

**USDA Notice of Funding Opportunity
2017 Borlaug Fellowship Program for
ASIA AND LATIN AMERICA: BIOTECHNOLOGY**

**NORMAN E. BORLAUG INTERNATIONAL AGRICULTURAL SCIENCE
AND TECHNOLOGY FELLOWSHIP PROGRAM
(BORLAUG FELLOWSHIP PROGRAM)**

**FISCAL YEAR 2017 NOTICE OF FUNDING OPPORTUNITY
for
ASIA AND LATIN AMERICA: BIOTECHNOLOGY**

Application Deadline: JUNE 16, 2017 @ 11:59 PM EDT

Email: BorlaugFellowships@fas.usda.gov

Website: <http://www.fas.usda.gov/programs/borlaug-fellowship-program>

Catalog of Federal Domestic Assistance Number (CFDA) – 10.777

USDA Funding Opportunity: Borlaug 2017 - Asia & Latin America - Biotechnology

This announcement is also being distributed through USDA's EzFedGrants system under the following funding opportunity numbers:

1. USDA-FAS-10777-0700-10.-17-0034, Costa Rica, Biotechnology
2. USDA-FAS-10777-0700-10.-17-0035, Mexico 1 (Fellow #1), Biotechnology
3. USDA-FAS-10777-0700-10.-17-0044, Mexico 2 (Fellow #4), Biotechnology
4. USDA-FAS-10777-0700-10.-17-0045, Panama, Biotechnology
5. USDA-FAS-10777-0700-10.-17-0038, India, Biotechnology
6. USDA-FAS-10777-0700-10.-17-0039, Indonesia, Biotechnology
7. USDA-FAS-10777-0700-10.-17-0040, Malaysia, Biotechnology
8. USDA-FAS-10777-0700-10.-17-0041, Sri Lanka, Biotechnology
9. USDA-FAS-10777-0700-10.-17-0042, Vietnam, Biotechnology

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**U.S. DEPARTMENT OF AGRICULTURE
FOREIGN AGRICULTURAL SERVICE
OFFICE OF CAPACITY BUILDING AND DEVELOPMENT
NORMAN E. BORLAUG INTERNATIONAL AGRICULTURAL SCIENCE AND TECHNOLOGY FELLOWSHIP
PROGRAM**

ISSUED BY: USDA Foreign Agricultural Service, Office of Capacity Building and Development

CATALOG OF FEDERAL DOMESTIC ASSISTANCE (CDFA) NUMBER: 10.777.

CDFA TITLE: Norman E. Borlaug International Agricultural Science and Technology Fellowship

NOTICE OF FUNDING OPPORTUNITY TITLE: Borlaug Fellowship Program 2017, Asia and Latin America, Biotechnology

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2. USDA-FAS-10777-0700-10.-17-0035, Mexico (Fellow #1), Biotechnology
3. USDA-FAS-10777-0700-10.-17-0036, Mexico (Fellow #4), Biotechnology
4. USDA-FAS-10777-0700-10.-17-0037, Panama, Biotechnology
5. USDA-FAS-10777-0700-10.-17-0038, India, Biotechnology
6. USDA-FAS-10777-0700-10.-17-0039, Indonesia, Biotechnology
7. USDA-FAS-10777-0700-10.-17-0040, Malaysia, Biotechnology
8. USDA-FAS-10777-0700-10.-17-0041, Sri Lanka, Biotechnology
9. USDA-FAS-10777-0700-10.-17-0042, Vietnam, Biotechnology

AUTHORIZING AND APPROPRIATION AUTHORITIES: The legislative authority for the Borlaug Fellowship Program is provided in 7 USC 3319j, Pub. L. 95-113, title XIV, §1473g, as added Pub. L. 110-234, title VII, §7139, May 22, 2008, 122 Stat. 1231, and Pub. L. 110-246, §4(a), Title VII, §7139, June 18, 2008, 122 Stat. 1664, 1992. Authority also comes from the National Agricultural Research, Extension, and Teaching Policy Act of 1977 enacted as Title XIV of the Food and Agriculture Act of 1977, 7 U.S.C. 3319(a), 7 U.S.C. 3318(b).

PROGRAM TYPE: New

AWARD TYPE: Cost Reimbursable Agreement for U.S. Universities

PROGRAM OVERVIEW, OBJECTIVES, AND PRIORITIES

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The United States Department of Agriculture's (USDA) Foreign Agricultural Service (FAS) announces the availability of funding through cost reimbursable agreements for the Norman E. Borlaug International Agricultural Science and Technology Fellowship Program (Borlaug Fellowship Program). These Fellows have been competitively selected based on research priorities, academic and professional accomplishments, commitment to Borlaug Fellowship Program goals, and leadership qualities. The Fellow's proposal and research plan appears at the end of this notice. USDA recommends that the program begin in the fall of 2017; however, priority should be given to a time that is appropriate for the Fellow's proposed research topic. The program's duration should be 12 weeks unless otherwise indicated.

Each Fellow has a specific research topic. Here is a summary of the applicants and a brief description of their research topics:

1. Fellow #1, (female); India; Technology and breeding tools for pest reduction
2. Fellow #2, (female); Sri Lanka; Focusing on high-yield, stress-resistant varieties of crops (red onion)
3. Fellow #3, (male); Indonesia; Gene expression and mapping of coconut/sugar palm
4. Fellow #4, (male); Malaysia; Genetic marker for high growth rate of goats
5. Fellow #5, (female); Vietnam; Improving cattle production; taste and texture of meat
6. Fellow #1, (male); Costa Rica; Developing a transient expression system for analysis of resistance genes against coffee rust
7. Fellow #3, (female); Mexico; Characterize the genetic diversity of at least 40 accessions of threatened wild peppers
8. Fellow #4, (female); Mexico; Manage corn as breeding, inbred to sequence DNA and protein extraction
9. Fellow #5, (male); Panama; Develop genetic diversity of rust fungus across infected coffee crops

Section VII provides each Fellow's proposal with background information and research plan.

This notice identifies the Borlaug Fellowship Program deadline, legislative authority, eligibility and proposal requirements, funding restrictions, cost share requirements, allowable and unallowable costs, reporting requirements, program purpose and priorities, focus areas and recommended topics, application and submission information, application review, selection and notification process, agency program contact information, and mailing address.

FEDERAL AWARD INFORMATION

AVAILABLE FUNDING: Up to \$40,000 for each award

PROJECTED NUMBER OF AWARDS: up to 9

PERIOD OF PERFORMANCE: 2 years

An extension to the period of performance may be permitted in certain circumstances. The awardee must request an extension at least 90 days prior to the end of the period of performance, including a

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justification to explain why the statement of work cannot be completed during the original period of performance.

PROJECTED PERIOD OF PERFORMANCE START DATES: between July 1, 2017 and January 1, 2018

PROJECTED PERIOD OF PERFORMANCE END DATES: between June 30, 2019 and December 31, 2019

FUNDING INSTRUMENT: Cost Reimbursable Agreement

DEADLINE: Applications must be received by June 16, 2017 by 11:59 p.m. Eastern Daylight Time. Applications received after this deadline will not be considered for funding.

ELIGIBILITY CRITERIA

ELIGIBLE APPLICANTS: Public and state controlled institutions of higher education.

FAS will accept proposals from U.S. state cooperative institutions or other colleges and universities and minority serving institutions (MSIs). Proposals from smaller academic institutions, MSIs (in particular American Indian, Alaska Native, Pacific Islander, Hispanic, Asian American, and African American institutions) are especially encouraged to apply.

A proposal from a consortium of organizations must be submitted as a single proposal with one U.S. institution serving as the lead and all other organizations as team members, when applicable. An individual mentor must be identified for each Borlaug Fellow. A single mentor may not host two fellows simultaneously. The Principal Investigator (PI) and mentor must hold a position at an eligible U.S. institution.

FAS reviews proposed project costs to make certain those costs are reasonable and allowable per applicable federal regulations. This program is subject to the provisions of 2 CFR Part 200, grant, cooperative, joint venture, and cost-reimbursable agreement recipients/cooperators (including, universities, non-profits, States, Cities/Counties, Tribes, for-profits, and foreign organizations) are subject to Title 2 of the Code of Federal Regulations and other legal requirements, including, but not limited to:

1. 2 CFR Part 25, Universal Identifier and Central Contractor Registration
2. 2 CFR Part 170, Reporting Subaward and Executive Compensation Information
3. 2 CFR Part 175, Award Term for Trafficking in Persons
4. 2 CFR Part 180 and Part 417, OMB Guidelines to Agencies on Government wide Debarment and Suspension (Nonprocurement)
5. 2 CFR Part 200, Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards, as adopted by USDA through 2 CFR part 400.

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University indirect costs for cost reimbursable agreements are limited to 10% of direct costs in accordance with 7 USC 3319a. A cost share or cost match is not required. Management and Administration (M&A) Costs are not allowable. In addition to the above mentioned, all recipients are subject to the Federal Award's general terms and conditions, project narrative, and budget narrative, as well as the applicable authorization used to issue the Federal Award.

In addition to the above mentioned, all recipients/cooperators are subject to the Federal Award's general terms and conditions, project narrative, and budget narrative, as well as the applicable authorization used to issue the Federal Award.

Section I: FUNDING OPPORTUNITY DESCRIPTION

A. PROGRAM DESCRIPTION

The Norman E. Borlaug International Agricultural Science and Technology Fellowship Program promotes food security and economic growth by increasing scientific knowledge and collaborative research to improve agricultural productivity. This program targets promising, early- to mid-career, English-speaking scientists and policymakers from developing or middle-income countries. Fellows spend 8-12 weeks in the United States and work one-on-one with U.S. scientists in their field. Mentors coordinate the Fellows' training, and they visit the Fellows' countries for 5-10 days within 6-12 months after completion of the training in the U.S. to continue collaborative efforts.

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

B. PROGRAM RESPONSIBILITIES OF HOST INSTITUTIONS

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.

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.

Mentor Roles

- 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.

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- 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 semi-annual 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.

Mentor Follow-up Visit

- The mentor visit is an essential and unique part of the Borlaug Fellowship Program. The reciprocal visit is required, not optional.
- 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 PI **must** consult with USDA/FAS **prior** to finalizing plans or purchasing plane tickets for the reciprocal visit. Mentor's travel information must be provided for emergency contact purposes and country clearance (if required by the 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.

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.

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.

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- 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.

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 shall be calculated based on 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.

Emergency Health Insurance

- The host institution will purchase emergency health insurance for the Fellow for the duration of stay, as required for all J1 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.

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.
- 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.

Fellowship Program

- The host institution will provide educational materials and supplies to each Fellow necessary for their full participation in the fellowship.

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- 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 to a local farm, processing plant, private industry, or other related industry 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. USDA/FAS will provide a report template.

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:
 - Explain and demonstrate local bus/transportation options
 - Explain cultural and legal expectations
 - USDA will provide a welcome and orientation packet for mentors

Progress Reports

- The Principal Investigator or Mentor will submit **semi-annual** progress reports. The Principal Investigator or Mentor will use Performance Progress Report (SF-PPR) to submit semi-annual progress reports.
- The Principal Investigator or Mentor will submit a final report to USDA/FAS within 30 days after the Mentor visit. USDA/FAS will provide additional guidance and a template for the final report.
- Reports should include the following:
 - Summary of activities, accomplishments, and any problems encountered or overcome
 - Photographs, when possible
 - Completed program evaluations and action plan
- An invoice cannot be paid if a progress report is past due, and will not be paid until the required report has been received.

Financial Reporting

- Financial reports will follow the Uniform Administrative Requirements for Grants and Agreements, 2 CFR Part 200.
- Invoices will use the Request for Advance or Reimbursement (SF-270).

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- Invoices will be submitted electronically to SF-270InvoicesMailbox@fas.usda.gov and copied to the USDA/FAS program manager and USD/FAS program assistant.
- A summary of expenses that aligns expense totals to the agreement's budget line items must be included.
- A detailed breakdown of expenses must be included with SF-270. Payment will not be processed without supporting documentation.
- A final invoice must be submitted within 90 days of the end of the period of performance for the agreement.
- Costs must be reported in accordance with the regulations that govern the agreement, and must follow the applicable Federal cost principles 2 CFR 200. The institution cannot be reimbursed for costs that are contrary to the specific terms of the agreement or are outside its scope.
- A Federal Financial Report (SF-425) must be submitted semi-annual and within 90 days of the end of the period of performance for the agreement.
- An invoice cannot be paid if a financial report is past due, and it will not be paid until the required report has been received.

SECTION II: APPLICATION AND SUBMISSION INFORMATION

A. ADDRESS TO REQUEST APPLICATION PACKAGE

This announcement contains all instructions and links to all forms required to complete the application. All applications must be submitted as PDF or Word documents. No mailed or facsimile submissions will be accepted. Email address is: BorlaugProposals@fas.usda.gov.

B. CONTENT AND FORM OF APPLICATION SUBMISSION

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

- Complete SF-424 Application for Federal Assistance for a single Borlaug Fellow. USDA/FAS cannot accept applications for multiple fellows in a single application.
- 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.

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- Identify the expected skills or knowledge to be acquired by the Fellow at the end of the program
- Provide a program budget using Standard Form -424A- Budget Information Non Construction Programs, including a detailed budget worksheet (see page 12).
- Provide a budget narrative. All line items should be described in sufficient detail to enable FAS to determine that the costs are reasonable and allowable for the project in accordance with federal regulations.
- If attendance at the World Food Prize in Des Moines, Iowa during October 2017 is feasible, then the Fellowship may be extended one additional week, not to exceed 13 weeks, to ensure the Fellow receives up to 12 weeks of training.
 - 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.
- Complete [AD-3030, Representations Regarding Felony Conviction and Tax Delinquent Status for Corporate Applicants](#).
- Complete [AD-3031, Assurance Regarding Felony Conviction or Tax Delinquent Status for Corporate Applicants](#)
- Complete the Host University Administrative Checklist on university administrative policies
- **If not submitting applications through the ezFedGrants portal at <https://grants.fms.usda.gov>, Submit all application materials as attachments to a single email.**
 - The primary document submitted in response to this REI with all information requested should be titled *Statement of Work*.
 - Include all application information that is not a specific form in a single PDF document.

Successful applicants will be required to submit all relevant national certifications and compliance documents prior to awards being issued.

C. UNIQUE ENTITY IDENTIFIER AND SYSTEM FOR AWARD MANAGEMENT (SAM)

All applicants are required to:

1. Be registered in SAM before submitting its application;
2. Provide a valid DUNS number in its application; and
3. Continue to maintain an active SAM registration with current information at all times during which it has an active Federal award or an application or plan under consideration by a Federal awarding agency.

FAS 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 FAS 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.

FAS is using ezFedGrants, which is an electronic grants management system. Applicant(s) with electronic access are to submit their applications electronically through: <https://grants.fmmi.usda.gov>. As stated above before you can apply, you must have a DUNS number, be registered in SAM, and have access to the ezFedGrants website.

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Applicants are encouraged to register early. The registration process can take approximately four 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>. 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. Failure to register with SAM will result in your application being rejected during the submissions process.

D. 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 GrantorHelpdesk@fas.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 GrantorHelpdesk@fas.usda.gov.

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 GrantorHelpdesk@fas.usda.gov.

FAS 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 FAS is ready to make a Federal award, FAS 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.

E. SUBMISSION DEADLINES AND TIMES

Submit all application materials in a single email. Include all application information that is not a specific form in a single PDF document. The following forms are required: SF-424, SF-424A, AD-3030, and AD-3031. The primary document submitted in response to this NOFO with all information requested should be titled *Statement of Work*.

Funding opportunities will be distributed through ezFedGrants and advertised via the USDA/NIFA listserv. All proposals must be submitted to through the ezFedGrants portal at

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<https://grants.fms.usda.gov> or the email address below with all required forms. Proposals not submitted by the stated deadline will not be accepted. Borlaug Fellowship Program Proposal Email: BorlaugProposals@fas.usda.gov and Sarah.Librea@fas.usda.gov

F. FUNDING RESTRICTIONS

Allowable Costs:

To help in this review and to expedite the award process, budgets must include a narrative detailing all line items. The categories listed below are examples of some of the more common items found in project budgets. All 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.

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.

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

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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; capital improvements; thank you gifts, and other expenses not directly related to the project are not allowed.

G. OTHER SUBMISSION REQUIREMENTS

All applications must be submitted electronically as indicated above.

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HOST UNIVERSITY ADMINISTRATIVE CHECKLIST

Please complete the following checklist concerning the university's policies on providing per diem funds to exchange visitors. This information is for USDA internal use only and does not determine your eligibility to serve as a host institution.

Host University Policies	YES	NO
Will the mentor listed in the proposal be present for the majority of the fellowship?		
Will the mentor be able to spend time meeting with fellow individually each week?		
Will the university be able to provide per diem within the first week of the Fellow's arrival?		
Will the university be able to provide fully furnished lodging with kitchen facilities?		
Does the university withhold federal tax on the participants' per diem and housing? * If so, you must list this expense as a separate line item on the budget.		

* Note that 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). No taxes should be withheld from Borlaug Fellows since they are exempt.

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Budget Worksheet

Host Institution:

Estimated Dates:

NOFO#/Country/Fellow#

SF-424 Category	Line Items	Rate	Days	Subtotal
Fellow's Logistical Expenses				
TRAVEL/Housing	1. Lodging			
TRAVEL	2. Meals and Incidentals			
OTHER	3. Federal Tax			
TRAVEL	4. Medical Insurance			
TRAVEL	6. Local Transportation			
TRAVEL	7. Airfare - International			
TRAVEL	8. Airfare - Domestic (If Applicable)			
			Subtotal	_____
Fellow's Professional Development				
TRAVEL	1. Field Tours			
SUPPLIES	2. Educational Materials and IT Expenses			
SUPPLIES	3. Shipping Materials			
			Subtotal	_____
Host Institution Fees				
PERSONNEL	1. Training Coordinator (Salary)			
FRINGE BENEFITS	1.b. Training Coordinator (Fringe Benefits)			
PERSONNEL	2. Mentor Fee			
FRINGE BENEFITS	2.b. Mentor (Fringe Benefits)			
SUPPLIES	3. Laboratory Expenses			
			Subtotal	_____
World Food Prize Symposium (Oct. 2017; If Applicable)				
TRAVEL	1. Domestic Transportation			
TRAVEL	2. Lodging			
OTHER	3. Conference Fee			
			Subtotal	_____
Mentor Follow up Activity (5-10 Days)				
TRAVEL	1. Mentor Airfare – International			
TRAVEL	2. Mentor Domestic In-Country Travel (If Applicable)			
TRAVEL	3. Lodging			
TRAVEL	4. Meals & Incidentals			
SUPPLIES	5. Supplies for Trainings/Workshops			
			Subtotal	_____
			Total Program Costs	
INDIRECT			Indirect Costs/Overhead (10%)	
			Total Request	

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Section III: Application Review Information

All proposals are carefully reviewed by USDA/FAS Program Officers and other FAS staff against the criteria listed below, including others who are experts in a particular field, as appropriate.

A. REVIEW CRITERIA

- **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 length of the program. The budget should include appropriate cost savings where available. Salary and fringe benefits expenses should not be excessive.

B. REVIEW AND SELECTION PROCESS

Other factors may also be taken into consideration such as regional diversity and MSI status in the review process. After review by appropriate offices, it is expected that all applicants will be notified within 2 months after the closing date for applications.

Section IV: Award Administration Information

A. AWARD NOTICES

Applicants should expect to be contacted by program staff for clarification and additional discussion on any budget related issues before final determination of successful applicants. Any notification by the program office regarding the selection of an institution is not an authorization to begin performance. No pre-award costs can be charged. The notice of award signed by the Deputy Administrator of USDA/FAS/OCBD is the authorizing document. This document will be sent by electronic mail to the university. Both parties must sign this document before the agreement is in force. Unsuccessful applicants will be notified of the status of their application by email.

B. ADMINISTRATIVE AND NATIONAL POLICY REQUIREMENTS

Certifications regarding debarment Suspension, Drug Free Workplace, Felony Conviction and Tax Delinquent Status, and other national administrative assurances and policies are required. The

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cooperator must adhere to administrative requirements, cost principles, and audit requirements as contained in 2 CFR Part 200, Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards.

All successful applicants for all cost reimbursable agreements are required to comply with Standard Administrative Terms and Conditions, which are available online at:

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 ezFedGrants GMO 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.

C. REPORTING REQUIREMENTS:

Primary Investigators are required to submit mid-term and final Fellow's performance reports on the U.S. portion of the Borlaug Fellowship. A final mentor's visit report including a final evaluation should be submitted no later than 30 days after the completion of the mentor visit.

- Financial reports will use SF-425.
- Progress Reports will use SF-PPR.
- Invoices will use SF-270.

Progress Reports

- The Principal Investigator or Mentor will submit **semi-annual** progress reports. The Principal Investigator or Mentor will use Performance Progress Report (SF-PPR) to submit semi-annual progress reports.
- The Principal Investigator or Mentor will submit a final report to USDA/FAS within 30 days after the Mentor visit. USDA/FAS will provide additional guidance and a template for the final report.
- Reports should include the following:
 - Summary of activities, accomplishments, and any problems encountered or overcome
 - Photographs, when possible
 - 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.

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After these reports have been reviewed and approved by *Program Division*, 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.

Section V: Agency Contact

Applicants can direct questions or request help before the deadline for submission of the application for these funding opportunities via the contact information below:

- Borlaug Fellowship Proposals General Email: BorlaugProposals@fas.usda.gov
- Borlaug Asia/Latin America: Sarah Librea, (202) 720-2018 or Sarah.Librea@fas.usda.gov
- Borlaug Asia/Latin America: Tanya Hinnant, (202) 720-3382 or Tanya.Hinnant@fas.usda.gov

Section VI: Other Information

The USDA Borlaug Fellowship Program began in 2004. More than 750 Fellows from 64 countries have been trained to date. Additional program information is available at <http://www.fas.usda.gov/programs/borlaug-fellowship-program>.

Related Requests for Expressions of interest will be distributed by region and topic including: Asia, Eastern Europe, Latin America, North Africa, East/ Sub-Saharan Africa. This will be posted on the NIFA listserv.

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Section VII: Borlaug Fellow Proposal and Research Plan

Fellow Reference Number	Country	Gender	Fellowship Length (weeks)	Research Focus
1	India	Female	12	CSA: Beneficial soil microbes to produce drought-tolerant crops
2	Sri Lanka	Female	12	CSA: Gene-trait in rice related to salinity tolerance
3	Indonesia	Male	12	CSA: Evaluate effects of the CSA practices and factors to scale it
4	Malaysia	Male	12	GRA: Wetting-dry rice production system to sustain rice yield and reduce methane emissions through improved water and nutrient management
5	Vietnam	Female	12	Improving cattle production; taste and texture of meat
6	Costa Rica	Male	12	Developing a transient expression system for analysis of resistance genes against coffee rust
7	Mexico	Female	12	Characterize the genetic diversity of at least 40 accessions of threatened wild peppers
8	Mexico	Female	6	Manage corn as breeding, inbred to sequence DNA and protein extraction
9	Panama	Male	12	Develop genetic diversity of rust fungus across infected coffee crops

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Appendix 1: Detailed Borlaug Fellow Proposal and Research Plan

Fellow 1 – India

1. The goal of my research is to gain theoretical and practical knowledge of the gene editing techniques used for genome modification of insects and apply this knowledge for developing novel solutions for insect pest control

2. The following research objectives will help in achieving the goal

1. Identify a specific agricultural pest for standardization of the methods.
2. Identification of target genes (gene expression and phenotype annotations)
3. Bioinformatic analysis
4. Generation of donor constructs for gene targeting and editing
5. Development of Insect transformation (embryo staging, microinjection)
6. Mating schemes for transformant recovery and maintenance
7. Molecular confirmation of gene targeting
8. Design Relevant Assays for confirmation of phenotypes

3. Sap-sucking insects as pests and vectors of plant pathogens represent an increasing threat to agriculture. Therefore breeding crops for insect-resistance is of primary interest to our company as well as my research program. Insect resistant traits in crops have come from a variety of sources. For example conventional plant breeding takes advantage of existing plant characters and natural genetic variations for developing varieties/hybrids, whereas the crop genetic modification (GM) approach relies on use of genes from variety of sources including plants and micro-organisms to impart insect resistance. The research in my lab uses both approaches for developing insect traits in crops.

The general workflow for evaluating insecticidal proteins for GM plant development includes: 1) cloning and characterization of insecticidal genes from plants and microbes 2) protein production and evaluation of insecticidal activity against target insect pest by diet bioassays 4) evaluate insect responses (mortality, growth inhibition, reduced fecundity) 5) Select candidate genes that adversely affect insects for plant transformation and transgenic product development. I have successfully completed POC of at least five candidate genes for product development. Another research project is on insect RNAi technology (POC in whiteflies, brown planthoppers): In this project the Insect gene sequences are analyzed for identification of potential targets for gene silencing. RNAi constructs are developed and evaluated for gene silencing in insects through dsRNA/si RNA feeding assays (In vitro). For establishing an effective method for large scale RNAi in crop plants different RNAi constructs expressing insect sequences are tested in model/target plants and transgenic plant are evaluated, and the silencing of the targets in insects and insect survival are quantified.

Using native resistance: This project focuses on germplasm evaluation for native resistance traits towards cotton sucking pests. Novel recombinant lines (allele stacking) are created through crosses and the insect-resistant germplasm is characterized further to decipher the resistance

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mechanism and inheritance of the resistance. The approaches used involve genetics, functional genomics, plant phenotyping and analysis of insect life tables for the identification of target genes/pathways conferring plant resistance. The project deliverables are: development of new breeding lines, characterization of genes/pathways conferring resistance to sucking pests and trait-linked markers for marker-assisted selection.

4. During the fellowship I wish to connect and work with insect scientists on project(s) that use advanced technologies such as (genome modification/gene editing in insects). My research interest is on insect pests of crops and in the past decade, I have evaluated different approaches both conventional (host plant resistance) and advanced technologies (genetic engineering, RNAi approaches) for developing insect traits in crops. I am currently working on genome edited traits in crops for improving crop productivity and this process involves target selection, rational edit design (analysis of targets and off-targets) and establishing technical proof of concept in rice and tomato. During my PhD research at the University of Nebraska-Lincoln, USA, I got the opportunity to work on New World screwworm, *Cochliomyia hominivorax* in the USDA-ARS Lab, Eradication of screwworm is one of the greatest entomological success and subsequent developments in screwworm research (genetic sexing strains, RIDL technology) are quite commendable. Gaining hands-on experience in insect gene editing and insect transformation methods at a reputed lab under the supervision of a mentor in the US will help me in using these technologies to develop solutions for specific crop pests of interest to our company and also develop effective research collaborations.

5. This fellowship will help me in gaining theoretical and practical knowledge of the state of the art technologies available for developing insect traits in crops and thus address the agricultural productivity problems. By working in a host lab, I can network with other researches and post training, I can apply the learnings to my current research projects.

Action Plan

Week 1: Identify an insect system (mosquito/spodoptera/others) for study and select target genes (novel targets/existing targets such as genes targeting reproduction or other traits)

Planned Outcomes: Identify 2-3 target genes for evaluation, Requirements: Insect strains and rearing facility

Week 2: Bioinformatic analysis- for gene expression and phenotype associations

Planned Outcomes: Identification of specific gene variants and associated phenotypes based on Annotations Requirements: Software tools for data analysis, access to proprietary databases, databases in the public domain

Week 3: Generation of constructs (donor constructs)

Planned Outcomes: Assembling the donor constructs containing regions of homology to the target Locus Requirements: Gateway vectors, markers-GFP/RFP

Week 4: Generation of constructs

Planned Outcomes: Generation of CRISPR constructs

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Requirements: Promoters, Cas9 gene, vectors, restriction enzymes

Week 5-6 Microinjection of embryos and selection of transformants, crosses

Planned Outcomes: Transformed insects

Requirements: Microinjector

Week 7-8: Containment of insects transformed with a gene drive

Planned Outcomes: GM insects generation advancement through crosses

Requirements: Insectary that is compliant with Arthropod containment Guidelines Level 2

Week 9-12: Molecular confirmation of gene targeting, assays for Phenotyping (for example fertility assays)

Planned Outcomes: Confirmation of editing through PCR sequencing and confirmation of phenotypes

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Fellow 2 – Sri Lanka

1. The goal of my research is to analysis diversity of cluster onion and its major fungal pathogens in Sri Lanka

2. Objectives of this research are study on genomic variation of *A. cepa* in Sri Lanka and evaluate genetic diversity, study on genomic variation of *Fusarium* spp. and *Alternaria porri* and develop genomic libraries, and Identify genetic markers suitable for species identification of *Fusarium* spp. and *Alternaria porri*.

3. Cluster onion or shallot, *Allium cepa* L., belongs to the family Alliaceae and is one of the important condiments in Sri Lankan dishes. A partial to complete tendency toward asexual reproduction is observed on true shallot species. There are a large number of cultivars or varieties of cluster onions that differ in flavour, size, yield, and disease tolerance grown by Sri Lankan small-holder farmers as cash crops. Efforts are under way to develop shallot cultivars that offer superior agronomical properties and these varietal development programmes could greatly benefit from genetic markers suitable for marker-assisted breeding. In addition to selective breeding, molecular markers can also be used for varietal identification, diversity analysis, colour and quality improvement, genetic mapping, and male sterility analysis (Joshi et al. 1999). Diploid bulb onion contains eight pairs of chromosomes and about 16 Gbp genome. In spite of advances in sequencing technologies, sequencing of onion genomes is a challenge due to large genome size.

A variety of genetic markers have been used for studies on cultivar identification and diversity in Alliums (Klaas and Friesen, 2002). For diversity analysis, RAPD (randomly amplified polymorphic DNA) and ISSR (inter-simple sequence repeat) markers have been used due to its low cost and simplicity. With RAPD markers it has showed that *A. roylei* as the closest relative of *A. cepa*, questioning the current classification of *A. cepa* in the section Rhizideum (Susan et al.,1993). A number of simple sequence repeat (SSR) or microsatellite markers identified from expressed sequence tags (EST) of *A. cepa* have been used in genetic studies (e.g. Baldwin et al. 2012, 2014; Duangjit et al. 2013; McCallum et al. 2002). Although these studies use *A. cepa* cultivars from several regions of world, the cultivars grown in Sri Lanka have not been tested with any of the published genetic markers. The purpose of this proposal is to receive a training in SSR-based genetic analysis that will facilitate validation of currently available SSR markers for *A. cepa* cultivars grown in Sri Lanka. In addition, genetic markers for the fungal pathogens of onions causing serious economic damage in Sri Lanka, specifically the bulb rot pathogens *Fusarium* spp., and the purple blotch pathogen *Alternaria porri*, will be developed and characterized during the proposed fellowship. The training and the genetic tools developed during this fellowship will facilitate current breeding programs as well as genetic characterization of *A. cepa* cultivars and two of its major pathogens in Sri Lanka.

4. Polymerase chain reaction (PCR) primers for *A. cepa* SSR markers will be obtained from public databases and used for validation with the genomic DNA isolated from the *A. cepa* collections available in Sri Lanka. Single nucleotide polymorphism (SNP) markers linked to SSR markers and ESTs. SNP markers from genomic and mitochondrial DNA will be evaluated for DNA bar coding to

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demarcate accessions. Genomic DNA isolated from the major fungal pathogens of onion in Sri Lanka, *Fusarium* spp. and *Alternaria porri* will be used to develop novel genetic markers to facilitate species identification and population characterization and explore the possibility of developing PCR-based method for species identification.

5. Molecular study on major onion fungal pathogens is very limited in local conditions. With this fellowship it will help to use novel technologies to enhance the molecular studies. Further more research can be diverting towards the verity development for resistant breeding. Those geneticall studies will be useful to reduce the loss due to disease infection of national onion production. With that it will be helpful to enhanced agricultural productivity and reduce the cost of importation for economic development, and also it will help to food security in my country

Action Plan

In order to achieve the research goal and objective, the activities have planned as following:

1. Prior to beginning of the fellowship

- a) Collect *Allium cepa* germplasm available in Sri Lanka and extract genomic DNA. Preserve germplasm collection for propagation and further studies
- b) Isolate and culture *Fusarium* spp. and *Alternaria porri* from diseased samples for DNA extraction. Preserve fungal pathogen cultures for further studies.
- c) Synthesize PCR primers for SSR markers using primer sequence data available in public databases.

2. First week of the fellowship

- a) Optimize PCR conditions and perform SSR primer validation on *A. cepa* genomic DNA collections from Sri Lanka.
- b) Begin construction of genomic DNA libraries from *Fusarium* spp. and *Alternaria porri* for isolating genetic markers. Submit genomic libraries to a core facility for sequencing.

3. Second to fourth weeks of the fellowship

- a) Identify SSR marker loci suitable for Sri Lankan accessions of *A. cepa* and perform a population genetic analysis to evaluate genetic diversity. Identify any potential genetic marker (or combinations of markers) suitable for demarcation of cultivars.
- b) Start PCR amplification of *A. cepa* genomic DNA with primers for selected SSR makers. Multiplex fluorescent tagging will be employed to analyze up to 4 different loci per lane of genetic analyzer. Submit pooled SSR amplicons to the core facility for analysis.
- c) Develop skills in assembly of high-throughput sequence data using bioinformatics software (CLC Genomics WorkBench, SNP & Variation Suite (SVS)). Develop skills in SSR and SNP marker identification using sequence data from *Fusarium* spp. and *Alternaria porri* (SSR Finder).
- d) Develop and purchase PCR primers for SSR markers identified from *Fusarium* spp. and *Alternaria porri*.

4. Fifth to eighth weeks of the fellowship

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- a) Validate SSR and SNP markers developed for *Fusarium* spp. and *Alternaria porri* by PCR and nucleotide sequencing. Identify genetic markers suitable for species identification.
- b) PCR amplify, clone, and sequence ribosomal RNA (rRNA) internal transcribes spacer (ITS) regions and mitochondrial DNA cytochrome oxidase 1 and 2 genes (CO1 and COII using universal primers to identify DNA regions suitable for DNA barcoding (species and strain identification) of *Fusarium* spp. and *Alternaria porri*.
- c) Complete SSR marker amplification from Sri Lankan *A. cepa* accessions (continued from 3a) and estimate genetic diversity and other population genetic parameters. Evaluate data to identify any markers or combinations of markers suitable for identification of specific cultivars of *A. cepa*.

5. Ninth to Twelfth weeks of the fellowship

- a) Wrap up SSR marker analysis of Sri Lankan *A. cepa* accessions and summarize data for reporting and publication.
- b) Complete genetic marker development and select genetic markers suitable for species/strain identification and population studies of *Fusarium* spp. and *Alternaria porri*.

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Fellow 3 – Indonesia

1. The goal of my research is to familiarize with: NGS whole genome data analysis (de novo assembly, read mapping, variant calling and annotation), SSR and SNP markers development based on NGS whole genome data, and validation and genetic diversity analysis of the target perennial crops using the developed markers.

2- Conduct De-Novo Assembly, generate read mapping, variant calling and genome annotation using the generated NGS whole genome data of coconut or/and sugar palm
- Develop SSR and SNP markers based on the generated NGS whole genome data,
- Validate effectiveness of the generated molecular markers information and evaluate genetic diversity of the coconut or/and sugar palm samples from Indonesia using the validated markers.

3. Coconuts and Sugar Palms are two major tropical perennial palms from Indonesia. Coconuts are important crop to Indonesia since it produce many useful products. Indonesia is the coconut centers of origin. Recently, interest for coconut cultivation has increased significantly because of the newly develop and more profitable product of coconuts are entering markets. Therefore, Farmers and private companies are looking for coconut seedling having certain superior phenotypes.

Sugar palms are the source of raw material for production of biofuels (ethanol). The sugary sap (neera) directly tapped from male inflorescences at age of 6-12 years is utilized to produce palm sugar, traditional wine and biofuel (ethanol). The ethanol content in the fermented sap may be used as a good source of biofuel. Biofuel can be used as an alternative to fossil-fuel since it is renewable and environmentally friendly.

Sugar palm is one of the many promising biofuel crops attracting many scientists in Indonesia. it grows in many places in South Sulawesi and is traditionally commercialized at Banti Murung National Park, Bulusaraung. Limited genetic information about sugar palm is available but we assume that natural populations exhibit high genetic diversity. Further studies need to be done for this important crop to support future sugar palm improvement.

Breeding and variety improvement for coconut and sugar palm are very difficult because of very long juvenile periods (5-7 years for coconut and 6-12 years for sugar palm). Development of molecular markers will assist future breeding and cultivar improvement of coconuts and sugar palms. Availability of markers associated with important phenotypes, such as early flowering and high yield characters are beneficial for supporting breeding programs.

Genetic diversity could be analyzed using DNA-based markers. Once the DNA-based markers are identified and linked to phenotypes, they can be used as indirect indicators of phenotypes and as tools for selecting coconut or sugar palm progenies in breeding programs. Such DNA markers can be generated in the form of microsatellites (SSR) and single nucleotide polymorphism (SNP) markers. Both markers can be used to evaluate genetic diversity since they are relatively abundance across the plant genomes, highly reproducible and high polymorphism levels. Therefore, the SSR and SNP markers will be useful to support breeding activities of target crops.

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Next generation sequencing (NGS) enable to resequence a whole or targeted region of plant genome to develop molecular markers for genetic analysis studies. The NGS is extremely high throughput technologies producing billions of DNA sequences at once at a fraction of the cost of previously develop Sanger sequencing method. Another advantage is the ability to identify sequences flanking the SSR regions which can be used to design primers and develop SSR markers. Thus, development of reliable SSR and SNP markers for coconut and sugar palm can be developed using the NGS and subsequently be used for genetic diversity analysis.

In previous activities, my professor at Bogor Agricultural University (IPB), Indonesia (Prof. Dr. Ir. Sudarsono, MSc.) has generated whole genome data of coconut and sugar palm using Illumina NGS. From those activities, at least 120 GB raw read of the genome sequence data have been generated. However, we have not been able to conduct downstream analysis of the whole genome data because of the computing power limitation. Meanwhile, downstream analysis of the NGS genome data is very important in order to extract useful information from the data. In addition to generating basic knowledge for the coconut and sugar palm genome, such results of coconut and sugar palm whole genome downstream data analysis will be useful for supporting breeding and cultivar development of coconut and sugar palm in Indonesia. Therefore, it is important to continue downstream data analysis of the available raw NGS whole genome data of coconut and sugar palm for developing SSR and SNAP markers.

4. I hope during the fellowship I could have the chance to get experiences on the downstream analysis of the whole genome data because the computing power is not a limitation in the host institution. Once the downstream analysis is completed, I intend to have the chance to develop SSR and SNP markers based on the assembled whole genome and evaluate the genetic diversity of coconut or/and sugar palm from Indonesia. As a researcher in forest tree biotechnology, I have applied SSR and RAPD markers for analyzing forest crops. I have been intensively discussing the whole genome NGS downstream analysis with Prof. Sudarsono at IPB. However, since there is only limited massive computing infrastructure, I have not been able to complete the analysis. As a host institution for my propose Borlaug Fellowship, I have contacted Dr. Dapeng Zhang from USDA ARS. Dr. Zhang has been positive about my proposed research proposal. By working in Dr. Zhang laboratory, I should be able to gain beneficial knowledge and practical application in the complete downstream analysis of the coconut or/and sugar palm whole genome NGS.

5. By acquiring the ability to do downstream analysis of NGS whole genome data and development of molecular markers, I will be able to contribute to the application of genome analysis to support breeding of coconut and sugar palms. By combining genomic data analysis and molecular marker development based on the genomic data, the effectiveness and efficiency of the breeding activities of tropical perennial crops may be improved. Therefore, it will enhance agricultural productivity, economic development and food security in Indonesia.

Action Plan

1) WEEK 1

a. Name of activity : Lab orientation and initiation of NGS downstream data analysis (de novo assembly)

Start : 1

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Finish : 7

Duration : 7 days

Goal : familiarize with contact persons, lab facilities and computer infrastructure facilities.

Initiating permit to access computing facility and NGS data analysis (de novo assembly)

2) WEEK 2

b) Name of activity : DNA isolation from sampled coconuts and/or sugar palm, SSR primer synthesis and continuation of NGS data analysis (de novo assembly and generating read map)

Start : 8

Finish : 14

Duration : 7 days

Goal : getting DNA from leaf samples and continuing NGS data analysis

3) WEEK 3

c) Name of activity : SSR analysis from sampled coconuts and/or sugar palm and continuation of NGS data analysis (generating read map and variant calling)

Start : 15

Finish : 21

Duration : 7 days

Goal : PCR amplification of the sampled DNA coconuts and/or sugar palm to generate SSR markers and continuing NGS data analysis (variant calling)

4) WEEK 4

d) Name of activity : Continuing SSR analysis from sampled coconuts and/or sugar palm and continuation of NGS data analysis (continuation of variant calling, identification of SSR and SNP loci)

Start : 22

Finish : 28

Duration : 7 days

Goal : PCR amplification of the sampled DNA coconuts and/or sugar palm to generate SSR markers and continuing NGS data analysis (variant calling, identification of SSR and SNP loci)

5) WEEK 5

e) Name of activity : Primer design from newly identified SSR sequences and continuation of NGS data analysis (identification of SSR and SNP loci and genome annotation)

Start : 29

Finish : 35

Duration : 7 days

Goal : Primer design from newly identified SSR sequences and continuation of NGS data analysis (identification of SSR and SNP loci and genome annotation)

6) WEEK 6-7

f) Name of activity: Validation of effectiveness of newly identified SSR primers, analysis of SNP marker and completion of NGS data analysis (genome annotation)

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Start : 36

Finish : 49

Duration : 14 days

Goal : Validation of effectiveness of newly identified SSR primers, analysis of SNP marker and completion of NGS data analysis (genome annotation)

7) WEEK 8-12

g) Name of activity : DNA amplification of all DNA samples using the designed SSR and SNP primers, SSR and SNP Genotyping and report

Start : 50

Finish : 84

Duration : 35 days

Goal : Amplifying all DNA samples, obtaining genotype data and final report done

Note : PCR amplification will be performed using the touchdown-PCR Sensoquest Thermocycler (Germany) with total volume of 12.5 μ L, containing 2 μ l of template DNA, 1.25 μ L of each primer pair (forward and reverse primers), 6.25 μ L of PCR mix (KAPA 2G Fast Biosystem) and 3 μ L of ddH₂O. The PCR amplification cycles will be conducted under the following steps : one cycle of initial denaturation at 94 $^{\circ}$ C for 60 seconds, 35 amplification cycles at 94 $^{\circ}$ C for 15 seconds (template denaturation), different temperature for each primer pair for 50 seconds (primer annealing), 72 $^{\circ}$ C for 60 seconds (primer elongation) and a final extension at 72 $^{\circ}$ C for 15 minutes. SSR fragment sizes will be calculated by the CEQ 8800 Genetic Analysis, and SNP will be genotyped using Fluidigm EP1 system.

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Fellow 4 – Malaysia

1. The goals of my research is to develop a new improved local goat breeds “Katjang”.

2.1 To apply the whole genome commercial separated markers panel to determine the ancestors of mixed breed of the current Katjang goat for the establishment of purebred Katjang.

2.2. To develop custom SNP Panel to identify molecular markers that linked to heat stress and disease resistance.

3.The demand for goat meat has increased over the year since 2000 in Malaysia. This is reflected by the increased in the number of goat slaughtered from 37, 653 to 63, 638 in 2012 and 2014 respectively (Ministry of Agriculture, 2014). However, according to Ministry of Agriculture, this contributed to only 18.1% of the national SSL rate. Therefore, proactive efforts are needed to increase our national SSL rate for the goat meat production. There are about 4500 farmers and entrepreneurs currently rearing goat in Malaysia but 75% of them are small-scale farming. Improper breeding and selection due to the lack of knowledge and information in the genetic backgrounds is one of the factors contributing to the slow growing of Malaysia’s goat farming industry. The goat population in Malaysia is comprised of the indigenous Katjang goat with several exotic goats such as Jamnapari, Boer, Kalahari, Saanen and Savanna goat. The Katjang goat has a high tolerance to the local environment and high resistance to most of local disease but it has low meat quantity. Thus, several attempts have been made to cross Katjang goat with the exotic introduced breeds to develop goat breed with both high local environment tolerance and meat quantity. However, disorganized and uncontrolled crossbreeding activities have caused inbreeding and loss of genetic diversity. Several studies also stated that the indigenous Katjang goats were being excluded in breeding efforts which lead to the extinction of the Katjang purebred. Through the discussion with Malaysian Department of Veterinary Service had claimed the pureness of Katjang goat about 70% based on the phenotypic data recorded.

This study therefore aims to apply the commercial whole genome markers SNP panel (50K SNP Panels) to determine the ancestors of mixed breed of the current Katjang goat for the establishment of purebred. In addition to the data, we also aimed to produce trait linked marker for heat stress and disease resistance through genome wide association study. Thus with all the information, the purebred Katjang goat population will be used in goat breed improvement program in Malaysia by crossing the local breeds Katjang with Boer. This expected to produce the improved Katjang breeds with higher meat quantity. The advancement of SNP genotyping technology (Illumina iScan) in MARDI has allowed the application of high throughput genotyping to this study resulting more powerful resolution in achieving the targeted objectives. However, there is lacking of expertise in Malaysia that can provide training on those analysis required for this project especially genome wide association study (GWAS). Therefore this training will serve as platform for this project to be realized with a potential of collaboration with expertise from United States of America.

4. As a researcher under animal genetics and well trained in handling Illumina iScan, I had lead projects focused mainly on marker development in various traits of aquaculture and livestock species by applying whole genome analysis SNP panel. The proposed proposal for Borlaug

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fellowship program involved in field of animal genetic and animal breeding, aimed to identify the genomic region and to explore gene pathway of heat stress and disease resistance related gene that will give an advantages for us to introgress those genes into hybrid breeds. The genotype information that we produced will assist us on determining which individuals that we can choose for development of improved local Katjang breeds through marker assisted breeding.

5. In Malaysia, agricultural sector especially livestock industry is one the key sectors that ensures its ability to provide sufficient food for the increasing population. Although Malaysia is blessed with fertile soil, abundant rainfall and suitable climate for food production, Malaysia is still a net food importer and never achieved a food trade balance. According to Malaysian Ministry of Agriculture in 2014, the goat meat production has contributed to only 18.1% of the national self-sufficiency level rate. On the other hand, this fellowship program provides training opportunities to fellows from developing countries like Malaysia. This training will provide exposure in working with experienced mentor at a selected university in the field of agriculture especially in animal genetics. This hands on training program will enable Malaysian researcher to bring back new technologies and approaches to increase meat production to fulfill the food demand and ultimately increased our self-sufficiency level.

Action Plan

1) First Week University and laboratory orientations and staff introductions.

2) Second Week Experimental Design

3) Third Week DNA extraction and Quality Control (Theory and Hands-On)

a) The DNA from all blood samples will be extracted using modified CTAB Method.

b) The quality control for the DNA will be further conducted using agarose gel electrophoresis system (DNA qualification) and Fluoroskan Ascent FL (DNA quantification).

4) Fourth Week Genotyping on High Troughput Platform (Theory) Examples on Illumina iScan

a) The process consist of 3 days of beadchip process before Illumina iScan will be used to scan the beadchips. The beadchip process will be divided into 3 major activities which is:

i) Whole Genome Amplification - The DNA samples are denatured and neutralized to prepare them for amplification. The denatured DNA is isothermally amplified in an overnight step. The wholegenome amplification uniformly increases the amount of the DNA sample by several thousandfold without introducing large amounts of amplification bias.

ii) DNA Fragmentation - The amplified product is fragmented by a controlled enzymatic process that does not require gel electrophoresis. The process uses end-point fragmentation to avoid overfragmenting the sample.

iii) BeadChip Hybridisation - The BeadChip is prepared for hybridization in a capillary flow-through chamber. Samples are applied to a BeadChip and divided by an IntelliHyb[®] seal (or gasket). The loaded BeadChip is incubated overnight in the Illumina Hybridization Oven. The amplified and fragmented DNA samples anneal to locus-specific 50-mers (covalently linked to one of up to one million bead types) during hybridization. One bead type corresponds to one locus.

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- 5) Fifth Week to Sixth Week Development of trait specific SNP Panel for Purebred Katjang development.
- a) Genotyped data will be viewed using Genome Studio software and will be screened and filtered under strict criteria of data quality control such as Gen Train Score, Cluster Separation, Call Frequencies, Heterozygous excess, Minor Allele Frequencies and many more.
 - b) All the final genotyped data will be used into secondary analysis software such as Package for Elementary Analysis of SNP Data V1.0 (PEAS), SPSS18, STRUCTURE V2.3.3, Cluster Matching and Permutation Program (CLUMP), Distruct, PHASE V1.2, MEGA V5.0, Golden Helix SNP Variation Suite V8.1 (SVS), Arlequinn 3.1 and GenAlex. Each of the software will be used to analyse:
 - i) Package for Elementary Analysis of SNP Data V1.0 (PEAS) - Statistical analysis, Calculate allele frequencies and genetic distance between breeds.
 - ii) SPSS18 - Genetic structure
 - iii) STRUCTURE V2.3.3 - To detect subtle breeds population genetic structure
 - iv) Cluster Matching and Permutation Program (CLUMP) and Distruct - To provide much finer control of the graphic plot of Q produced from STRUCTURE software.
 - v) PHASE V1.2 - To estimate haplotypes for each individuals
 - vi) MEGA V5.0 - To construct phylogenetics trees using Neighbour Joining algorithm
 - vii) GenAlex - Statistical Analysis and identifying unique SNP markers for each breeds. Principle Component Analysis (PCA)
 - viii) Golden Helix SNP Variation Suite V8.1 (SVS) - Identifying unique SNP markers for each breeds.
 - ix) Arlequinn 3.1 - To analyse Molecular variance (AMOVA) for each breeds
- 6) Seventh to Eleventh Week Genome Wide Association Study on marker development for heat tolerance and disease resistance trait.
- a) Single marker analysis
 - b) Animal model (BLUP)
 - i) Introduction
 - ii) Animal model
 - iii) Genetic relationship
 - iv) BLUP with R
 - v) Sire Dam Model
 - c) Linkage Disequilibrium
 - i) Significant Test of LD
 - ii) Multiple testing adjustments for pValues
 - iii) Linkage Disequilibrium measures
 - d) Haplotype Analysis
 - i) Infer haplotype with Simwalk software
 - e) Haplotype Association
 - i) Import haplotype data into SAS
 - ii) Join Haplotype with phenotypic data
 - iii) Haplotype association with 0-1 disease trait
 - iv) Haplotype association with quantitative trait (Heat tolerance)
 - f) Random Forest (RF)
- 7) Twelfth week Introduction to Genotyping by Sequencing

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Fellow 5 – Vietnam

The goal of this research is finding out the relationship between the quality of beef cattle carcass and the forage crops and grasses.

The beef cattle in Vietnam are valuable. Although pork, fowls and aqua-products are still the main protein sources of Vietnamese the consumption of beef are increasing. The beef cattle play an important role in Vietnam due to its high protein content and its specific flavor and taste. Beef also plays quite important role in the Vietnamese culinary. Beef exists in traditional pho, Hue beef noodle or in foreign dishes such as beefsteak or ragout. Up to the 2014, the consumption of beef is of 6 percentages of Vietnamese daily diet. However, the demand on beef quantity and beef quality are increasing. Now in the menus of the premium restaurants to the small restaurants, beef is on the most premium dishes. It may due to the import of beef from foreign countries such as Australia, European countries shifts the customer choice to the better flavor and taste, tenderer texture of beef.

The beef cattle industry in Vietnam is based on the two main sources of beef cattle. The first one is the domesticated cattle, named Yellow or Laisind breeds, which may be fed and sold by farmer or may be fed by the feedlotter before being sold to the market. The domesticated beef cattle is approximately five million cattle. The second source is the imported feeder cattle. The feedlotter import the beef cattle from many countries to Vietnam. The number of beef cattle imported to Vietnam in the 2015 reaches approximately 400000 beef cattle. The domesticate beef cattle insufficiently supply to the domestic demand. The feeding methods applied for domesticated beef cattle are recently ineffective to reach the production and quality of beef carcass. The increasing import in beef cattle carcass and beef cattle to Vietnam has change the customer choice and inhibit the domestic beef cattle husbandry.

The quantity and the quality of the feedstuffs for beef cattle in Vietnam are the challenge for beef cattle industry. In Vietnam, the feeding stuffs are from forage crops and grasses such as dried stuffs or silage from corn, cassava leaves, rice straw, sugar cane... Recently, some grasses such as Pennisetum purpureum, Centro cavalcade, Bracciarria ruzizensis...are now increasing in planting areas for feeding source. However the limits in big farm and large lands for grasses growth inhibit the development of beef cattle industry. In addition, the quality of beef depends on the beef cattle and therefore depending on the cattle's diets and feeding. The quality of domestic beef cattle should be increased throughout the changes in feed stocks and feeding methods.

In this research, the carcass characteristics, chemical composition, texture and sensory attributes of beef from cattle that fed with vary forage crops or grasses. The forage crops or grasses should be various in growth conditions and species. The nutritional value of these feed stocks will be analysis including the nutritional value of the forage crops or grasses, the composition of the fed diets. The wholesale rib cut of beef cattle will be collected at each carcass and then be fabricated into steak. The carcass characteristics, chemical composition, texture of the beef carcass will be analyzed. The USDA yield grade, USDA quality grade are determined. The near Infrared spectrometer are supposed to use for basic composition analysis of the beef steaks while the cooking loss and the texture analysis instrument will be used for determine the texture of beef

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steak. The composition of fatty acids in steak will be determine using gas chromatography method. pH and color of steak will be analysis using pH meter and colorimeter. The sensory attributes of beef steak will be evaluated. The customer acceptance and the descriptive sensory analysis will be the means to evaluate the sensory attributes of beef steak. the 9-point hedonic scale will be applied for customer acceptance test for determination of the overall acceptability average.

During this fellow program I hope to find out the relationships among the nutritional value of the forage crops or grasses and the production and the quality of beef carcass. The effects of grass feed stocks and forage crop feed sstock to the texture and sensory attributes of steaks should be clarified. Thus the results can bring the new approach for Vietnam farmer and feedlotter to increase the production, the quality of the beef cattle and thus they increase their incomes. The advises and instruction form the mentor will be the excellent knowledge supporting source for me to solve the above problem. Under the supporting of The Borlaug Fellowship, the study will be implemented effectively.

Action Plan

Week 1: To discuss to the mentor, works with lab staffs, lab equipments. Preparing for sample collection

Week 2: To collect samples

Needs of the connection and cooperation of the stockers or farmers , the commercial feedlots that follow the various subjected feed diets, diet compositions

Connection and cooperation of the slaughter houses

Needs consumable collecting and storage devices, cooler

Should collect the right samples with suitable amount

Week 3: To prepare sample - and make steaks

Needs consumable devices and cooler for making steak.

Vacuum packaging machine, packages, refrigerator and storage chambers

Week 4: To prepare sample - and make steaks to analyze the beef carcass quality

Needs consumable devices and cooler for making steak

Vacuum packaging machine, packages, refrigerator and storage chambers

Week 5: To analyze the beef carcass quality

Needs chemicals, analytical standard chemicals, Near Infrared Spectrometer , pH meter, texture analysis instrument, gas chromatograph inserted with flame and ionization detector and column

Week 6: To analyze physical and chemical properties of the beef steak

Week 7: To analyze physical and chemical properties of the beef steak and evaluate the results

Week 8: To prepare for the sensory test

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Needs of oven and cooker, kitchen wares

Week 9: To analyze the customer acceptance
Needs of help in penalist invitation, at least 180 people
Needs of oven and cooker, kitchen wares and bland foods

Week 10: Descriptive sensory test
Needs of help in penalist invitation, at least 20 people
Needs of oven and cooker, kitchen wares and bland foods

Week 11: Analyze the sensory data
Needs of Compusense software

Week 12: To interpret the data, make conclusion and evaluate the study

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Fellow 6 – Costa Rica

Fellow #1 (Male); Costa Rica; brief proposal description – Development of a transient expression system for the functional analysis of resistance genes against coffee rust in *Coffea arabica* L. It is expected to develop a simple and efficient *Agrobacterium*-mediated assay in *Coffea arabica* for transient expression of genes conferring resistance against fungal diseases. The developed system could be used to investigate the function of genes of expressed in the leaves of coffee and evaluate the possibility to use them to confer resistance against coffee rust.

The rust disease caused by the fungus *Hemileia vastatrix* is considered one of the pests that cause severe damage and economic impact. This disease has affected 49% of the cultivated area of coffee in Central America and has forced the pruning of 28% of the coffee plantations in the region, despite the measures taken in each country to combat it. In Costa Rica, the damage caused by rust has been severe and according to estimates of the Costa Rican Coffee Institute (ICAFFE) this disease affect crops in 60 counties in the country, especially in some 60 000 hectares of the 90,000 planted in 2012, which representing 64% of the coffee plantations in the country.

In plants, WRKY proteins are a group of transcription factors existing as a gene superfamily that play important roles in development and regulation of defense response pathways. In this sense, the VvWRKY1 of grapevine was overexpressed in tobacco, and the transgenic plants exhibited reduced susceptibility to various fungi. These results suggested the possible role for VvWRKY1 in grapevine defence against fungal pathogens. Recently, five WRKY transcription factor members were identified in coffee and they might play important roles as regulators of pathogen resistance responses and could be useful for improving coffee tolerance to various biotic stresses, such as leaf rust. Importantly, the CaWRKY1 gene from *Coffea arabica* is induced by several biotic and abiotic stresses, including challenge by the rust fungus *Hemileia vastatrix*. These findings open the possibility to overexpress CaWRKY1 transcription factors to confer resistance against coffee leave rust.

Therefore, the development of an efficient, simple and fast transformation protocol for testing WRKY transcription factors function in coffee is very desirable. The method of agroinfiltration is highly efficient in *N. benthamiana* and permits proteins of interest to be produced transiently in plant cells opening the possibility to use this approach for the evaluation of genes involved in the resistance against coffee rust in *Coffea arabica* L. leaves.

General objective

To develop a transient expression system for the functional analysis of resistance genes against coffee rust in *Coffea arabica* L.

Methodology

Plant material: *Coffea arabica* L. seeds will be germinated directly in soil and grown in climate-controlled chambers with a 16-h day/8-h night photoperiod at $21 \pm 1^\circ\text{C}$.

T-DNA constructs for in planta expression: Full length coding sequences for WRKY transcription factor genes. The resulting PCR fragments will be cloned into the vector pGEM-T-EASY following standard molecular techniques.

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Finally, the target genes in pGEM-T-EASY will be transferred into T- DNA vector pL1F-1, and fused to CaMV 35S promoter and Nos-terminator.

Transient expression in coffee: T-DNA plasmids will be transformed into *Agrobacterium tumefaciens* strain GV3101::pMMP90 by electroporation. The agrobacteria will be cultured on LB agar plates containing appropriate antibiotics. The plates will be incubated for 2 days at 28°C and colonies will be tested for insertion by PCR using gene specific primers. Transformed single colony will be used to inoculate a 5 ml culture of LB medium plus appropriate antibiotics and cells will be grown for 24 hours at 28°C and 180 rpm. This pre-culture will be used to inoculate a 50 ml culture and grown for another 16 hours at 28°C. The OD (600 nm) will be adjusted to 0.5 in a total volume of 20 ml. Agrobacteria will be harvested by centrifugation at 1800 × g and 4°C for 90 min. The cell pellet will be resuspended in 0.25 volumes LB medium, 0.25 volumes sterile water and 0.5 volumes of infiltration buffer (10% w/v sucrose, 20 mM glucose, 8.6 g/l Murashige & Skoog basal salt mixture) to reach a final volume of 20 ml. 20 µM Acetosyringone will be added to the agrobacteria suspension and the mixture will be immediately infiltrated into leaves of coffee plants by pressing a 1 ml syringe against the abaxial side of the leaf. Transformed plants will be returned to climate- controlled chambers for further analysis.

Disease evaluation: The resistance will be characterized by inoculation of the abaxial leaves (resistant, susceptible and agroinfiltrated leaves) with 20 drops of urediniospores suspension of the *H. vastatrix*. The inoculated leaves were transferred to an acrylic box, containing a 1.0 cm thick foam, saturated with distilled water and covered with nylon mesh. The gerbox were kept at the dark for 48 h at 22 ± 2_C, and then, exposed to a photoperiod of 12 h light, 12 h dark at 22_C, at constant saturated humidity within the boxes. Symptoms will be assessed 45 days after inoculation according to the following scale: 1— absence of symptoms; 2—small chlorotic lesions; 3 —median chlorotic lesions, without spores formation; 4—chlorotic lesions, with small urediniospores formation (urediniospores occupying 25% of the lesion area); 5—sporulation occupying among 25 and 50% of the lesion area; and 6—sporulation occupying 50% of the lesion area.

Expected results

It is expected to develop a simple and efficient *Agrobacterium*-mediated assay in *Coffea arabica* for transient expression of genes conferring resistance against fungal diseases. The developed system could be used to investigate the function of genes of expressed in the leaves of coffee and evaluate the possibility to use them to confer resistance against coffee rust.

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Fellow 7 – Mexico

I am interested in characterizing the rare genotypes of wild peppers using the GBS approach in order to identify these “rare genotypes”, relate them to their phenotypes, and select these genotypes to conserve in situ as well as ex situ.

The wild pepper (Chiltepín), *Capsicum annum* L. var. *glabriusculum*, is considered the ancestor of the modern cultivated chili and bell pepper. Its distribution is from Colombia to the Southwestern United States [a]. Chiltepin has a high phenotypic plasticity and a resistance of pathogens; however, the most important trait is its fruit shape [a]. In Central and Southern Mexico, chiltepin has a pointed and elongated fruit shape; contrasting from the Northwestern Mexico’s chiltepin that has small round berries [b]. The harvesting of wild populations of chiltepin in the Northwestern Mexico have led the local decline and even extinction of local populations, loss of habitat and damage due to the livestock and collectors [c, a]. Harvesting the fruits of chiltepin in their natural habitat is an important economic activity for people mainly in the Sonoran desert area; and this practice has conducted to the overexploitation of this species. Nowadays, the importance to rescue wild species as primary genetic resources for improvement in biotic and abiotic stress tolerance, for nutritional composition of crops as well as yield production is becoming an important issue for plant breeders and conservation biologists [d]. In that sense, molecular markers are being widely used for decades to analyze genetic diversity within and among populations to obtain specific information about the biology of the species and develop long-term strategies [e]. However, the rapid advances in high-throughput genotyping platforms has revolutionized the field of plant genomics, plant breeding and the way populations are genotyped [f] by the next-generation sequencing (NGS). One of this NGS technology is genotyping by sequencing (GBS) that has been developed as a rapid and robust approach that allow a targeted fraction of the genome rather than the entire genome, even in species with little or no previous genomic information or large genomes [f, g]. Recently our lab group and a group of farmers from Sonora State that are worried about the loss of chiltepin populations in their natural habitat have paid attention about this problem. We conducted a field sampling to the Sonora highlands and collected some accessions of wild chiltepin, which have different fruit colors; however, these wild populations are declining.

Materials and Methods

Biological sample

Forty accessions of wild pepper from Northwestern Mexico.

Methodology

- a-) DNA extraction of at least 40 accessions of wild pepper from Northwestern Mexico.
- b-) Library preparation (all steps for GBS: digestion, ligation adapters, PCR)
- c-) Library assembly
- d-) Sequencing on Illumina MiSeq platform
- e-) Filtering the raw data
- f-) Analyze DNA sequence alignment and data for genetic diversity
- g-) Selection of “rare genotypes” of wild pepper for conservation strategies

Goal of the project and expected results

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To identify and select rare genotypes of wild pepper and take strategies to conserve these populations and help to reforest and fighting to the decrease of these wild pepper populations in the Northwestern Mexico. As far as I know, there are no studies using GBS in wild peppers and it will be the first one for identifying and conserving the rare genotypes for wild peppers.

Week 1: University and laboratory orientations and facilities. Staff introduction.

Week 2: Growing plants from wild pepper seeds. Prepare all the material for using in the greenhouses (Sunshine® substrate Mix 3 and Vermiculite Specialty GRACE, 38 square hole cell seedling starter trays, growth chamber, pots). Work meeting with the US mentor and plan the next week work.

Week 3 and Week 4: While the pepper seeds are beginning to grow, prepare all the material for DNA extraction (DNeasy Plant Mini kit-QIAGEN, nitrile gloves, centrifuge, -80°C freezer, -20°C freezer, centrifuge, vortex, micropipettes from different volumes, tips for micropipettes, autoclave sterilizer, alcohol, isopropanol, DNase free water). Literature review about Genotyping by sequencing. Learning about bioinformatics software. Work meeting with the US mentor and plan the next week work and results of the week.

Week 5: Taking leaf sample from peppers in the greenhouse. DNA extraction of the samples, DNA quantification and DNA quality by gel electrophoresis (gel electrophoresis chamber, power supply, agarose, Gel Red-Biotium, Imaging system with transilluminator, Nanodrop®, eppendorf tubes). Maintenance of the plants in the greenhouse. Work meeting with the US mentor and plan the next week work and results of the week.

Week 6: Prepare all the samples to make the libraries for GBS and submit to the Sequencing services in any institution in US. Learning about bioinformatics softwares. Maintenance of the plants in the greenhouse. Literature review about Genotyping by sequencing and peppers. Work meeting with the US mentor and plan the next week work and results of the week.

Week 7: It will be necessary to take courses about bioinformatics and use of the different software to analyze all the data. Waiting the results from genomic services. Maintenance of the plants in the greenhouse. Work meeting with the US mentor and plan the next week work and results of the week.

Week 8: Learning bioinformatics. Learning of using TASSEL-GBS pipeline (Glaubitz et al. (2014) TASSEL-GBS: A High Capacity Genotyping by Sequencing Analysis Pipeline. PLoSONE 9(2): e90346. doi:10.1371/journal.pone.0090346) and GBSX (Herten et al, 2015. a toolkit for experimental design and demultiplexing genotyping by sequencing experiments. BMC Bioinformatics 2015:16:73) Computer with at least 8-16 GB RAM. TASSEL-GBS is a pipeline for users that do not have large clusters and very useful for many laboratories in developing countries. Maintenance of the plants in the greenhouse. Work meeting with the US mentor and plan the next week work and results of the week.

Week 9: Phase I: Analyzing the raw data from genomic services. Analyze the data with different software to GBS such as TASSEL-GBS, Freebayes, GBSX and comparing the results. Maintenance of the plants in

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the greenhouse. Work meeting with the US mentor and plan the next week work and results of the week.

Week 10: Phase 2: SNP calling and filtering. Beginning the preliminary results. Maintenance of the plants in the greenhouse. Work meeting with the US mentor and plan the next week work and results of the week.

Week 11: Phase 3: SNP discovery and final filtering. Preliminary results that could relate with the genotypes. Maintenance of the plants in the greenhouse. Prepare the material for store the seeds and/or samples material under -80°C freezer or at room temperature. Work meeting with the US mentor and plan the next week work and results of the week.

Week 12: Collect and store material and/or seeds. Label all the material for further studies. Final preliminary results and work meeting with the US mentor and discuss the results. Future perspectives and plan proposal for the US mentor visit to Mexico.

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Fellow 8 – Mexico

Some PI in corn lead maize be stronger against Fungi, PI usually are tested against some other pathogens. Studying PI proteins/DNA Maize in silico & at lab we can be ready in our own institution to write projects & obtain grants to build a lab, & study PI of maize from origin land and test it against some other pathogens.

I was study twenty three Maize: from Northeast of México, inbred lines: T-38, T-41, T-42, LRB-16, LRB-18, LRB-137, LRB-10, LRB-14; single cross hybrids: LRB-16xLRB-18, H-435 (T-38 x T-42), H-436 (T-41 x T-42); three way cross hybrids: H-437 [(T-41 x T-42) x LRB-10], H-439 [(T-38 x T-42) x LRB- 14], H-440 [(LRB-16xLRB-18) x LRB-137]; open pollinated varieties: V-454, CPSRC3; VS-409 and VS-440, were obtained from INIFAP-Campo Experimental Río Bravo. Composites of fourth (C4), fifth (C5), and sixth (C6), cycles of selection for drought tolerance from population Poblacion Amplia Base Genética (PABG) were obtained from INIFAP-Campo Experimental Iguala in Iguala, Guerrero (South of México). H-347, SB302 were obtained from INIFAP-Campo Experimental Bajío, in Celaya, Guanajuato (Central of México). LRB-16, LRB-18, LRB-137, LRB-10, VS-409 VS-454, VS-440 (Northeast of México), C4, C5, and C6 (Iguala, Guerrero, Mexico) are tolerant to drought. This was a qualitative descriptive study and was designed to identify a TI-14 kDa protein that potentially confers resistance to attack by *A. flavus*. Results: Just two of these maize varieties not expressed TI-14 kDa proteins. Perspectives: Further screening of this experiment will enable rapid identification of resistant maize germplasm for use in agriculture in Mexico and maybe we can use against other human concern as disease or other pathogens.

week one: obtain general information about maize about how to grow and what it needs to grow, how manage corn as breeding, inbreds, hybrids, open-pollinated corn , learn about security information about work in lab in USA

week two: learn how to an easy classification of corn and how to made labels of samples, and manage samples at lab and in silico.

week three: learn how to make extraction of DNA of maize and sequencing; learn how to made extraction of proteins and sequencing

week four: learn how to identified Protease inhibitors proteins from other proteins and sequencing

week five: learn how to use the bio/chemical informatics tools

week six: make a bio/chemical informatics analysis with the PI protein/DNA sequence obtained in this training. statistical analysis of the info obtained report of the activities

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Fellow 9 – Panama

The goal of my research is to generate new knowledge that can be used to generate novel strategies against coffee rust *Hemileia vastatrix* and will allow the strengthening of scientific research collaborations between scientists from the USA and Panama in order to address this agricultural problem of regional importance.

Objectives General

To genetically characterize specimens of *Hemileia vastatrix* collected in Panama.

Specifics questions to be addressed

1. Do different coffee varieties, commonly planted in Panama are infected by different genotypes of coffee rust?
2. What is the most commonly found genotype of coffee rust in Panama and how this compare to genotypes found in other regions?

The coffee fungus pathogen *Hemileia vastatrix* caused around 55% harvest damage across Latin America in 2012-2013, representing total losses estimated in USD 499 million. Due to the highly adaptable nature of *H. vastatrix* to new coffee varieties, the breeding for rust resistance has declined over as a means to control the disease. Gradual breakdowns of resistance have been documented in many of the coffee improved varieties in several countries. The socio-economic and environment impacts of the epidemics of coffee rust, recently related to climate change, are important reasons to study the genetic diversity of *H. vastatrix*.

This project will determine the genetic diversity of the rust fungus *Hemileia vastatrix* across infected coffee crop plantations in order to understand what races or genotypes of this pathogen occurs in Panama, and whether it is the same or different races responsible for the recent epidemic. The level of pathogenicity on coffee leaves was the standard method to identify *H. vastatrix* physiological races. Later, the genetic diversity of coffee rust was assessed mostly using molecular markers such as RAPDs and AFLPs. Currently, it is unclear how many physiological races or genetic variants of *H. vastatrix* are circulating in Panama. Here I propose to use specific gene sequences to characterize the genetic diversity of *H. vastatrix* across infected coffee crop plants in Panama. The emergence of new rust variants, along with the potential for new races to become epidemically spread at local and global scale, is a serious and constant threat that should be understood.

Objectives

I- General: To genetically characterize specimens of *Hemileia vastatrix* collected in Panama.

Actions

A- Collecting fungal spores

Responsibility: Scrapings of *H. vastatrix* spores from coffee leaves, and placed into conical flask contained dH₂O

Outcomes: Obtain several specimens of *H. vastatrix* from different coffee variety in Panama Timeline:

Month 1

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B- Performing DNA extraction

Responsibility: Extraction of genomic DNA from each spore collected Outcomes: Extracted *H. vastatrix* DNA

Timeline: Month 1

C- Characterizing the *H. vastatrix* specimens

Responsibility: Amplifying the LSU rDNA region and SSR markers Outcomes: Specimens of *H. vastratix* amplified and sequenced Timeline: Month 1-2

II- Specifics:

1- Do different coffee varieties, commonly planted in Panama are infected by different genotypes of coffee rust?

2-What is the most commonly found genotype of coffee rust in Panama and how this compare to genotypes found in other regions?

Action: Phylogenetic analysis

Responsibility: Analyzing the molecular genomic data using the phylogenetic software's CIPRES

Outcomes: Trees describing the relationships between *H. vastatrix* specimens

Timeline: Month 2-3