Feed the Future: Innovation Lab for Integrated Pest Management Trip Report

Country(s) Visited: Kenya- Nairobi

Dates of Travel: January 8- June 30 2019

Travelers' Names and Affiliations: Gezahegne Getaneh Damessa, PhD student on Chickpea Integrated Disease Management

Purpose of Trip: Molecular laboratory work

Sites Visited: icipe-Nairobi Molecular laboratories

Description of Activities/Observations:

Month 1:

Trainings on the Basic laboratory work and precondition activities

- Visited all functional laboratories with the contact personnel: It was guided by the molecular lab heads, Dr Jandouwe Villinger and Mr Kabii, and we have visited all the labs and assigned personnel whom we might use and contact on our subsequent works.

- Training on molecular laboratory management: this training was held by the Molecular Biology lab head on all basic laboratory management including safety rules, disposal of waste, working bench and equipment cleaning and how to share resources with others.

- Training on use of basic molecular lab instruments: It was done by laboratory head and we were acquainted with all necessary laboratory equipment operations.

- Training on sample Genomic DNA extraction, handling, Polymerase Chain Reaction and gel documenting: Steps in molecular laboratory were exercised with practical session to the level of working alone.

Month 2:

Activity one: Genetic diversity and population structure of *Ascochyta rabiei* from Ethiopia

Objective: To characterize chickpea ascochyta blight pathogen diversity in Ethiopia

Working condition Optimization for primers

Purchase of primers and Amplification of internal transcribed spacer (ITS) region:

Specific primers for ITS, Mating type and Microsatellite were ordered and purchased based on pre-proposed document. The primers were reconstituted to the working conditions and kept in separate cool place (-30^0C). Optimization of Polymerase Chain Reaction (PCR) conditions for ITS region of 173 samples, the whole genomic DNA that was extracted in Ethiopia and 154 samples were amplified successfully for the further work.

Month 3:

Optimizations of the PCR working conditions of 20 Simple Sequence Repeat (SSR) markers for selected representative samples were done. Out of 20 primers, 19 were successfully amplified. After Optimization, Screening for the polymorphism was done on selected samples from representative geographic locations. As a result, seven primers were found highly polymorphic and selected for further analyses.

High Resolution Melting (HRM) analyses of microsatellite markers

Representative Samples were tested on HRM to check the isolates polymorphism and the results were not satisfactory to analyze using bioinformatics tools

Sequencing of amplified ITS region:

The amplified single specific bands of representative isolates of the pathogen were eluted and subsequently purified using gel extraction kit (Bangalore Genei, India) and sequenced. The alignment of multiple sequences and pair wise alignment were developed using BioEdit version 7.0.59.

From different geographical location 14 samples were sequenced for their ITS region and analyzed with their similar GenBank sequences. Additionally 96 samples were amplified and sent for sequencing for further molecular characterization. Eleven samples were cleaned and used for further nuclear DNA analyses as Ethiopian isolates.

Month 4:

Simple sequence repeats (SSR) analysis:

= Optimization (the PCR working conditions) of the primers was done on few samples. After optimization, the screenings of all the twenty different SSR markers were done for their polymorphic condition for 13 samples from different geographical locations. The selected seven SSR primers were sent for genotyping and 96 samples collected from different ascochyta blight hotspot areas of the country. Seven primers with selected 96 samples (7*96=672), a total of 672 reactions, were amplified for genotyping.

Ethiopian chickpea blight (*Didymella rabiei*) Mating type characterization.

Activity two: Mating Type Groups of *Ascochyta rabiei* (Teleomorph: *Didymella rabiei*), the Causal Agent of Chickpea Blight in Ethiopia

Objective: To verify the mating type of *A. rabiei* in Ethiopia

= Sampling, isolating and DNA extraction of the pathogen done in Ethiopia.

= Optimization of the PCR working conditions to some selected samples were done effectively.

= Amplification of the 154 samples was done. As a result, both mating types were found in Ethiopia but MAT1-2 were the dominant and MAT1-1 were found only in few places. Selected samples from both MAT1-1 and MAT1-2 were sequenced and blast to the GenBank to confirm the mating types.

In the current study, chickpea blight prevalence was found lower in major chickpea production areas of the country but the significance of its incidence was different over seasons, which implies its potential of occurrence with favorable environmental conditions. The majority of the fields were free of chickpea blight; only 24% and 7% of

the chickpea field were found infected by chickpea blight disease in the first and second season, respectively.

= Draft paper prepared.

Month 5:

DNA analyses

Exercise on different molecular data analyses (diversity, haplotype and DNA polymorphism) and their application with the bioinformatics group members of *icipe*. The genetic diversity of chickpea ascochyta blight was analyzed using the nuclear ribosomal inter transcribed spacer (ITS1 and ITS4), the fungal barcode. The analyses were made intra and inter collection of Ethiopian isolates (n=11strains) and additionally with other global collections (n=29 strains). Genetically, the global collections grouped into ten haplotypes while Ethiopian isolates alone grouped into five haplotypes. The grouping did not clearly follow their geographic origin but distributed distantly with each other. The haplotype diversity of (0.472) and (0.475) were observed from Ethiopian and global collections, respectively. The Tajima' D resulted in negative values for both datasets and were not significant for Ethiopian collections (-1.44519ns) but it was significant for global collection (-2.23081; ** $P < 0.01$) which shows a population change in the past, consistent with the population expansion model. The observed bimodal distribution pattern suggests that the samples originated from populations at demographic equilibrium. The current studies verified the genetic diversity of Ethiopian population intra and inter counties.

Month 6:

Bioinformatics Training

One day training was organized by *icipe* bioinformatics team focusing on DNA data management. Informally we trained on molecular data analyses, interpretation and reporting.

SSR marker amplification for genotyping

Ten samples of amplified SSR marker were sent to Illinois University for genotyping to confirm working conditions and as a result the FAM labeled primers were confirmed to successfully attach to the selected primers. Finally, 96 samples were sent for genotyping and the result is being analyzed.

Two draft papers were prepared on Mating type distribution and ITS diversity.

Suggestions, Recommendations, and/or Follow-up Items:

Suggestion:

I suggest establishing mini molecular laboratory in Ethiopia with close supervision of *icipe* Nairobi team that will help students to manage their work without traveling to Nairobi for routine molecular works.

List of Contacts Made:

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