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Report on Hands-on Training workshop on detection of Genetically modified Organisms (GMOs), Mikocheni Agricultural Research Institute (MARI), Dar es Salaam 17-20th April 2012

April, 2012

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1. BACKGROUND

The Environmental Management Act Cap 191 of 2004, Section 69 of the Act requires to put in place Biosafety procedures and measures to ensure that the research, development, handling , trans-boundary movement , transit, use , release and management of GMOs are undertaken in a manner that prevents or reduces risks to human and animal health, biological diversity and the environment. In the view of the above, the Government of Tanzania, through the Vice President's is implementing the National Framework with the purpose of ensuring the safe application of modern biotechnology in the country.

One of the main objectives of the NBF is to build human and infrastructural capacity within the principal regulatory bodies, to efficiently and effectively carry out biosafety related. In the light of this background, Vice Presidents Office (VPO) in collaboration with Mikocheni Agricultural Research Institute (MARI) organized a five days hands-on training on detection of genetically modified organism (GMOs) at ARI MIkocheni, Dar Es Salaam 17th April to 20th April, 2012. The hands-on practical training course attracted twelve participants and was conducted at the laboratory premises of MARI by facilitators from the Mikocheni Agricultural Institute and the University of Dar Es Salaam.

The objective of the workshop was to impart both theoretical and practical knowledge of the molecular techniques used in the detection of GMOs to the technical staff of national regulatory agencies, universities and national agricultural research institutes. The molecular techniques workshop will gave regulators hands-on experience with DNA and protein detection protocols for GMOs and provided a critique of the strengths and weaknesses of these detection systems.

2. OBJECTIVES OF THE WORKSHOP

2.1. Main Task/Objective

The main objective of the workshop was to impart both theoretical and practical knowledge of the molecular techniques used in the detection of GMOs to the technical staff of national regulatory agencies, universities and national agricultural research institutes.

2.2. Specific objectives

The specific objectives of the workshop were:

a) To develop and strengthen capacity of participants in molecular GMO detection procedures with emphasis on DNA and Protein based technologies;

b) To give participants hands-on experience with different protocols for GMOs detection and provide a critique of the strengths and weaknesses of these detection systems;
c) To enhance participant's knowledgeable on the most efficient use of materials, reagents and equipment required for GMO detection
d) To facilitate close and effective collaboration and linkages among the relevant regulatory agencies for implementation of the national biosafety legislations.

3. PARTICIPANTS/BENEFICIARIES

The workshop was attended by 12 participants (Annex 4) from 10 different institutions as shown in Table 1 below

Table 1: A List of institutes that benefited from the workshop

Sn	Institution	Mandate	No of participants
1	Tropical Pest Research Institute (TPRI)	Regulatory	1
2	Tanzania Bureau of Standard (TBS)	Regulatory	1
3	Tanzania Official Seed Certifying Institute (TOSCI)	Regulatory	1
4	Government Chemist Laboratory Agency (GCLA)	Regulatory	1
5	KIzimbani Research Institute	Regulatory and policy	1
6	Mikocheni Agricultural Research Institute (MARI)	Research	2
7	Tanzania Food and Drug Authority (TFDA)	Regulatory	1
8	Sokoine University of agriculture (SUA)	Research and Training	1
9	University of Dar Es Salaam (UDSM)	Research and Training	1
10	Plant Health Service of the Ministry of Agriculture	Regulatory	1
	Total		**12**

4. METHEDOLOGY

The hands-on practical training course was conducted at the laboratory premises of MARI by facilitators from the MARI and UDSM. The five days workshop consisted of lectures and hands-on practical sessions given by resource persons working in the fields of genetic transformation, biosafety and in areas of DNA and protein based detection methods. The handouts of the presentations were made available to all participants during the workshop.

4.1. Lecture sessions

In order to prepare the participants for the hands on practical sessions, the resource persons gave a series of introductory lectures on detection of GMOs. The topics covered in the lecture sessions were relevant to the needs of the participants and covered the basic principle and procedures required for genetic transformation and detection of GMOs and GM products (Annex 2). In the view of the current debate on biosafety and biopolicy, participants were also given a lecture on the status of National Biosafety framework for Tanzania. Main topics that were covered in the lecture sessions include the following:

- Basic molecular biology principles;
- Flow of genetic information, gene expression and protein sythesis;
- Sampling procedures for grain food and feed;

- Genetic modification and overview of available GM crops;
- DNA-based qualitative and quantitative detection methods (PCR, Real time PCR, and micro array technology);
- Criteria for validation of detection methods;
- Use of commercially available test kits and their validation; and
- Principles of protein based GMO detection methods (ELISA and Western Blot)

4.2. Hands-on practical sessions

Participants were divided in groups of four each working independently. The hand-on practical training was conducted in three parts/components. The first part (ca 1 day) was devoted to preparation of stock solutions/ buffers and DNA extraction. DNA was extracted from leaves of sweet potatoes and cassavas. All participants were able to extract DNA of good quality. The second session (ca 1 day) was used for DNA based GMO detection methods. The participants used polymerase chain reaction (PCR) because it is the one currently in use for DNA based qualitative and quantitative detection methods. In this training two PCR experiments were conducted on cassava DNA. The first PCR (a control) targeted the house keeping gene (invertese) while the second PCR targeted the cassava satellite molecule (in place of a transgene). The final session (of ca 1.5 day) was used for protein based detection methods. This analytical method uses DAS ELISA with antibodies raised against the protein encoded by the introduced gene.

The handouts of the presentations were made available to all participants during the workshop. In addition, the participants were provided with a CD-ROM containing all the training materials.

5. GENERAL OBSERVATIONS, CHALLENGES AND RECOMMENDATIONS FOR THE FUTURE

5.1. General

The training workshop was well attended and all participants stayed for the whole duration of the training. The workshop ran smoothly throughout both lecture and practical sessions. Participants were active, attentive and very enthusiastic about learning new skills and technologies. What was so striking was the way participants demonstrated their increased understanding of the issues and procedures presented to them. The training schedule provided adequate time for participants to grasp practical procedures and to share ideas and experiences.

5.2. Feed back from the participants

At the end of the workshop, questionnaires (Annex 3) were distributed to the participants to obtain their evaluation of the training methodology, training materials and to obtain views on how improve future workshops. Overall, the participants were positive in their feed back and considered the workshop to have met its objectives. The participants considered the schedule of the workshop and technical arrangement of the practical sessions to be well organized. Most participants highly rated the professionalism and effectiveness of the resource persons. However, some participants felt that the time

allocated for the practical session was inadequate. Detailed results of the participant's evaluation are presented in Table 2.

Table 2: RESULTS OF THE WORKSHOP EVALUATION

Sn	Question	Response/results
1	How well did the **topic** of the workshop meet your needs?	The score ranged from 4 to 5 Score of 4 = 4 participants, Score of 5 = 6 participants
2	How well did the **content** of the workshop meet your needs?	Ranged from 4 to 5 Score of 4 = 3 participants Score of 5 = 7 participants
3	How well did the **Delivery** of the workshop meet your needs?	Ranged from 4 to 5 Score of 4 = 4 participants Score of 5 = 6 participants
4	How good were the resource persons?	Ranged from 4 to 5 Score of 4 = 8 participants Score of 5 = 2 participants
5	How would you rate the hands –on activities	Ranged from 4 to 5 Score of 4 = 3 participants Score of 5 = 7 participants
6	How would you rate the speed of delivery	Just right : All participants
7	Level of course content	Just right : All participants
9	What part of the workshop did you learn the most from?	PCR= 8 participant Dev of GMO= 6 participants DNA extraction = 5 participants Practical sessions = 4 participants
10	What things could be improved in the future workshop?	More time for practical= 5 participants More theory =4 participants Advanced procedures = 2 participants
11	What topics would you like covered in similar workshops in the future?	RT PCR = 6 participants, ELISA = 5 participants, Interpretation of PCR data = 2 participants, Sequencing = 2 participants
12	What things will you change in the way you do your job based on what you learned in this workshop?	Sampling procedures= 5 participants Good lab practices = 4 participants Data analysis = 4 participants
13	Any other comments/ suggestions?	Welldone, = 8 participants More practice = 3 participants retraining course = 2participants follow-up visit = 2 participants Train more researcher in the future= 1 participants

5.3. Workshop outputs/benefits and future needs

In general, the organizers and participants were satisfied with the outcome of the workshop. The overall impression is that the workshop VPO training on GMO detection was a success.

The workshop provided adequate opportunity for the participants from Tanzania to gain knowledge and build capacity in various techniques required for GMO detection. The training has raised significantly the national capacity for GMOs detection as prior to the workshop Tanzania had very few people with such skills.

The participants came from the leading regulatory and research institutions of Tanzania. After having stayed for five days together and discuss matters of mutual interest during this period, these trainees are now in a position to continue their dialogue and form a network for future collaboration. In addition, the opportunity provided by the workshop for the participants to get to know the resource persons was important. Participants were advised to maintain contact with the resource persons for future advice or/or consultation.

It would also be helpful if in future a follow up mechanism is established to monitor how each institution is practicing the skill and knowledge acquired/gained from the workshop. It is further recommended that similar training/retraining programs be organized so as to give participants ample time to practice and grasp the GMO detection procedures.

Figure 1: Group picture- day one with the guest of honor

Figure 2: Group picture of participants in the GMO detection laboratory

Figure 3: Dr Ningu giving speaking to the participants

Figure 3: Participant loading PCR products

Figure 3: Dr Ningu giving closing remarks on the last day of the workshop

ANNEXES

ANNEX 1: List of equipments, reagents and consumables used for GMO detection

(A) For PCR based protocols

1.0 DNA extraction	**3.0 PCR reaction**
Trizma hydrochloride	10 X reaction buffer
EDTA	DNA polymerase
Chloroform	DNTP
Isopropanol	MgC12
Mercaptoethanol	Specific primers – for CRY gene
PVP-40	Template DNA-transgenic samples
Ammonium acetate	Controls
Sodium hydroxide	Eppendorf tubes (1.5 & 2.0 ml)
Sodium acetate	Polycarbonate plates- in 48; 96
CTAB or SDS	PCR reaction tubes (0.2-0.5 µl)
Isoamyl alcohol	Thermocyler (PCR machine)
Phenol and or Octanol	Yellow tips (10-100µl)
Ethanol	Blue tips(100-1000µl)
Acid washed sand or Liquid nitrogen	White tips(1000-5000µl)
Mortals and pestles	
Centrifuge and centrifuge tubes	
Water bath	
Spectrophotometer	
2.0 Electrophoresis	**Miscellaneous/general equipment**
Ethidium bromide	Photographic equipment
Agarose	Laminar flow cabinet
Orange G/Bromophenol blue	Autoclave
Kb ladder	Vortex machine
Lambda (λ DNA)	Gloves
Glacial acetic acid	Lab coats
Gel casting trays and combs	Pipette
Ellectrophoresis apparatus and power pack	
UV transilluminator	

(B) For Protein Based protocol -Direct <u>Sandwich Elisa Method</u>

Buffer/reagent	**Materials**
PBS	8.0g NaCl 0.2 g KH_2PO_4 1.15g Na_2 HPO_4 0.2 KCL Make to 1L distilled water adjust to pH 7.4 with HCL or NaOH
PBS-T	PBS 1L + 0.5 ml Tween 20 re-adjust the pH to 7.4
Antibody buffer	PBST 1L + 50 g Skimmed milk
Coating buffer	1.59g Na_2CO_3 2.93g $NaHCO_3$ Make up to 1 L by distilled water adjust to pH 9.6 with HCL or NaOH
Extraction buffer	PBST 1L + 20g PVP-40000 re-adjust the pH to 7.4
Pre-absorbent	1g healthy plant tissue + 5mls conjugate buffer. Grind, filter (store and freeze in 1 ml aliquots for use as needed
Conjugate buffer	Extraction buffer + 0.2% skimmed milk 0.02% NaN_3 (optional) Make up to 1L in PBST adjust to pH 7.4 with HCL or NaOH Make sure the PVP dissolve completely
Substrate buffer	96 mls Diethanolamine 85mls distilled water with HCL to adjust to pH 9.8 with NaOH
p-nitrophenyl phosphate (pNPP) substrate tablets (5mg)	Dissolve 15 mg (tablets) into 22 mls of substrate buffer enough for plate as each well requires 200 uls
96 well Microtitre plate	
Yellow tips	10-100uls
Blue tips	100-1000uls
White tips	1000-5mls
ELISA sample bags	4x5x 1000 counts
Roller press	
Microtitre plate reader	With 630_{nm},405_{nm} filters
Capturing antibody monoclonal	Specific to protein in question E,g Bt-Cry1Ab/Cry1Ac
Detecting antibody Conjugated monoclonal	Specific to protein in question
Neg+positive controls	

ANNEX 2: Program for the VPOs training workshop on GMO detection

Agricultural Research Institute (MARI), Dar Es Salaam , Tanzania
(17th to 20th April 2012)

Date	Time	Topic/Event	R/ person
Day 1 (Tues) 17/04/12	8:30 – 9:00	Registration	All
	9:00 – 9:05	Welcome and Self Introductions	O/In charge, MARI
	9:10 – 9:30	Opening Speech	Assistant Director, Environment
	9:30 – 9:35	Workshop objectives	Thomas Bwana
	9:35– 10:00	Status of Biotechnology in Tanzania applications	Dr Mneney
	10:00 – 10:30	Status of Biosafety in Tanzania & the need for capacity building in GMO detection	Mr Bwana/Kamukuru
	10:30-10:35	Photo session	ALL
	10:35 – 11:00	Tea and Coffee Break	
	11:00-11:30	Genetic modification and GMO applications	Dr Mneney
	11:30-12:00	Overview of GMO applications and benefits	Dr Mneney
	12:00- 12:30	GMO detection –methods	Dr Tairo
	12:30-13:00	GMO detection methods –ctd	Dr Tairo
	13:00-14:00	Lunch break	ALL
	14:00-15:45	Practical: preparations of stock solutions	Dr Tairo/Rajabu
	15:45-16:00	Afternoon tea/coffee break	ALL
	16:00-1700	Practical: DNA extraction buffer	Dr Tairo/Rajabu
Day 2 (Wed) 18/04/12	09:00-10:00	Overview of Sampling and extraction methods	Dr Tairo
	10:00-10:30	PCR- basics, primer selection & Lab practice	Dr Tairo
	10:30-11:00	Tea and Coffee Break	ALL
	11:00-13:00	Practical –DNA extraction	Dr Tairo/Rajabu
	13:00-14:00	Lunch break	ALL
	14:00- 15:45	DNA quantification and Electrophoresis	Dr Tairo/Rajabu
	15:45-16:00	Afternoon tea/coffee break	
	16:00-1700	DNA quantification and Electrophoresis	Dr Tairo/Rajabu
Day 3 (Thur) 19/04/12	09:00-10:30	Set and Run PCR for GMO detection	Dr Tairo/Rajabu
	10:30-11:00	Tea and coffee break	ALL
	11:00-12:00	Overview alternative methods: Protein based	Dr. Tairo
	13:00-14:00	Lunch break	ALL
	14:00-15:45	Running agarose gels and interpretation	Dr Tairo/Rajabu
	15:45-16:00	Afternoon tea/coffee break	ALL
	16:00-1700	Running agarose gels and interpretation	Dr Tairo/Rajabu
			Dr Tairo/Rajabu
Day 4 (Frid) 20/04/12	09:00-10:30	ELISA: Overview: sample extraction, loading , running ELISA, reading results	Dr Tairo
	10:30-11:00	Tea and coffee break	

	11:00-11:30	Trouble shooting	Mneney/Tairo/Rajabu
	11:30-12:00	Experience of GMO detection in Tanzania and in the region (SANGl)	Dr Tairo/Muruke
	12:00- 12:30	The Tanzania Centre of Excellency for biosafety	Dr Mneney/VPO
	12:30- 13:00	End of course test	Dr Mneney/Tairo
	13:00-14:00	Lunch break	ALL
	14:00-15:00	General discussion	ALL
	15:00-15:10	Course Evaluation & Admin Issues	Dr. Mneney
	15:10-15:30	Closing ceremony - Certificates and closing remarks	Director for Environment – VPO
	15:30-15: 35	Vote of thanks	Representative of the Participants
	15:45-16:00	Afternoon tea/coffee break	ALL
	16.00	Departure	ALL

ANNEX 3: Workshop Evaluation Form for VPO/ UNEP GMO Detection workshop

THANK YOU for attending the workshop and for helping us to make these workshops better.

1. How well did the **TOPIC** of the workshop meet your needs?
   (CIRCLE RESPONSE: 1 =Very poor; 5 = Excellent) 1 2 3 4 5

2. How well did the **CONTENT** of the workshop meet your needs?
   (CIRCLE RESPONSE: 1 =Very poor; 5 = Excellent) 1 2 3 4 5

3. How well did the **DELIVERY** of the workshop meet your needs?
   (CIRCLE RESPONSE : 1 =Very poor; 5 = Excellent) 1 2 3 4 5

4. How would you rate the hands-on (practical) activities?
   (CIRCLE RESPONSE : 1 =Very poor; 5 = Excellent) 1 2 3 4 5

5. How would you rate the resource person's knowledge in the subject?
   (CIRCLE RESPONSE: 1 =Very poor; 5 = Excellent) 1 2 3 4 5

6. How would you rate the resource person's style of teaching?
   (CIRCLE RESPONSE: 1 = Very poor; 5 = Excellent) 1 2 3 4 5

7. How would you rate the speed of the delivery?
   (CIRCLE RESPONSE: 1= Too slow, 2= Just right, 3= too fast) 1 2 3

8. Was the workshop above or below your current skill level?
   (CIRCLE RESPONSE: 1= below, 2= Just right, 3= above 1 2 3

9. What part of the workshop did you learn the most from?

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10. What things could be improved in the future workshop?

11. What topics would you like covered in similar workshops in the future?

12. What things will you change in the way you do your job based on what you learned in this workshop?

13. Any other comments/suggestions?

ANNEX 4: Participants list for the VPOs Training workshop on GMO detection

Agricultural Research Institute (MARI), Dar Es Salaam , Tanzania (17th to 20th April 2012)

S/N	Name of Participant	Institution	Contacts
1	Rebecca Mawishe	Plant Health services (Kilimo)	rebeccamawishe@yahoo.com pps@kilimo.go.tz Tel no: +255 22 2865642 Celphone no: +255 784216240
2	Catherine Luanda	Tanzania Food And Drugs Authority(TFDA)	catherine.luanda@tfda.or.tz catherineluanda@yahoo.com +255 716617262
3	Christina Kidulile	Mikocheni Agric. Research Institute (MARI)	tyneebls@gmail.com +255 714874792/767 874791
4	Kelvin Shitindi	University Of Dar Es Salaam -UDSM (DMBB)	K.shitindi@yahoo.com +255 0714 817744/767 817744
5	Lilian Gabriel	Tanzania Bureau Of Standards (TBS)	lyngebo@yahoo.com Lilian.gabriel@tbstz.org +255 715845500
6	Elias Mulima	Goverment Chemist Laboratories Authority (GCLA)	eliasmlima@yahoo.com +255 713419612
7	Abdalla Salum	Zanzibar - Kizimbani	Abdallasalim200@yahoo.co.uk +255 777497312,
9	Adolf Gerald Saria	Tanzania Official Seed Certification Institute (TOSCI)	adolf8358@yahoo.com +255 754493551, 788259019
10	Benignus Ngowi	Tropical Pesticides Research Institute (TPRI)	Benastras_x@yahoo.com +255 754399000
11	Protas Deogracious	Sokoine University of Agriculture (SUA)	protasdeo@yahoo.com protas@suanet.ac.tz +255 765991133

12	Rajabu Cyprian	MARI/Resource person	ckitona@yahoo.com +255 754757121
13	Dr Fred Tairo	MARI/Resource person	fredtairo@gmail.com
14	Dr Emmarold Mneney	MARI/Resource person	emneney@gmail.com 0754387662

ANNEX 5: Sample of the certificate issued to the participants

UNEP

Certificate of Participation

This is to certify that

Protas Deograceous

Participated in the hands-on training workshop on

Detection of Genetically Modified Organisms

Under the Project

National Biosafety Framework Implementation Phase

Mikocheni Agricultural Research Institute, Dar-Es-Salaam, Tanzania. 17th - 20th April, 2012

Officer In-Charge
ARI– Mikocheni
Dar Es Salaam, Tanzania

Director of Environment
Vice President's Office
Dar Es Salaam, Tanzania