tr>
<tr>
<td>Week</td>
<td>Activity Description</td>
<td>Expected Outcome</td>
<td>Notes</td>
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<tr>
<td>8</td>
<td>Lectures and Practices on Phytosanitary Treatments for quarantine pests</td>
<td>Have got knowledge and technologies on the available phytosanitary treatments for</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Field visit to Pre-shipment Phytosanitary treatment facilities, Doing Arthropod Pest Risk Analysis</td>
<td>Have got knowledge and technologies on the available phytosanitary treatments for</td>
<td>A laptop if possible</td>
</tr>
<tr>
<td>10</td>
<td>Doing Arthropod Pest Risk Analysis</td>
<td>Improve skill and confidence to complete PRA</td>
<td>A laptop if possible</td>
</tr>
<tr>
<td>11</td>
<td>Doing Arthropod Pest Risk Analysis</td>
<td>Improve skill and confidence to complete PRA</td>
<td>A laptop if possible</td>
</tr>
<tr>
<td>12</td>
<td>Making Complete PRA for one selected imported fruit from USA to Myanmar Closing Ceremony</td>
<td>Finish complete PRA for one selected imported fruit from USA to Myanmar</td>
<td>A laptop if possible</td>
</tr>
</tbody>
</table>
Proposal

Pheromone-based population management of Ephestia cautella: food safety and security through environmental-friendly pest management

1. The goal of my research is to learn the pheromone-based pest management methods as environment and consumer-safe pest management alternative to the synthetic neurotoxic management practices currently in use, and thereby to ensure the food security and safety.

2. The above goal will be achieved by:
   i) Comparing the effect of two pheromone compositions (straight ZETA and the blend of four components) on mating disruption in E. cautella.
   ii) Studying how the efficacy of MD in E. cautella can be maximized.
   iii) Determining if the MD alters with the type of grains/food.
   iv) Testing MD under real warehouse conditions (Different structures/complexity, density of traps)
   v) Testing if MD efficacy can be enhanced when pheromone is used with different kairomones (Food oils)

3. Rice is the staple food of Sri Lankans. At present, Sri Lanka produces 1,500,000 MT of paddy and imports 500,000 MT per annum. For Sri Lankans, the daily per capita availability and energy from cereals are 469.37 g and 1627.20 calories, respectively. To meet the consumer demand year around, as the production mainly seasonal, paddy has to be stored. However, the annual post-harvest losses of paddy reach 9.2 billion LKR (US$ 60 million). Insects cause 80% of the loss during paddy storage. Ephestia cautella is a major pest of stored paddy, other cereals and processed products. Its high population densities deteriorate stored food quantitatively and qualitatively including pathogenic microbial infection and allergic reactions in human.

According to FAO specifications, food safety, food security and risk management link together. Use of insecticides/fumigants in pest management accompanies poisoning from direct exposure, negative impacts on environment, presence of pesticide residues. Hence pest management using reduction/elimination of synthetic pesticides is sought. ISO 6322-1:1996(E) and SLS (Sri Lanka Standards) I528-I: 2016 highlight the importance of having minimum (permissible) quantities of pesticide residues/toxins in the grains stored. In Sri Lanka, food safety is regulated through the Food Act No. 26 of 1980 and Amendment Act No. 20 of 1991. Contamination of food in Sri Lanka by chemicals and presence of pesticide residues in food have been of increasing concern in the recent years. High rate of renal failure disease in the North-Central province of Sri Lanka, where my university is located, is linked to use of agrochemicals. Therefore, development of biorational pest management alternatives is timely needed.

Ephestia cautella females release a sex pheromone consisting of principally (Z,E)-9, 12-tetradecadienyl acetate (ZETA) and (Z)-9-tetradecenyl acetate (Z9-14: OAc) at 14:1. An analogue alcohol, (Z,E)-9,12- tetradecenol (ZETOH), when combined with ZETA, generally enhances responses of males of moths of the subfamily Phycitnae. However, ZETOH has the opposite effect of inhibiting responses by the almond moth, Cadra cautella.

Orientation of male moths towards the female is disrupted by high concentrations of synthetic pheromone. This process is termed mating disruption (MD). In my laboratory in Sri Lanka, we completed a substantial preliminary study on MD of E. cautella in September, 2017 (Sammani et al., 2017). We found that MD of E. cautella increases with the synthetic pheromone
The maximum MD (75%) is at 4.5 mg of ZETA. MD varies with the population size; maximum at the intermediate population size than the highest or the lowest population size. Presence of air flow increases the MD, possibly due to the increase in the dispersion of pheromone plume.

4. What you hope to accomplish during your fellowship?

The above basic findings on MD were completed using the minimum facilities available locally. However, further experiments are required to understand grey areas before recommending this valuable technology for safe protection of stored food in facilities. The next experiments need the close monitoring of a scientist who has been using this technology as well as access to well-equipped laboratory, warehouse and, processing plants having the structures (such as complexity) required/designed for such studies.

As a similar research program is currently on-going at Dr. Charles Burks’s laboratory in Parlier, CA, our country will be immensely benefitted if the proposed research can be conducted there. Dr. Burks has obtained agreement from Dr. Kent Daane (kdaane@ucanr.edu), at University of California, Berkeley to co-host my appointment.

5. My Scientific background relate to the goals of my proposal:

I have been a stored-product entomologist in the last 15 years. My PhD at the University of Manitoba, Canada (2007-2011) was on ‘Effects of methoprene on Tribolium castaneum (Coleoptera: Tenebrionidae)’. Methoprene is an insect hormone analogue and therefore is a safe chemical (LD50 rats is 34,500 ppm).


Also refer attached files, Wijayaratne and Fields 2010; Wijayaratne et al., 2012a; Wijayaratne et al., 2012b).

I continue to work using reduced-risk chemicals, including the US$ 50,000 grant received in 2016. The skills acquired by me by working with entomologists in Canada, Israel, Thailand, Egypt, India, Iran and Nigeria, and my experience on moth research (Refer, Wijayaratne and Fields, 2012) would provide me ideal foundation for this new research using pheromones, another insect-specific compound.

6. How will working with a mentor in the U.S. help you to achieve your research goals?

Comprehend the latest techniques used in the protection of stored food from pests to meet the food safety.

Enhanced agricultural productivity, Economic development and Food security in my country. Innovations in safe food protection will reduce the risk, cost and he labour associated with the hazardous insecticides toxic to the nervous and respiratory systems of insects and human. This will augment the marketability, market value, income and profit earned by the producers. As our country has obstacles such as scarcity of funds and technology as well as climate change affecting the crop production, protection of the harvested yield is extremely important. The Sri Lankan government also emphasizes postharvest protection in agriculture. This proposed research directly links to these current needs and priorities of the country to enhance the food security and food safety.

Action Plan

Work plan to the proposed study (Pheromone-based population management of Ephestia cautella: food safety and security through environmental-friendly pest management)

Objectives of proposed research
1. Compare the effect of two pheromone compositions (straight ZETA and a blend of the two most important components) on mating disruption in E. cautella.
2. Study how the efficacy of MD in E. cautella can be maximized.
3. Determine if the MD alters with the type of grains/food.
4. Testing MD under real warehouse conditions (Different structures/complexity, density of traps)
5. Testing if MD efficacy can be enhanced when pheromone is used with different kairomones (Food oils)

Procedures and Approaches
Preliminary experiments have already been completed in Sri Lanka (Sammani et al., 2017). The objectives 1 and 2 will be tested during the fellowship. The objectives 3, 4, 5, possible limitations that may emerge when the developed technology is practiced, will be achieved during the follow-up period when the US mentor visits Sri Lanka.

New experiments will be used to examine the response of Ephestia cautella males to the two pheromone compositions (single component ZETA and a blend of ZETA and Z9-14:OAc), and the effect of these on orientation of males to calling of E. cautella females to maximize the mating disruption. A candidate for optimal blend for mating disruption will be selected prior to the visit. The experiments for objective 1 will be conducted over an initial 5-week period, and those for objective 2 will be conducted over a second 5-week period. Objective 3 will be done as a follow-up activity in Sri Lanka. An additional week at the beginning and end of the stay will allow time for preparation, wrap-up and planning follow-up experiments in Sri Lanka coinciding the visit of US mentor.

(weeks 2-6). The first experiment will use chambers with about the same internal area as the 0.6 m cubes described by Sower et al. (J. Chem. Ecol. 1:335-342, 1975), but constructed with cylindrical polyvinyl chloride (0.76 m diameter). These chambers will be built with a closed recirculating ventilation system, with filters on both at the intake and outlet. Additional ports will be constructed for introduction and removal of pheromone dispensers, and introduction of moths. Either ZETA or the blend of two components will be placed in these chambers according to the schedule below.

Treatment and mating schedule for Ephestia cautella maintained on a 10 hour scotophase
Treatment Pre-exposure (hours) for males Recovery (hours) Females present (hours)
1 6 0 4 (either ZETA or the blend still present)
2 6 0 4 (No ZETA or the blend)
3 4 2 4 (No ZETA or the blend)
4 2 4 4 (No ZETA or the blend)
5 0 6 4 (No ZETA or the blend)

All experiments will use a density of two male-female pairs per chamber. At the end of the scotophase, adults will be recovered and females will be dissected to determine if there are spermatophores in the bursa copulatrix (indicative of mating). Treatments 1 and 5 essentially serve as positive and negative controls. If the composition of pheromone (ZETA Vs. blend of two components) is an important aspect of mating disruption, then it is expected that mating would be suppressed at varying degrees. The two pair density is ca. 1.1 pairs per 1 m² of side and top. Sower et al. (1975) noted that females did not mate with pre-exposed males to synthetic pheromone unless they came within 1-2 cm of each other. Ten chambers will be constructed, so
that one replicate of each treatment can be run each day. Presuming four replicates in time each week, twenty experiments can be obtained in 5 weeks (weeks 7-11). The second experiment will use the same apparatus to determine how the mating disruption efficacy can be maximized. The experiment will test whether off-blends are effective to invoke a non-competitive/mixed mechanism to manage higher population densities as often found in warmer environments (Sri Lanka). Either 1, 2, 3, 4 or 5 male-female pairs of E. cautella will be examined (i.e., densities of 0.56, 1.11, 1.67, 2.22, and 2.78 pairs per 1 m² of side and top). Theory and some evidence indicate that mating disruption should be less density-dependent for a non-competitive mechanism than for a competitive mechanism.

Data will be analyzed with a generalized linear mixed model with binomial distribution, with mating rate as a response variable, the pre-exposure or density factor as a fixed variable, and night as a random variable. Logistic regression models is also a possibility.

Anticipated Benefits
Ephestia cautella and other stored-product moths with similar pheromone blends (Plodia interpunctella, Ephestia keuhniella, and Ephestia elutella) are pests of global importance. Understanding mating disruption and methods to increase its efficacy for these species therefore has a great potential for the protection of stored food. The impact of this research will definitely benefit Sri Lanka to augment its food security through safe pest management systems with the introduction of this most recent technique.

Facilities and capacity
Dr. Charles Burks laboratory at the San Joaquin Valley Agricultural Sciences Center, Parlier CA, USA has the experience and equipment to conduct the research described. Availability of two climate-controlled outbuildings of ca. 70 cubic meters each, GC-MS (to quantify pheromone), wind tunnel and EAG apparatus for ancillary studies are additional resources for this study. As Dr. Kent Daane (kdaane@ucanr.edu), at University of California, Berkeley has agreed to co-host this study, the facilities at the University will also be available thus ensuring readiness of all the resources for this proposed study.

Proposal
1. Goal
To develop risk assessment models to estimate dietary intake of pesticides using national consumption data.
2. Specific objectives
2.1 To compile available update data on pesticides residues in Thai foods.
2.2 To develop aggregate and cumulative risk assessment models for estimating dietary intake of pesticides.
2.3 To assess exposure to pesticides from food consumption of Thai population using updated national consumption data (2016) and pesticides residues (measured pesticides residues or maximum residue limit established by Codex or USDA or EU).
2.4 To evaluate health risk of pesticides intake using aggregate and cumulative risk assessment approaches.
3. Background information
Thailand is one of the leading exporter of agricultural products in the world. In Thailand, agriculture is an engine of economic growth and a major comparative advantage in the international trade. However, Thailand depends heavily on pesticide usage for protecting crops and increasing yield. During 2007 to 2012, Thailand was ranked as one of the top five counties in terms of annual pesticide consumption. There was an increasing trend of pesticides importation from approximately 110,000 tons in 2007 to approximately 172,000 tons in 2013. The overuse of pesticides leads to increasing health risks for farmers and consumers.

Risk assessment consists of four processes: hazard identification, dose-response assessment, exposure assessment and risk characterization. Hazard identification depends on the availably toxicological data to assess whether chemicals can cause adverse health effects in humans. Dose-response assessment characterizes the relationship between the dose of exposed and the adverse effects. Exposure assessment is conducted to determine the extent of exposure or intake. Risk characterization is the final process to assess the adverse effects in human. Exposure assessment and risk characterization depend on food consumption pattern/behavior and chemical levels/residues of individual country.

Traditionally, risk assessments are conducted based on individual pesticides or so-called aggregate risk assessments. This approach estimates the possibility for people to be exposed to a single chemical through all relevant food commodities. However, people are exposed to multiple pesticides in daily life; therefore, cumulative risk assessment approach has been developed. This approach evaluates the potential for people to be exposed to more than one pesticide at a time from a group of pesticides that share a common mechanism of toxicity. There is an increasing interest in conducting cumulative risk assessments due to the possibility of multiple exposures could cause unanticipated adverse effects on human health. Currently, United States Environmental Protection Agency (US EPA) performed cumulative risk assessments for five groups of pesticides.
Risk assessment models for estimating dietary intake of pesticides have been developed by a few international organization including the European Food Safety Authority (EFSA) and US EPA. Maximum residue limits (MRLs) of individual pesticides and particular food intake have been applied to calculate pesticides exposure levels. However, it is well recognized that the food consumption data vary from country to country. As a result, each country needs to develop its own pesticides risk assessment models based on the national food consumption and MRLs or measured pesticides levels in domestic foods.

A few working groups in Thailand including the Bureau of Quality and Safety of Food, Department of Medical Science, Ministry of Public Health have conducted risk assessment of pesticides from food consumption. They have studied the dietary intake of pesticides using total diet studies and aggregate risk assessment approach. However, no data available on estimated dietary intake of pesticides in Thai population using updated national food consumption data or by using cumulative approach. In addition, pesticides risk assessment models for Thai population have not been developed. To protect consumers in Thailand, pesticides risk assessment models should be developed, and aggregate and cumulative exposure assessments of pesticides need to be studied. The outcomes of the proposed study would be helpful in understanding the exposure to pesticides from food consumption and determining the maximum residue limits (MRLs) of pesticides in various food commodities for safe consumption. The results will be disseminated to national food safety authorities for effective risk management.

4. I hope to learn and practice probabilistic and cumulative risk assessment approaches in order to develop skills to complete the proposed study. During the fellowship, I will be able to develop aggregate and cumulative risk assessment models for estimating dietary intake of pesticides. The developed models will be applied for estimating pesticides intake from food consumption and characterize the risk of pesticides exposure of Thai people.

I have knowledge and experience in conducting probabilistic risk assessment and have applied a commercial software (@RISK, Palisade cooperation) in my research studies. My research team at the Institute of Nutrition, Mahidol University has received a number of grants related to food safety and risk assessment of food additives and heavy metals from Thai FDA and the Ministry of Agriculture and Cooperatives. However, we have not conducted risk assessment of pesticides by using cumulative approach. The cumulative risk assessment is a complicated process and very limited resources are available in Thailand. Additionally, I would like to learn how to incorporate cooking factors, variability and bioavailability information into pesticides risk assessment. Therefore, if I have been given an opportunity to work closely with an expert in pesticides risk assessment, I will be able to accomplish the proposed study and disseminate the outcomes to national food safety authorities. The knowledge and experience obtained during the training will be disseminated to my colleagues who are working in the risk assessment area, and apply to other risk assessment studies in the future which will benefit Thailand.

1. Thailand is an agricultural country and one of the leading food producers in the world. Nowadays, food safety is one of the most concern in the world trade. To meet the local and international food security aspects, not only the food production and quality but also the safety of the agricultural produce has to be considered. Comprehensive and profound knowledge in risk assessment
is a useful approach to support regulatory decision makings in order to protect consumers, ensure national food security and compete in the world trade.

**Action Plan**

1st week
Action step: University and laboratory orientations and staff introductions  
Needed resource: University and laboratory facilities  
Outcome: Knowing the University and laboratory facilities/staff/rules/limitations

2nd week
Action step: Learning and practicing on probabilistic risk assessment approach (Intermediate and advanced levels)  
Needed resource:
- Computer  
- Probabilistic risk assessment software  
- Internet access  
Outcome: Having profound knowledge and skill in using probabilistic risk assessment approach

3rd–4th week
Action step: Learning and practicing on cumulative risk assessment of pesticides  
(including how to incorporate cooking factors, variability and bioavailability information)  
Needed resource:
- Computer  
- Probabilistic risk assessment software  
- Internet access  
Outcome: Having profound knowledge and skill in conducting cumulative risk assessment of pesticide

5th-6th week Action step:  
- Studying how to develop pesticides risk assessment models  
- Design and develop aggregate and cumulative risk assessment models  
Needed resource:
- Computer  
- Internet access  
Outcome:  
- Having knowledge and understanding in developing pesticides risk assessment models  
- Having pesticides risk assessment models

7th week Action step:  
- Compiling available update data on pesticides residues in Thai foods  
- Compiling available update data on MRL established by Codex or USDA or EU  
- Preparing the available national food consumption data  
Needed resource:
- Computer  
- Probabilistic risk assessment software  
- Internet access  
Outcome:  
- Having a set of data on measured pesticides residues in Thai food and MRLs in various commodities  
- Having food consumption data ready for exposure assessment
8th-9th week
Action step: Conducting exposure assessment of pesticides from food consumption of Thai population using the models developed
Needed resource:
- Computer
- Probabilistic risk assessment software
- Internet access
Outcome: Results on exposure to pesticides from food consumption of Thai population

10th week
Action step: Characterization of risk of exposure to pesticides from food consumption
Needed resource:
- Computer
- Probabilistic risk assessment software
- Internet access
Outcome: Result on health risk of exposure to pesticides from food consumption of Thai population

11th–12th week
Action step: Summarizing the results, discussing and writing the report
Needed resource:
- Computer
- Internet access
- Statistical program e.g. SPSS, minitab, etc.
Outcome: Report on developing pesticides risk assessment models, and risk assessment of pesticides from food consumption of Thai population.
Fellow #15, Vietnam, Female/NOFO: USDA-FAS-10777-0700-10.-18-0027

Proposal

(1) Goal: The goal of my research is to determine the contamination rate and antibiotic resistance profiles among Salmonella spp. isolated from chicken processing chain in Vietnam.

(2) Research Objectives

- To identify the antibiotic use and knowledge of antibiotic resistance among chicken farmers in Vietnam
- To determine the contamination rate and antibiotic resistance profiles among Salmonella spp. isolated from chicken farm, chicken slaughterhouse and retail market
- To detect antibiotic residue in chicken meat product

3. Most human Salmonella outbreaks are associated with the consumption of contaminated animal derived products (WHO, 2005). Poultry products have always topped the incidence of sources for salmonellosis in many developing countries (Yang et al, 2011). Contamination with Salmonella in poultry products can occur at multiple steps along the food chain, which includes production, processing, distribution, retail marketing, handling and preparation (Dookeran et al, 2012). The increase in antibiotic-resistant Salmonella has been a worldwide problem over recent decades (White et al., 2002). Antibiotics are widely used in animal husbandry for several purposes including therapeutics, prophylaxis and growth promotion. The use of antibiotics in livestock may increase the antibiotic-resistant bacteria in human via the food chain.

In Vietnam, antibiotics use is very common in animal husbandry without control. The farmer can easy buy Veterinary drugs without prescriptions. In fact, the farmer can buy the antibiotics for prevention or control animal disease based on their experiences or by recommendation of the seller. Antibiotic residues in animal products, seafood is also causing antibiotic resistance in humans. The Ministry of Agriculture and Rural Development (MARD) of Vietnam has proposed the national action plan for management of antibiotic use and antibiotic resistance in livestock and aquaculture in Vietnam for the 2017-2020 period. Therefore we want to conduct the study on: “Antibiotic resistance profile of the Salmonella isolated from chicken production chain in Vietnam”.

The hypothesis of this study is: Salmonella isolates from chicken meat will be resistant to antibiotics used for feed addition in chicken farms.
4. To be a Borlaug fellow will help me work on my interesting topic and it is very hot issue in Vietnam right now. With support from a US mentor, I can improve my research proposal for more intensive and quality. I love the way the Borlaug fellowship brings US mentors to visit fellow's institute, It will be very good chance for US Mentor to know more about the situation in Vietnam. After that, both fellows and US mentor can improve more about research proposal or may also have new ideas for future collaboration.

5. With support from Borlaug Fellowship, this is the first such survey study along chicken processing chain and the result from this study can be informed to the policy makers (MADR, Government) as ideas or guidelines about antibiotic resistance and antibiotic residue.

The study will take place in some provinces such as Ha Noi, Bac Giang and Nghe An province where chicken husbandry is very common. The study will be conducted for around 7 months (because the study follows the chicken processing chain. The chickens were raised in the farm for 3-5 months. We do the survey, then take the sample from farms, follow to the slaughterhouse and market). Propose time: August 2018 to February 2019.

The contents of study will be:

1. KAP (Knowledge, Attitudes and Practices) survey of antibiotic use and antibiotic resistance of chicken farmers in Ha Noi, Bac Giang and Nghe An (100 farms per province).

2. Isolate Salmonella: collect 10 farms in each province after KAP survey. Through the chain from farm to slaughterhouse, slaughtering point and retail market, take the samples, including: 30 feces of chicken in farm; 30 chicken carcasses swab in slaughtering point and 30 chicken meats in retail market. Total of 270 samples will be collected from three communes for isolation of Salmonella.

3. Salmonella strains will be tested for antibiotic resistance, list of antibiotics base on the antibiotics use from survey data.

4. Antibiotic residue will be tested on chicken meat collect from retail market.

5. Data analysis and writing a report.

So, during 12-week fellowship period, below is weekly plan of proposed research activities and planned outcomes:

1. First week: university and laboratory orientations and staff introductions.

2. Second week: learn how to establish a good questionnaire survey for chicken farmer on antibiotic use and antibiotic resistance, focus on KAP (Knowledge, Attitudes and Practices).

3. Third week: learn how to enter data and analyze the data from questionnaire survey.
4. Fourth week to sixth week: learn how to collect sample and Lab work on Salmonella isolation.

5. Seventh week to Eighth week: learn how to test for antibiotic resistance? Method? May be learn more about Molecular for Salmonella antibiotic genes if possible.

6. Ninth week to Tenth week: learn how to detect antibiotic residue in meat

7. Eleventh: work with US Mentor about research proposal, write more proposal detail

1) Goal: The goal of my research is to examine some scientific based of aquaponic research for further practical application on the improvement of local food security issues as well as mitigate environmental problem in the Mekong Delta, Viet Nam.

(2) Research Objectives
2. The specific objectives are:
   - to determine scientific based needed to effectively study and manage about nutrient balance between the aquaculture and plant, as well as nutrient recycling pathway for optimum growth of plant and vegetable
   - to introduce to the farmers, officers about the aquaponic systems as well as to deal with climate controlled environments.
   - to transfer developed technologies via workshops and extension publications that will build community support and improve workforce quality in the areas of aquaculture and sustainable agriculture.

3. Rationale and research background
   Global warming is challenging for environmental, social and economic issues around the World. It drives the need for developing new technology to solve the issue of food production and consumption requirement (FAO, 2014). Studies have showed that aquaponics would be a promising technology for sustainable food production and biosecurity system. Aquaponics combines two technologies between recirculating aquaculture systems (RAS) and hydroponics (plant production in water, without soil) in a closed-loop system (plants use the waste produced by the fish, thereby continuously cleaning namely RAS and hydroponics [Rakocy et al. 1992, 2004, 2006]. Therefore, it would applicable and widely used in developing country if this technology is developed and being transferred to the society.

Viet Nam is one of the most 5 countries has seriously affected by climate change such as drought, flood, salt water intrusion, and so on. These problems affected on both agriculture and aquaculture in the area, especially in the Mekong Delta (MKD). Farmers used to lose their business on aquaculture farms, paddy field, vegetable and facing to food security as well. On the other hand, due to economic purposes, intensive aquaculture is relative increasing in the area. These issues would cause environmental problem. Although researches have been done to deal with waste water treatment from aquaculture activities but there are not feasible and unreliable methods due to cost effective. Currently, we have tried to conduct some basic research on aquaponic and the results seem to be reliable for food production of fish and plant biomass. However, it also includes many issues such as balancing between fish tank and the plant growing beds and ammonium, nitrate as well. Thus, these issues need to be addressed if the technology is to contribute to more production systems. However, there is a lack of knowledge about aquaponic in Viet Nam. Therefore, there is need of study on the effectiveness of integrated aquaculture and hydroponic for the sustainable agriculture in the MKD, Viet Nam for sustainable food production system in the region.

4. With the purpose of my research proposal, during the fellowship (if awarded) period, I hope to work closely with Professor (Mentor) in the U.S to learn more about scientific based needed
for aquaponic food production system such as water chemistry analysis, system design, nutrients pathway issues.

Here are my experimental design during and after finishing fellowship program:

Study site and location. This research activity will be carried out at the host University in the U.S and College of Aquaculture and Fisheries, Can Tho University, and some provinces which located in Mekong Delta, Viet Nam.

Research activity and output 1: Experimental trial on application of different local aquatic species integrated with various hydroponic plant and vegetable.
The purpose of this experiment is to determine scientific based needed to effectively study and manage about nutrient balance between the aquaculture and plant, as well as nutrient recycling pathway for optimum growth of plant and vegetable in the MKD conditions.

Experimental design
1. Experiment 1 (Exp. 1): Nutrient remediation and nutrients dynamic in aquaponic system at the host University in the U.S and repeating in Viet Nam when return
2. Experiment 2 (Exp. 2): Optimal fish to plant ratios for a controlled environment in aquaponic system
3. Experiment 3 (Exp. 3): Comparison different hydroponic sub-system in aquaponic system

There are three different indigenous fish species including of snakehead, swarm eels and tilapia will be applied because those fish species are commonly raise in the area for daily food consumption and economic purposes.

Research activity and output 2: Pilot trials
The purpose of conducting pilots is to introduce to the farmers, officers about the aquaponic systems as well as to deal with climate controlled environments.

After completing the above experiments with achieved all basic results on balancing system between fish and plants, Pilot trials will be conducted at least 3 farms modeling at different locations in the Mekong Delta, Viet Nam. The pilot will be associated with farmers in observation of Department of Aquaculture and fisheries at the provincial level.

Research activity and output 3: Training and technology transfer to end users.
While holding aquaponic pilots for farmers, a training on how to set up and operation aquaponic system will be carried out for at 50 people including farmers and Fisheries officers as well as conducting demonstration workshops (n=3) on farm field demonstrations. The results will play an important role for future extension and application of aquaponic in the area.

With the above research outcomes, I need Mentor advise me during and after finishing the fellowship period in the U.S.

5. If I have an opportunity to get Borlaug fellowship, this endeavor will help me to 1) improve my research field in food security by doing and acting directly with Mentor about the research proposal. The success of this research will contribute significantly to (i) novel methods for the
sustainable cultivation of fish and vegetable in efficient of the system, (ii) local food security issues in the MKD, Viet Nam, (iii) Transfer developed aquaponic technology to appropriate end users; 2) to enhance my networking with scientists, researchers, policymakers and regulators in the U.S and other countries.

The following assuming 12 week activities plan:
1. Week 1: Arrive the U.S set up accommodation, laboratory orientations, meeting the Mentor to discuss on detail activity, making detail plan for the 12 week period
2. Week 2: Experimental design on aquaponic basic system (Nutrient remediation and nutrients dynamic) at host university: fish and plan
3. Week 3 - week 7:
   - Conducting/running experimental trial: take care of the experiment by daily feeding fish and laboratory work on water chemistry.
   - Finishing the first cycle of trial, writing report
   - Progress report, presentation on achievement
4. Week 7. Discussion with the Mentor on research proposal for conducting research in Viet Nam when returning home
6. Week 7- 11:
   - Repeating the second cycle for the experiment, daily work on feed, taking care of fish and plan
   - Continue to work on research proposal and final review with the Mentor.
7. Week 8 - week 12
   - Continue to conduct the second experiment, lab work
Final report and presentation; Return Viet Nam
Proposal

1. The goal of my research proposal is to elucidate the patterns of gene expression and phenotypic changes of M. roreri strains with contrasting virulence, during the infection of T. cacao L.

2. To estimate the disease incidence and severity caused by M. roreri strains with contrasting virulence in a susceptible cacao genotype.
   - To evaluate the shift from biotrophic to necrotrophic stage of Frosty Pod Rot (FPR) using histological analyses.
   - To evaluate the expression patterns of candidate effector genes previously identified in the transcriptome of M. roreri during the shift from biotrophic to necrotrophic stage.
   - To compare differential gene expression and morphologic changes between both M. roreri virulent strains during disease development in T. cacao.

3. Cacao (Theobroma cacao L.) is an important cash crop that supports the economic activity in regions affected by difficult socio-economic conditions. One of the limited factors that affect the crop productivity is the infections of plant and pods by fungal pathogens, such infections not only affect the plant but can also affect the quality of the final product, resulting in big economic losses.

In Colombia, the main disease that limits the cacao yield is Frosty Pod Rot (FPR) caused by Moniliophthora roreri. FPR is responsible for the largest economical losses because it infects the pod, affecting both the production and the quality of the product.

M. roreri, the asexual state of a Basidiomycete, attacks only the pod and has two distinct stages. In the biotrophic phase, the infected pods do not present symptoms, however, after 14 to 21 days, the tissue growth is affected and the fruits develop malformations; during this stage, the fungus delays the host defense response and continues with the necrotrophic phase, leading to sporulation over necrotic tissue (Meinhardt et al., 2014).

In the biotrophic phase, the fungus genes encode proteins involved in the attack of plant tissue through the breaking down of cell wall components to invade the intercellular space (Meinhardt et al., 2014; Bailey & Meinhardt, 2016). The complex expression profiles have been determined in both stages and during biotrophic/necrotrophic shift between tolerant and susceptible cacao clones. Nevertheless, few studies have focused on the effect of genetic variability of different M. roreri isolates over T. cacao disease.

Mr can adapt to different environment conditions so that diverse populations of the fungus are established in several geographic regions in Colombia (Jaimes et al., 2016). According to published results (Phillips-Mora et al., 2007; Jaimes et al., 2016), in Colombia the collected M. roreri isolates show a high level of genetic diversity probably due to high mutation rates or high gene flow within and among different geographic locations. Considering Mr as an asexual fungus and its differentiation between populations, mutation rates and subsequent evolution can affect the virulence of the strains (Jaimes et al., 2016), allowing the adaptation to enhance resistant or tolerant host (Ali et al., 2015). However, so far there is no information about plant disease measurement (incidence and severity) or about differential expression of pathogenic factors in isolates with distinct level of virulence. For the above, it is necessary to conduct experiments to elucidate the patterns of gene expression and phenotypic changes of M. roreri strains with contrasting virulence.

4. During the last two years I have worked with cacao, focus in postharvest processes, particularly, in understanding the process of fermentation. During these projects, talking to...
farmers and being in the field, I have realized that FPR is one of the most important limiting factors in Cocoa production. My academic training in plant pathology and the real problems on plant diseases have made me aware about the phytopathology problems in cocoa crops and the concern of producers who do not obtain the desired economic benefits. Thus, I am interested in learning deeply about interactions occurring in the disease caused by this complex pathogen. I would like to generate useful information to develop strategies to decrease the prevalence of the pathogen, especially in regions where small family farmers are most affected. The proposal presented for the Borlaug fellowship aim to improve the understanding of Cocoa phytopathogens, specifically what pathogen effectors (genes) are involved in the establishment, spread and necrosis, during FPR development. In order to do so, I plan to use strains with contrasting levels virulence. The strains will be selected according to unpublished results of research conducted previously in Corpoica. Understanding the difference in expression pattern between strains could allow us to: determine the temporal pattern of expression of different strains, identify genes that are expressed in both strains during the infections and correlate gene expression with phenotypic changes observed during the histological analysis. This information would permit in the future the development of resistant cocoa clones through genetic improvement, in regions of predominance of M. roreri.

To address my research goals, I would suggest carrying out this fellowship at Beltsville Agricultural Research Center USDA/ARS, taking into account the research and extensive experience with cocoa pathogens of many researchers. Particularly important is the work of Dr. Bryan Bailey, who has a vast experience in this crop and continue addressing many projects to understand the “complex relationships” that occur between T. cacao and its pathogens. During the fellowship, I want to apply and improve my knowledge in molecular techniques, such as RNA extraction, cDNA preparation or RT-qPCR, data analysis and NGS sequencing. I also want to monitor the morphological observation of the pathogen during the process of infection and necrosis, using histology techniques. I could have then a better view of what is happening during this interaction.

5. Thanks to the creation of programs like Borlaug Fellows and international cooperation agreements, it’s possible getting support of researchers who want to improve their knowledge in specific research areas. In addition, they permit the establishment of collaboration between countries and, make possible the generation of high quality results in a short period of time. After Borlaug Fellowship, further collaboration between Corpoica and USDA will be essential to continue investigation in the most promising current crop in Colombia.

Action Plan
The proposed research program has been planned in collaboration with Corpoica’s researchers to ensure that it can be accomplished within the stipulated fellowship period.

Timetable

Week 1: Presentation of the mentor and discussion of the proposed research program with research group. Get familiar with the laboratory, work instructions and staff introduction.

Week 2-3: Get familiar with previous work developed in the laboratory and get orientation to work with genomic and transcriptomic data to select the genes of interest that will be quantified
through qPCR. Learn more skills in data analysis through bioinformatic tools. Design specific primers for qPCR assays.

Week 4-5: Observation of slides with infected tissue previously prepared and standardization of other possible histology techniques. Standardization of qPCR.

Week 6-7: Make expression analysis assays using qPCR. Data analysis.

Week 8-9: Possible preparation of cDNA libraries. Learning more techniques applied in current experiments in the laboratory related with plant-pathogen effector analysis.

Week 10-11: Possible sequencing of RNA prepared libraries and data analysis. Training in other molecular techniques or work methodologies in experiments in progress.

Week 12: Writing of report and socialization of results obtained during Borlaug Fellowship.
(1) **Goal:** The goal of my research is to evaluate the inclusion of novel traits in genetic breeding programs of dairy cattle to manipulate recombination rates, which would allow breeders of elite animals to increase their likelihood of producing animals with extremely high genetic potential.

(2) **Research Objectives**
This study will calculate the recombination rate heritability in dairy cattle and evaluate the impact of recombination level on different economically important traits and inbreeding in order to determine the possibility of including this trait in genetic breeding programs. The objectives of this study are to calculate the heritability of recombination rate in dairy cattle and to evaluate the impact of recombination level on different economically important traits and inbreeding in order to determine the feasibility of including this trait in genetic breeding programs in different Holstein cattle populations.

3.- Genetic gain is determined by four factors: a) the accuracy of the genetic evaluation, b) the generational interval, c) the selection intensity, and d) the genetic standard deviation of the trait in the population (Gonen et al., 2017). The first three factors have been improved through the development of mathematical models, the use of reproductive technologies and genomic selection (Wiggans et al., 2017; Gonen et al., 2017); while the fourth element has been improved through the exchange of genetic material, factor that is limited by the inbreeding level of dairy populations worldwide (Young and Seykora, 1996). An increase of the recombination rate of parental homologous chromosomes generates new combinations of existing alleles, improving allelic variation. Increasing recombination rate may be beneficial if the binding between two undesirable alleles is broken, which would improve the efficiency of selection, but may be also detrimental if favorable allele combinations are broken (Otto and Barton, 2001; Battagin et al., 2016). Studies have shown that the level of recombination can be transmitted from one generation to another, showing heritabilities of 0.15 in sheep (Johnston et al., 2015), 0.26 in cattle (Weng et al., 2014), and 0.30 in humans (Kong et al., 2014).

The recombination of genetic material ensures the generation of new genetic variants linked to progeny that maintains or increases genetic diversity, fosters evolution of populations (Wahls et al., 1998) and facilitate genetic improvement through selection (VanRaden and Sanders, 2003). The recombination rate between two loci is not randomly regulated (Jeffreys et al., 2005) and is largely determined by physical distance of loci in the chromosome; in addition, chromosomes have regions of frequent recombination known as hotspots (Neher et al., 2009). These hotspots are short regions where parental DNA strand exchanges are more common than in other regions (Lupski, 2004). Recombination potentially increases the efficiency of selection because it produces the combination of alleles, and decreases the statistical associations between different loci, decreasing linkage disequilibrium (LD) rates (Kouyos et al., 2006). Recent simulation studies (Battagin et al., 2016) explored the potential of manipulating recombination rates to increase the response to selection in breeding programs for cattle, demonstrating than an increase of recombination rate increases the overall response to selection, and can increase the efficiency of breeding programs and the genetic variance. However, to significantly increase the response to selection, the rate of recombination should have to be increased by 10 or 20 times. Other
authors (Gonen et al., 2017) also used simulation models and explored and quantified the potential effect of the transfer of hotspots on genetic gain, setting scenarios where they presented distinct locations of QTNs and hotspots in the genome. They found that by changing hotspots along the genome, genetic variance is increased by new combinations of alleles, resulting in greater increases in genetic gain. However, the benefit of changing hotspots for increased genetic gain was only observed when QTNs were found outside the hotspots. When the QTN is within the hotspots the genetic gain is diminished.

4.- During the fellowship I hope to learn and develop novel tools to evaluate the possibility of changing the rate of genetic recombination. During the last few years I have been working on genetic improvement of dairy cattle applying genomic information on different economically important traits, which has help me to understand the management of genetic and genomic data. Now, I would like to evaluate rate of recombination as a novel trait than could help accelerate the rate of genetic gain in dairy cattle and other livestock species. Working together with a US mentor (Dr. John Cole) and his research group who have expertise on the use and manipulation of genomic data, will enable the learning and development of new techniques to reach the proposed goals. Previously, during my PhD Studies, I carried out an internship at USDA and I had the opportunity to work with Dr. Cole’s research team, which without a doubt will speed up my integration to the work team. My experience and the previous contact with this research team, make me ideally suited for attending this fellowship that offers exiting challenges and the opportunity to develop professionally.

5.- The Borlaug fellowship will contribute to increase the likelihood of producing animals with higher genetic potential through the use of recombination rate as a selection trait, which will complement the work than Dr. Cole and Mr. Li Ma, from the USDA and the University of Maryland are currently doing. If recombination rates are heritable and if these have an impact on the expression of genes related to productive traits, it will be possible to modify the expression of genes related to economically important traits in dairy cattle and could be possible to extend the development in livestock production.

Work Plan

Before the Borlaug fellowship period, it will be necessary to evaluate techniques and methods than could be applied to reach the main objective. The stay in the USDA is planned weekly.

Week 1. Meeting with the working group to present the reviewed techniques and/or methods, and evaluate the possibility of applying any of them or another proposed by the team. Determine the set of animals which will be used in the first US Holstein training and collect pedigree and genomic date of those animals.

Week 2-4. Apply the method(s) to calculate the recombination rate in the test group and present progress to the working group.

Week 5-8. Calculate the genetic additive component (heritability) of recombination rate in dairy cattle using many genetic models and present results to the working group.
Week 9-12. Evaluate the effect on the recombination rate level on the expression of economically important traits used in the US dairy cattle.

Week 12. Show the results and determine if it is necessary to include new analysis to complete the study and how this will be performed.

After the stay, I’ll complete analysis and results, and will evaluate the recombination rate level in the Mexican dairy cattle. During Cole’s visit to Mexico, the obtained results in different countries will be compared and discussed. Results will be presented in scientific congresses and publications.
Proposal

1. The goal of my research is to understand genetic and chemical interactions in the microbial communities that lives in the cacao pod, the cacao fruit microbiome, in order to select species of microbes (fungi or bacteria), or combination of them, with capacity to inhibit the growth of cacao pathogens Moniliophthora roreri and Moniliophthora perniciosa, and to identify effective strategies to use beneficial microbes to control diseases caused by cacao pathogens under farm conditions.

2. Objectives

Research Objective 1: To identify whether cacao genetics has an effect on the pod microbiome community composition. Question: Do different cacao genetic varieties (genotypes) have distinctive microbial communities?

Research Objective 2: To identify patterns of microbial community changes in relation to cacao pod development. Question: Are there different microbial taxa associated with different developmental stages of the cacao fruit?

Research Objective 3: To generate knowledge about cacao pod microbiome dynamics after treatment of pods with beneficial microbes that inhibit the growth of Moniliophthora species. Questions: What happens with other members of the microbial community when we inoculate the pods with high dosages of a beneficial microbe?, What happens to these inoculated pods when they are exposed to Moniliophthora roreri?

Research Objective 4: To identify profiles of metabolites that can be associated to cacao pod developmental stages, specific microbial community composition, and cacao resistance to Moniliophthora. Question: What chemical compounds or molecules are produced in the microbiome of the cacao pod that can be responsible for stopping damage caused by species of Moniliophthora?

3. The chocolate tree, Theobroma cacao, harbors highly diverse microbial communities (microbiomes) that includes species that cause diseases and species that increase resistance of cacao against pathogens. In particular, the communities of endophytic fungi, those that asymptotically colonize plant tissues internally, are very diverse and with members that inhibit the growth of pathogens. Part of my research has focused on identifying endophytic fungi species with capacity to control pathogens of cacao and on the identification of mechanisms by which endophytic fungi increase resistance of cacao against pathogens (Christian et al 2017; Mejia et al. 2003, 2008, 2014). Our research on cacao pod microbes has been done mostly using classical microbiology techniques, specifically the isolation, culture, and morphological identification of microbial species and have had the limitations that many microbial species can’t be cultured. Therefore we have had a limited view and understanding of the pod microbiome. Nonetheless, with these techniques we showed for the first time that endophytes are capable of reducing damage caused by cacao pathogens under laboratory, greenhouse and field conditions (Arnold et al 2003, Mejia et al. 2003, 2008). Recently there has been major advances in techniques that doesn’t require the culture of microorganisms to identify them in a given sample or tissue, in particular the use of next generation DNA sequencing technology (NGS) for metagenomic analyses of microbial communities. This technique allows an accurate characterization of all microbial community diversity composition across many samples, substrates and therefore improved resolution for our experimental research. Additionally it can be used for identification of genes expresed in particular conditions. This DNA sequencing
technology coupled with matrix assisted laser desorption ionization as a mass spectrometry imaging (MALDI-IMS) allows the identification of different metabolites present in a given sample. However these techniques relies on heavy use of bioinformatics and analytical methods. 

A main reason to compete for the Borlaug fellowship program is to get the opportunity to receive training while doing research on bioinformatics, NGS data analyses, and metabolomics in the laboratories of Dr. Rob Knight and Pieter Dorrestein at the University of California in San Diego (UCSD). Dr. Knight laboratory is one of the top laboratories in the world in microbiome research and he and his collaborators have developed some of the most used and efficient tools for analysing NGS data as it applies to microbial communities. I contacted Dr. Knight and he is willing to host my visit to his laboratory in 2018 to provide training in bioinformatic analyses of microbial communities of cacao. I also contacted Dr. Pieter Dorrestein who runs one of the top laboratories in the world in metabolic network profiling and use of MALDI-IMS, is willing to co-host my visit to UCSD and to provide training and help with metabolite analyses of the samples. Dr. Dorrestein is a close collaborator of Dr. Knight, and also have done collaboration with my home institution INDICASAT in Panama (e.g. Boya et al 2017).

4. Expected Accomplishments
I hope to accomplish bioinformatics training and analyses of NGS and MALDI-IMS data that will allow us to generate new knowledge about crop microbiomes and how to use them in agriculture for plant protection.
I am a microbiologist by training and my research is about microbes of cacao. The proposed research is clearly connected to my research goals.
Working with very accomplished mentors in microbiome and metabolomic research will help me accelerate discoveries in this field and to publish results obtained, hopefully in prominent research journals

5. Currently the cacao production of Panama is very low despite having high quality cacao material and enough suitable land to substantially increase its production. But the main limiting factors are two diseases: Moniliasis caused by Moniliophthora roreri and Witch’s Broom disease of cacao caused by Moniliophthora perniciosa. The proposed research is planned to be carried out at a farm in Bocas del Toro region, the main producing area of cacao in Panama and where Moniliasis occur. The knowledge generated is expected to be translated directly to cacao farm conditions in Bocas del Toro and to other cacao regions of Latin America. Neither Moniliasis or WBD of cacao occur in Africa. This research is a step forward towards preparedness for controlling potential devastating epidemics of these diseases in Africa and Asia, the main producing areas of cacao.

Action Plan
The general plan is to run experiments in Panama that will address questions associated to objectives 1-4 and to send samples to the laboratories of Drs. Knight and Dorrestein for us to analyze the samples there and to draft a manuscript that will report the findings.
Some baseline information for this project has been generated. Specifically, endophytic fungi that lives asymptotically within cacao pods were isolated and are evaluated in vitro, in laboratory, for their capacity to inhibit the growth of cacao pathogens. Further previous research by our group has shown that endophytes of cacao pods and leaves can decrease pathogen development in leaves and fruits of cacao (Arnold et al. 2003; Christian et al 2017; Mejia et al. 2003, 2008, 2014).
The proposed research activities includes the execution of experiments in Panama, in a selected farm in Bocas del Toro region, were pods of cacao from the field experiments to answer questions associated to objectives 1-4 will be harvested for pre-processing in Panama and for shipping to the laboratory of Dr. Rob Knight and Pieter Dorrestein in UCSD. Samples will be sent to these laboratories ahead of my arrival, for generating data on the microbial community composition and metabolites of the cacao pod.

While the proposed activities are for the period of the fellowship in USA, in particular in UCSD, it will be very useful to have support for the activities conducted in Panama as I don’t have any funding support specifically for cacao research at the moment. This involves expenses of my assistant and myself of traveling to Bocas del Toro Region from Panama City to perform field experiments associated to research objectives 1-4, and for consumables for laboratory and field work. Also, I expect the fellowship to cover laboratory consumables and related expenses of the proposed activities in the laboratories of Dr. Knight and Dorrestein in UCSD. Finally, I want to comment that the following plan was done for three months of training and research in the USA, but I prefer to do the proposed activities in a period of two months in USA.

Week and Activity
Week 1: Arrival and introduction to the university, laboratory procedures, and to bioinformatics.
Week 2: Training in laboratory methods to generate metagenomics data.
Week 3: Training in bioinformatic pipelines for microbial community ecology.
Week 4: Training in metagenomics and time course data analyses.
Week 5: Data analyses to identify the role of cacao genetics on the cacao pod microbiome species composition (Research Objective 1).
Week 6: Data analyses to identify patterns of microbial community changes in relation to cacao pod development (Research Objective 2).
Week 7: Data analyses to identify changes in cacao pod microbiome species composition after inoculation with beneficial microbes that inhibit the growth of Moniliophthora roreri (Research Objective 3).
Week 8: Data analyses to identify profiles of metabolites in relation to cacao pod developmental stages, microbial community composition, and cacao resistance to Moniliophthora (Research Objective 4).
Week 9: Presentation of results to members of the laboratory and interaction with other faculty at the University of California. Presentation of results to USDA/Borlaug Program.
Week 10: Work of manuscript draft.
Week 11: Work on manuscript draft.
Week 12: Week to complete any needed necessary work.
Proposal

1. The goal of my research is to recover the productive capacity of the High Andean pastures and improve the life quality of small and medium producers. The specific objectives of the present research are: to determine the quality and floristic composition of the High Andean pastures, to identify the main water sources and to implement the techniques of rotacional grazing systems in the intervention areas.

2. According to IV CENAGRO (2012), in Peru, the area of natural pastures is approximately 18 million hectares and it is the food base of about 85% of the camelidae, cattle and sheep population. However, due to overgrazing, most of these lands are degraded, whereby the production yields of meat, milk and fiber are low, limiting the economic income of the producers, reason why about 40% is under the poverty and extreme poverty level, because of this, population is expose to another problem like chronic malnutrition and anemia.

3. I believe that in my training in animal science bachelor, I look for applying livestock management techniques in the production systems that allow the use of natural resources in a sustainable way, putting in a balance between productive and conservation activities. I believe that the availability of food (forage) is the basis for livestock development, once this resource has been achieved, it will be possible to working designing breeding programs. For me it is too important to work with a mentor because his field experience, vision and knowledge, will allow me to design the best management strategy that it can be adapted to my country conditions, putting on to combine environmental, productive and social aspects in an optimal way.

4. The Borlaug Fellowship will allow me to have adequate tools and to include new scientific knowledge that i can use into the high Andean pastures management, with which I hope to improve the animal production of milk, meat or fiber, allowing the producers to insert their products in the national market, increasing their incomes, which it will allow them to acquire other products that meet their nutritional needs, thereby improving their quality of life, moving away from poverty.

Action Plan
Weekly Plan Proposal
Week 1: Laboratory orientations
Week 2: Livestock and environment
Week 3: Livestock and greenhouse gas emissions
Week 4: Climate Change and Livestock: Impacts, Adaptation, and Mitigation
Week 5: Livestock and foraging behavior
Week 6: Identification of Native plants
Week 7: Remote sensing and image interpretation
Week 8: Floristic and vegetation structure of a grassland plant community
Week 9: Ecological restoration
Week 10: Effect of grazing on floristic quality and soil properties
Week 11: Livestock development strategies
Week 12: Grazing plan in livestock systems
Fellow #21, Peru, Male/NOFO: USDA-FAS-10777-0700-10.-18-0033

Proposal
1. State the goal(s) of your research proposal in one sentence.
The goal of my research is to develop an innovative tool to evaluate silvopastoral systems in the Peruvian tropics.
2. Identify the specific research objective(s) that will achieve your goal.
- To identify examples of silvopastoral systems simulation models developed.
- To develop a simulation model that allows the understanding of the main interactions of silvopastoral systems.
3. Provide enough background information about your research, explaining it in terms that someone unfamiliar with your scientific field can understand.
Traditionally, livestock in the Peruvian tropics has been questioned because it is related with deforestation, low productivity, soil degradation high rates and its production is affecting environmental sustainability. Modernel et al., (2014) argue that to mitigate climate change, sustainable livestock systems must be promoted. In this sense, silvopastoral systems (SPS) are an example of resilient agriculture; in which trees interact with animals and forages under a system of integrated management that promotes the conservation of soil and water and a reduction in emissions of greenhouse gases (GHG). Also, SPS contribute to the agricultural production in the country. Among the benefits of the SPS are: The diversification of the products obtained, stress reduction due to the shade provided to animals, the increase of animal productivity, the recycling of nutrients and reduction of soil compaction (Arévalo et al., 1998), the supply of environmental services, among others. Despite some research have been done in the country on the analysis and design of silvopastoral systems (Alegre et al., 2012), there is still a little adoption of these systems in the field. This proposal aligns with the given priority "Resilient Agriculture" because benefits of these systems include improved livestock performance, water quality and wildlife habitat, recreational opportunities, along with long-term profits from sales of wood products.
Describe what you hope to accomplish during your fellowship. How do your research interests and scientific background relate to the goals of your proposal? How will working with a mentor in the U.S. help you to achieve your research goals?
During the fellowship I hope to develop a tool, or adjust an existing one, to improve the understanding of silvopastoral systems in the tropical area of Peru. Additionally, I am the coordinator of the field activities of a strategic research project on silvopastoral systems in the tropics of Peru, funded by the Peruvian government and the World Bank. So, during this fellowship I would like to develop specific expertise to support the objectives of the ongoing research project. My proposal is committed to the use of silvopastoral system modeling to demonstrate how these systems can promote the competitiveness of the agricultural and forestry production chains of the Peruvian tropics. Recently, the Peruvian government has presented its iNDC, which envisages a reduction of emissions equivalent to 30% in relation to the Greenhouse Gas (GHG) emissions of the projected Business as Usual scenario (BaU) in 2030. In the case of the agriculture sector, this considers measures such as the recovery of degraded soils with silvopasture in the Peruvian Amazon to mitigate 1,344 MtCO2eq from the intervention of 102,000 hectares of degraded land until 2030. A mentor in the U.S. will help in the development of a dynamic simulation model which integrates the main interactions of the SPS as a technique widely developed in the U. S. by various institutions as USDA (Dairy Gas Emissions Model –
DairyGEM, Integrated Farm System Model – IFSM) and universities (Cornell University, University of Florida, University of Wisconsin–Madison). In addition, silvopastoralism is also being developed by several research centers in the U. S. as an agroforestry practice to produce a high-value timber component, while providing short-term cash flow from the livestock component in a sustainable way, both in the U.S. and other countries worldwide.

5. How will a Borlaug Fellowship contribute to enhanced agricultural productivity, economic development, and/or food security in your country?
This proposal, specifically, will achieve the three aspects: The agricultural productivity will improve due to an efficient use of natural resources, there also will be an economic development, especially, on small farmers whom manage the majority of silvopastoral systems in the Peruvian tropics and the food security will be enhanced as a result of a sustainable production of milk (or dairy products) and meat.

Action Plan
1st – 3rd week: Introduction to silvopastoral modelling
- Presentation of ongoing research project on silvopastoral systems in Peru
- Literature searching
- Interviews with field experts
Outcome: Identification of examples of silvopastoral systems simulation models developed
4th – 6th: Development/Adjusting of a silvopastoral modelling tool
- Simulation lab work
- Literature searching
- Interviews with field experts
Outcome: Preliminary version of the silvopastoral modelling tool
7th-9th: Development of the beta version model of silvopastoral systems
- Simulation lab work
- Literature searching
- Interviews with field experts
Outcome: Beta version model of silvopastoral systems
10th-12th: Refinement of the silvopastoral modelling tool
- Simulation lab work
- Interviews with field experts
- Analysis and revision of the model Outcome: Silvopastoral modelling tool refined
Proposal

Mapping of gene-specific markers in sweet potato (Ipomoea batatas L.) Lam.) for performance under drought conditions.

Several studies have considered gene expression for storage root development in sweet potato (Kim et al. 2002; Noh et al. 2010; 2012; Firon et al. 2013). Solis et al. (2014) went a step further to look at genes associated with storage root development under drought stress. Most of these studies have however been carried out in just one or two genotypes but not in an actual breeding population that would be expected to create impact in food security. Sweet potato is an important food security crop especially for the poor and it is currently one of the most important bio fortified crops being used to combat vitamin A deficiency especially in sub-Saharan Africa (Low et al. 2017). Drought stress is an important abiotic stress that is currently unpredictable and expected to cause yield reductions especially in the developing world.

Methods towards sweet potato improvement for drought conditions would therefore be beneficial to cushion the farmers from the effects of climate change. Being among the crops with the highest yield per unit area, sweet potato is also expected to contribute towards the challenge of feeding about 9 billion people projected to be the global population in 2050. The international potato center (CIP) is committed to contribute towards global food security by ensuring resilient and nutritious sweet potato varieties developed to fit into farmers’ systems. Consequently, CIP develops various populations to achieve different objectives. One such population is a bi-parental mapping population between Beauregard (a US-developed famous variety that has been used in most of the above studies) and Tanzania (a famous African variety). The two parents differ for several traits including beta-carotene, dry-matter content, drought tolerance and sweet potato virus disease resistance, which are expected to be segregating in the progeny. This population is being genotyped and phenotyped for linkage and QTL analysis. However, since hexaploid sweet potato still does not have a complete reference genome, mapping polymorphisms known to be within reported genes will significantly help understand traits especially if such data can be compared with diploid reference genomes and gene expressions. It is proposed to study the allelic variations in several genes reported for drought tolerance in sweet potato and to relate those variations to sweet potato performance under drought conditions.

Specifically, primers specific to three genes found to be upregulated under drought as reported by the study of Solis et al. (2014) will be designed and used to amplify DNA of 350 progeny and the two parents of the mapping population. These genes are: IbHB2 which encodes a homeobox protein, IbCRF1 which encodes cytokine response factor 1, IbAREB which encodes abscisic acid-responsive elements-binding factor. Only three genes have been selected because of the volume of work involved in amplifying the entire mapping population. After amplification, the PCR products will be sequenced and sequence-specific variations will be identified and used for further downstream analysis with phenotypic data. This work will not only allow me to learn about molecular techniques of studying plants under abiotic stress but will also allow to develop gene specific markers.
that can be used in molecular-assisted breeding for various traits in sweet potato under drought and is expected to have impact on a global basis as per CIP’s mandate regions.

References
Firon, N. et al. (2013). Transcriptional profiling of sweet potato (Ipomoea batatas) roots indicates down-regulation of lignin biosynthesis and up-regulation of starch biosynthesis at an early stage of storage root formation. BMC Genomics DOI:10.1186/1471-2164-14-460

Action Plan

<table>
<thead>
<tr>
<th>Week #</th>
<th>Work Subject</th>
<th>Summary</th>
<th>Materials needed</th>
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<tbody>
<tr>
<td>Week 1</td>
<td>Finalize work plan.</td>
<td>Mentor will check work plan and provide feedback. Planning layout and Calendar of activities.</td>
<td>Two laptops</td>
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<tr>
<td>Week 2</td>
<td>Travel to LGU, arrange DNA shipment from CIP</td>
<td>Travel and meeting team at LGU Preparing genetic material and all things that will be involved in the experiment.</td>
<td>DNA of the B x T mapping population shipped to LGU from CIP</td>
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<tr>
<td>Week 3</td>
<td>Primer design and introduction to Lab methods</td>
<td>The primers for the three candidate genes to be designed Primer development Introduction to lab procedures and rules at LGU</td>
<td>Computer and primer design software,</td>
</tr>
<tr>
<td>Week 4 &amp; 5</td>
<td>Primer optimization to run on DNA samples</td>
<td>Conditions will be optimized for the developed primers to run on the DNA sample by using a few genotypes of the mapping population</td>
<td>Computer Lab consumables and PCR equipment</td>
</tr>
<tr>
<td>Week 6, 7, 8</td>
<td>PCR on the entire mapping population</td>
<td>PCR will be carried out on the entire mapping populations for the selected genes</td>
<td>Computer Lab consumables PCR equipment</td>
</tr>
<tr>
<td>Week 9, 10, 11</td>
<td>Sequencing of PCR products</td>
<td>The PCR products will be sequenced from the entire mapping population for the amplified gens</td>
<td>Computer Sequencing services</td>
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<tr>
<td>Week 12, 13</td>
<td>Analysis for allelic variations</td>
<td>The individual sequences from the mapping population will be aligned for each candidate gene and allelic variations will be determined</td>
<td>Computer Relevant alignment software</td>
</tr>
<tr>
<td>Week 14, 15</td>
<td>Data analysis to associate gene-specific allelic variations to traits</td>
<td>The allelic variations identified will either be mapped or used in association mapping to identify marker-trait associations</td>
<td>Computer Relevant mapping/association software</td>
</tr>
<tr>
<td>Week 16</td>
<td>Follow-up visit by mentor</td>
<td>Mentor will visit 6 months after the experimental phase to do final revision of manuscript before submission for peer review.</td>
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