FOR THE PROJECT

"IMPROVEMENT OF BANANA FOR SMALLHOLDER FARMERS IN THE GREAT LAKES REGION OF AFRICA"

REPORTING PERIOD: CONSOLIDATED REPORT FOR THE SECDOND YEAR FROM REPORT DATE 01ST OCTOBER 2016 AND FOR THE SUBSEQUENT PERIOD TO 31ST MARCH 2017

UNIVERSITY OF MALAYA

The Leader in Research & Innovation

CEBAR

Report outline

1. EXECUTIVE SUMMARY

This report summarizes the progress achieved by University of Malaya from 1st October 2016 until 31st March 2017 of the 5-year project on "Improvement of banana for smallholder farmers in the Great Lakes region of Africa". University of Malaya is principally involved in work package 3&4 (WP3 & 4) "Genetic and genomic studies for the development of molecular makers to be used in selection". The second instalment of the project budget amounting to USD 65,836.00 was received on the 8th of March 2017. This report focuses on the activities till the end of the reporting period. The Project has shown progress and the PhD candidate has completed his first semester in the Academic session 2016/2017.

2. PRIMARY OUTCOME AND PROJECT PROGRESS

Table 1 gives the Framework and Results Tracker as adjusted to the delayed start as previously reported

Table 1: Framework and result tracker

	Primary outcomes	*Inter-mediate outcomes*	*Outputs*	*YEAR 1*	*YEAR 2*	*YEAR 3*	*YEAR4*	*YEAR 5*
1	*Transfer of Diploid segregating population from Malaysia*	*Population indexed and sub-cloned*	*Segregating populations defined, indexed and shipped* *MTA signed*	*Review of populations and team*	*reconstitute a new segregating population of Musa acuminata ssp Mallaccencis.*	*Population defined and TC clones developed. Indexing carried out*	*PopulationTransfered to Uganda*	*Segregating populations established in both Malaysia & Uganda*
2	*Capacity building for PhD & MSc candidates*		*PhD & MSc Student trained & graduated*	*Application for PhD Submitted, Proposal Reviewed.*	*PhD Student (Uganda) registered* *MSc student (Malaysia) recruited*	*MSc (Africa) student recruited*	*N/A*	*All students complete*
3	*New Markers developed for FOC*	*Standard assays & marker iibrary*	*Markers selected & identified*	*Review of expertise*	*Markers identified IRAP, REMAP, ISSR, STMS and SNPs.*	*Publication*	*Analysis of data*	*Markers selected & identified.* *Publication*
4	*Application of Markers*	*Markers identified*	*Testing and analysis of populations*	*Identification and expt. design of study population*	*Testing of markers on 2 populations. One population from Malaysia. One populations from Uganda*	*Analysis of data*	*Testing of field populations* *Analysis of data Publication*	*Final Testing and analysis of field populations Publication*

Outcome : Preparation, Indexing and transfer of Segregating Diploid Population from UM

Challenges were initially faced in meeting this outcome and alternative strategies have since been identified and several adjustments have been made to our team to enable the execution and the following progress has been made.

i. Project team at UM

This project is managed by CEBAR, Centre for Research in Biotechnology for Agriculture University of Malaya with Co-PI for this project Prof Dr. Jennifer Ann Harikrishna Director of the centre and project manager (Dr. Teo Chee How). Dr Teo will be representing the group at the April 2017 meeting in Uganda. The collaborator for the work on segregating populations in the field - Dr Fatimah Kayat from Universiti Malaysia Kelantan (UMK) attended the coordination meeting in May in Arusha. Dr Fatimah was formerly with the banana group in UM but now heads the breeding programme in UMK. The population has been reconstituted for this project and will be used for the student project on MAS and for screening at IITA. One research assistant (RA) Ms Nurul Husna Baruddin has been employed to work on the Fusarium FOC Race 4 challenge on *Musa acuminata* subsp. *malaccensis* parents and F_1 open pollinated segregating population.

ii. Segregating population and marker systems available

A segregating population resulting from *a* diploid population has been reconstituted at the field plot in UMK Jeli, Kelantan in North East Peninsula Malaysia and will be made available for this project. The time lines for the readiness of the samples has been restructured. Additionally, several marker data sets from previous and current projects on FOC related projects have been made available to this project including ISSR, STMS, IRAP & REMAP Marker sets and new potential markers from an associative transcriptomics project currently being carried out at University of Malaya. The projects have been carried out through funding sources from UM. Additionally, analysis will be carried out using the molecular marker sets at UM with population DNA samples from 1 population maintained in Uganda. DNA was received in UM in February. Dr Teo and PhD student Ivan Arinaitwe also made a field visit to UMK in February 2017.

Outcome : Capacity Building : Registration of PhD student at University of Malaya

Candidate : ARINAITWE IVAN KABIITA, Uganda

The University Senate had approved the candidature application for a PhD and the visa process has also been completed. The candidate arrived in mid September and officially registered on the 7th October, Semester 1 Academic session 2016/2017. The candidature will involve a minimum 6-month initial residential period at University of Malaya to Feb 28th 2017 to fulfil candidature requirements and to structure the workflow. This involved lab work in UM and field visits to UMK. The work involves comprehensive training on laboratory technology in molecular biology, marker analysis and bioinformatics and will include molecular analysis of 2 populations currently maintained for the programme. The study will focus on the development of diploid segregating populations and markers related to FOC. The transfer of Tissue culture plants from the new population to Africa will follow the progress of the work and is projected for late 2017/early 2018.

Proposal defense by Mr Ivan Arinaitwe

Outcome : Molecular marker development for FOC.

Molecular markers specific for banana have been identified (IRAP, REMAP, STMS & ISSR). The primers for these markers have been designed and currently the markers are tested on segregating populations resulting from a diploid population of *Musa acuminata* subsp. *malaccensis* and F_1 population of Kokopo and Monyet crosses. Several ISSR markers have been applied on a F_1 population of Kokopo and Monyet crosses and their parents. Polymorphic bands were observed on some of the ISSR markers tested (Figure 1). New SSR primers will be designed from the flanking sequences of polymorphic ISSR bands. In addition, SSR loci that located near to putative FOC race 1 and race 4 disease resistance genes have been identified through bioinformatic analyses. Primers flanking the SSR loci have been designed and will be used to screen the segregating populations resulting from a diploid population of *Musa acuminata* subsp. *malaccensis* and F1 population of Kokopo and Monyet crosses.

No.	Primer		Parent with Polymorphic Band	Approximate Size of Band (kb)	Hybrids Clearly Containing Polymorphic Band	Hybrids Clearly Displaying Absence of Polymorphic Band	Comments
	Primer Type	Primer Sequence					
1	ISSR	CTC6T	Kokopo	0.85	TBD	TBD	polymorphic band missing in hybrids
2	ISSR			0.75	414,422,448,450,459a, 465,466,470,474,475,476,477	464, 472,473	Clear
3	ISSR	GTG6A	Kokopo	0.45	414,422,448,459a,466,472	TBD	polymorphic band confirmed
4	ISSR		Kokopo	0.65	450,459a,464	TBD	Clear
5	ISSR		Kokopo	0.55	422,464,466,470,473,474,476,477	414,450	large number of bands in vicinity and insufficient resolution.
6	ISSR	CAC6T	Kokopo	<.5	414,448,450,459a,472,476,477	422,464,465,473,474,475	Unclear recommended for rerun
7	ISSR		Kokopo	<1.0	TBD	TBD	
8	ISSR	CTC6G	TBD	1.2	TBD	TBD	Polymorphic band visible only in Hybrids
9	ISSR	ACC6G	Monyet	0.3	TBD	TBD	Low band intensity for parent
10	ISSR			0.85	TBD	TBD	
11	ISSR	ACC6C	TBD	-	TBD	TBD	-
12	ISSR	GTG6T	Kokopo	0.7	TBD	TBD	ow band intensity for Monyet, 465,473 and 47
13	ISSR		Monyet	0.25	TBD	TBD	
14	ISSR	TCG6A	none	-	TBD	TBD	No amplification in parents
15	ISSR	TCG6G	none	-	TBD	TBD	Low band intensity.

Figure 1: ISSR markers analysis on F_1 population of Kokopo and Monyet crosses and their parents. Polymorphic bands have been identified and will be sequenced to determine the flanking sequences of their corresponding SSR sequences.

3. SUMMARY OF RESULTS TO DATE

In Table 2, we give an overview of the progress of activities to date for the outputs with a milestone in year 2.

Table 2. Progress for University of Malaya for WP3&4 year 2.

	Outputs	Targets/ Milestones YEAR 2	Progress	Variance
1	Existing Segregating populations	MTA, Indexing and transfer of diploid segregating population	We were not able to send the TC population to Uganda as originally scheduled due to unforeseen circumstances. The project is being rescheduled to reconstitute a new segregating population that will be used for the study and will be part of the incoming PhD project in UM. The *Malaccencis* population has now been reconstituted and maintained in UMK Jeli.	50% achieved Reason for variance: A new population is being used for the study. It is expected that we will be able to be back on track by end of of year 2.
2	PhD Student at UM	i. Application for PhD Submitted. Ii Proposal Reviewed and offer made. Ii Student registered.	The University has approved the PhD application and the candidature had started in Semester 1 Session 2016/2017. Supervisors appointed: Prof Dr Rofina Yasmin Othman, Prof Dr Jennifer Ann Harikrishna and Dr Teo Chee How. Mr Ivan Arinatwe successfully registered on 7th October 2016 and completed the necessary courses needed for first semester. First semester Proposal defense for Mr. Ivan Arinatwe has been completed successfully	100% Achieved Application process and approvals including Visa approvals was lengthy. Proposal defense was completed on 11th January 2017 and endorsed by the faculty
3	Markers selected & identified	I. Commencement of PhD training in lab on molecular techniques ii. Assessment, Selection & testing of markers for standardisation	Laboratory training for use of DNA markers commenced. Markers will include IRAP, REMAP, ISSR, STMS and SNPs. Several ISSR primers have been tested on F_1 population of Kokopo and Monyet crosses and their parents. Activity for ii. is on going.	Revised
4	Testing and analysis of populations	i.Identification and experimental design of study populations ii. Testing and analysis	Samples of populations for analysis in the form of leaf material and DNAs for marker analysis were received in UM from: i. One segregating population of *m. acuminata* subsp. *malaccensis* from UMK Jeli ii. One Kokopo x Monyet F1 populations from Uganda	Revised

4. CHALLENGES ENCOUNTERED

The main challenge for the execution of the activities was the quality of the DNA samples received for MAS. Some of the DNA samples of F_1 population of Kokopo and Monyet crosses and their parents received from Uganda and IITA showed degradation when analysed using gel electrophoresis.

5. LESSONS LEARNED

Better communication with main WP team will need to be maintained by UM team.

6. WORKPLAN

The overall workplan for UM, including timeline and budget, for our contribution WP3&4 has been revised as presented in the last report. The evaluation of marker systems (ISSR, STMS, IRAP, REMAP and SNP) will be continued for two F1 populations (*Malaccensis* & Kokopo x Monyet) to obtain polymorphic markers for MAS. The contribution to WP 2 will be reassessed after discussions with the coordinator and team members during the April 2017 meeting in Uganda.

7. BUDGET SUMMARY

The initial budget received from the project is the first instalment of USD 2745.00 with expenditure recorded as in the preceeding report ending September 2016. The budget was used partly for Dr Fatimah to attend the 2016 Arusha meeting travel & covered some costs of our PhD student from Uganda. All associated work for the Project has been carried to using co-funding sources from University Malaya grants under UM-HIR & UMRG votes and included costs of i. setting of new field population, ii. Collating of marker data and iii. Travel cost internally by collaborator from UMK Jeli to Kuala Lumpur. The remaining 1st year & subsequent 2nd year fund disbursement was received by UM on 8th of March totalling USD 65,836.00 and the accounts will be reported in the next 6 monthly report as no new transactions was executed till March 31st 2017.

8. OTHER RELEVANT PROJECT INFORMATION

Annex 1 Budget received and expenditure of University of Malaya till March 31st 2017

ANNEX 1 BMGF IITA Great Lakes programme – UM Budget summary

BUDGET RECEIVED AND EXPENDITURE OF UNIVERSITY OF MALAYA (Does not include budget received on 8th March 2017 . No transactions on new budget till March 31st 2017)

Budget	Allocation (USD)	%	Commited (USD)	%	Available (USD)	%
Personnel						
Travel	2151.16	78.55	2,085.17	76.14	65.99	2.41
Capital Equipment						
Other direct costs						
Consumables	587.37	21.45	587.37	21.45	-	0
Grand Total	**2738.53**	100	2,085.17	97.59	65.99	2.41

IITA Statement					
Account Debited	**Account Name**	**Transaction Amount**	**Transaction Date**	**Beneficiary Information**	**Beneficiary Account**
10088691	International Institute of Tropical Agriculture	USD2745	4-Dec-14	Universiti Malaya	8001279998
10088691	International Institute of Tropical Agriculture	USD65,843.00	8-March-17	Universiti Malaya	8001279998

UM Statement						
University of Malaya Account No	**Transaction Date**	**Branch Code**	**Ref No.**	**Trans. Amount**	**Trans. Time**	**Actual Amount Credited and exchange rate**
8001279998	4-Dec-14	9825	20013694	RM9324.70	11:42:51	USD2740 @3.4050
8001279998	8-March-17	9825	2000507028	RM288,657.94		USD65836@4.3845

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