

Cassava Mosaic Disease Pandemic Mitigation in East Africa

**A SYSTEM-WIDE WHITEFLY IPM
AFFILIATED PROJECT**



First Quarterly Technical Report

Phase 3

October-December 2000

International Institute of Tropical Agriculture

February 2001

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I. Executive Summary

Quarterly Report

<i>Organization:</i>	International Institute of Tropical Agriculture	<i>Date:</i>	February 23, 2000
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Programme title: Emergency Programme to Combat the Cassava Mosaic Disease Pandemic in East Africa

Cooperative Agreement/Grant No: LAG-411-G00-3042-00

Country(ies)/Region(s): UGANDA, KENYA and TANZANIA

Disaster/Hazard: East African CMD Pandemic

Time Covered by This Report: October-December 2000

Activities Summary

Virus diagnoses were completed for samples collected from 15 districts in western Kenya during a diagnostic survey implemented in the third quarter of 2000. Results indicated that the pandemic associated virus, EACMV-Ug was present in 11 of the 15 districts covered in the survey. Whilst EACMV-Ug was detected in a sample from Migori, near to the Tanzania border, there was no clear evidence for a major expansion in the distribution of the pandemic associated EACMV-Ug.

In Uganda, a cumulative figure of 1,278 bags of CMD resistant material were ratooned in Rakai and Masaka and distributed to 1,131 vulnerable households. A new variety, I92/00067, was procured by IITA in collaboration with the National Cassava Programme and distributed to farmers within the community-based technology transfer centres. This material was used to establish more than 5 ha of village-based multiplication blocks in each of four technology transfer centres.

Secondary multiplication sites were established in Western and Nyanza provinces, western Kenya. 24 ha were established in Nyanza whilst 14.8 ha were established in Western province. A total of 127 ha of tertiary multiplication sites were also established in this quarter of the project. Sites to host the technology transfer centres were identified as Butere/Mumias, Kuria and Siaya districts in western Kenya. Training demonstrations were also conducted in both Western and Nyanza provinces with a total of 810 and 804 farmers trained in each province respectively.

In November, the multiplication plots at Ukiriguru and Maruku were ratooned and 230,165 cuttings obtained were distributed to contract farmers for further multiplication. The Uganda-derived clones at the open quarantine site were harvested and evaluated for yield potential, ability to produce planting material and reaction to pests and diseases. 10 of the best clones were selected for multiplication. A further 80 clones were selected for further evaluation. The remaining 420 clones were preserved in the germplasm for breeding purposes.

A scientist from Plant Protection Division, Tanzania, funded by the OFDA-CMD project was invited to attend a training-of-trainers workshop for FAO farmer field schools in Kagera region. 8 farmer groups were selected to participate in the technology transfer centre programme in Tanzania. 4 farmers from these groups were given 8 varieties from Ukiriguru for evaluation. In Mara region, 4 farmers were selected and given varieties TMS 81983, TMS 30337, TMS 42029, Lwakitangaza and the local Karinisi for evaluation.

The second regional stakeholders meeting was convened in Kampala, Uganda from the 24-26 October. The three day meeting was very successful and stakeholders were able to draw up an action plan for phase three of the project.

II. Programme Overview

A. Goal and Objectives of the Programme

Project Goal: To improve food security and alleviate poverty in the East African region through enhancing the sustainable production of cassava

Project Purpose: To strengthen sustainable production of cassava in areas of Uganda, Kenya and Tanzania most affected by the CMD pandemic through the exchange and development of cassava germplasm and the accelerated multiplication of CMD resistant varieties

Project Objectives:

1. **Monitoring and Diagnostics:** Provide up to date information on the extent of spread of the CMD pandemic and associated viruses and vectors in target areas, and develop forecasts and risk assessments to guide the implementation of control activities
2. **Multiplication:** Accelerate the multiplication of well adapted CMD resistant varieties and facilitate their distribution to farmers impacted by the CMD pandemic
3. **Germplasm diversification and exchange:** Increase the range of cassava materials available to farmers in areas targeted by the Project thereby reducing future risk of production collapse
4. **Training and technology transfer centres:** Provide options for the sustainable development of cassava production in the region through participatory germplasm evaluation and farmer training in pest and disease management and improved processing methods
5. **Project management, monitoring and impact assessment:** Build on links established with a wide range of stakeholders to facilitate effective co-ordination both within target zones and at regional level. Monitor Project impact.

B. Geographic Locations of Major Programme Activities

Country: Uganda

Site/Institution	District	County	Latitude	Longitude	Activity
IITA-ESARC	Mpigi	Kyadondo	0° 31.2' N	32° 32.7' E	Regional co-ordination
IFCD	Rakai	Kyotera			District-based co-ordination
DFI – Rakai	Rakai	Kooki	0° 40.5' S	31° 25.9' E	Multiplication
DFI – Masaka	Masaka	Kalungu	0° 18.3' S	31° 39.6' E	Multiplication/Germplasm
ICR	Rakai	Kakuuto	0° 48.5' S	31° 30.0' E	Germplasm

IITA-ESARC International Institute of Tropical Agriculture – Eastern and Southern Africa Regional Centre

IFCD Irish Foundation for Co-operative Development

DFI District Farm Institute

ICR International Care and Relief

B. Geographic Locations of Major Programme Activities (cont.)

Country: Kenya

Site/Institution	Province	District	Latitude	Longitude	Activity
KARI-Kakamega	Western	Kakamega	0° 17.0' N	34° 46.1' E	Co-ordination, Multiplication, Germplasm
KARI-Alupe	Western	Teso	0° 29.9' N	34° 7.5' E	Multiplication, Open quarantine, Germplasm
KARI-Kibos	Nyanza	Kisumu	0° 2.4' S	34° 49.0' E	Multiplication
FTC-Bungoma	Western	Bungoma	0° 36.0' N	34° 37.3' E	Multiplication
FTC-Siaya	Nyanza	Siaya	0° 5.0' N	34° 19.0' E	Multiplication
FTC-Busia	Western	Busia	0° 27.5' N	34° 6.9' E	Multiplication
FTC-Bukura	Western	Kakamega	0° 13.4' N	34° 37.3' E	Multiplication

KARI Kenya Agricultural Research Institute
FTC Farmer Training Centre

Country: Tanzania

Site/Institution	Region	District	Latitude	Longitude	Activity
Ukiriguru ARI	Mwanza	Mwanza	2° 43.1' S	33° 1.0' E	Co-ordination, germplasm
Maruku ARI	Kagera	Bukoba	1° 25.0' S	31° 46.8' E	Germplasm, Multiplication, Open Quarantine
Nyakasanga	Mwanza	Mwanza	2° 46.3' S	32° 56.6' E	Germplasm, Rapid multiplication
Masalakulangwa	Mwanza	Kwimba			Multiplication

ARI Agricultural Research Institute

III. Programme Performance

A. Progress in Attaining Programme Objectives

Objective # 1: Monitoring and Diagnostics

Kenya

- Virus diagnoses were completed for the CMD diseased samples collected from 15 districts in western Kenya during a survey carried out by staff from KARI-Kakamega in collaboration with staff from IITA-ESARC. The survey was conducted in western Kenya both to collect samples for diagnostics tests, which would identify the different cassava mosaic geminiviruses in the region, as well as to gauge the extent of spread of the CMD pandemic. Results of incidence of the major cassava pests and diseases as recorded from the survey were presented in the report on the July-September quarter. Maps of the results are provided in this report, however, as figs. 1-3. Virus diagnosis results are presented here.
- The virus diagnostic procedure comprised an initial PCR test using primers specific for ACMV and for EACMV-Ug, followed by RFLP analysis of full length virus fragments using the restriction enzymes: EcorV and Mlu1. 130 samples were collected from the 15 districts. Results indicated that the pandemic associated EACMV-Ug was predominantly present in all but 4 of the sampled 15 districts in Western and Nyanza provinces. In Nyanza province, EACMV-Ug was found in the districts of Kisumu, Migori, Nyando and Rachuonyo. Most of the samples collected contained EACMV-Ug (45 out of the total 130 samples) whereas the least frequent was EACMV: only three samples which were picked from Migori district were EACMV positive. EACMV-Ug was detected in only one district in South Nyanza, again Migori. This suggests little change in the status of the CMD pandemic in western Kenya. Detailed results for the virus diagnoses are presented in Annex 4.

Tanzania

- No surveys were conducted during this quarter of the project.

Indicator: CMD, EACMV-Ug spread maps; > 30 early warning farmers trained, Whitefly biotype map; > 30 impact survey sites Ug, Ke, Tz; radio bulletins, newspaper articles

Current Quarter's Measure: Diagnoses completed for > 100 samples collected from Western and Nyanza Provinces in western Kenya for diagnostic tests to identify the different geminiviruses in the region. Whitefly biotype characterization on-going
Maps developed for CMD pandemic spread, W. Kenya

Cumulative Project Measure: > 100 virus samples diagnosed, W. Kenya
Maps developed for CMD pandemic spread, W. Kenya

Objective # 2: Multiplication of CMD resistant varieties

Uganda

- The established multiplication sites continue to be maintained. Ratooning and distribution of resistant material to vulnerable households continued in this quarter of the new phase. A cumulative figure of 1,278 bags were ratooned and distributed to 1,131 vulnerable households.
- Some of the multipliers sold close to 60% of their SS4 material to buyers in the districts of Masaka, Rakai and Mbarara. A total of 230 bags were sold (Annex 1).
- A new variety I 92/00067 was procured by IITA in collaboration with the National Cassava Programme and this was distributed to farmers neighbouring the Technology Transfer Centres in the districts of Rakai and Masaka. 200 bags were sent and 50 bags allocated to each site to plant a total of 5ha for each of the four sites. The farmers who benefited are those who actively participated in the maintenance of the TTCs (Annex 1).

Kenya

- Two new primary sites were established in Nyanza during the long and short rains. Ratooning of cassava stems from existing primary multiplication sites yielded more than 1,300 bags of ministem cuttings. These were used to establish additional secondary and tertiary sites. Material for establishment of the secondary sites came from KARI-Kibos. In Nyanza Province, sites were established at Siaya, Kisumu, Rachuonyo, Homa Bay, Kuria and Migori. In all, 24 ha of secondary multiplication sites were established in Nyanza Province this quarter out of which 6 ha were SS4 and 18 ha Migyera. In Western province, 14.8 ha of secondary sites were established in Vihiga, Teso, Busia and Mount Elgon districts. 8.8 ha of this total was SS4 with the remaining 6 ha Migyera. 19.8 ha of tertiary multiplication sites were established during the quarter in Nyanza province, whilst in Western province the total tertiary multiplication area established was 107.2 ha.
- There was resprouting of the ratoon crop at KARI-Alupe due to heavy hailstorms. These fields were ratooned and the material given to farmers. The field was then top dressed with CAN fertilizer to allow rejuvenation of the ratoon crop.
- Maintenance of the 20ha of primary multiplication plots under the management of ICIPE in south Nyanza province continued during the quarter. This material will be ready for ratooning in the March/April 2001 planting season.

Tanzania

- During this quarter, all multiplication plots at both Ukiriguru and Maruku were maintained and kept weed free. All CMD infected plants were identified by regular field inspection and rogued.
- In November multiplication plots in both Ukiriguru and Maruku were ratooned and cuttings distributed to contact farmers/ FRGs/IPM/N groups and FFS. Material from Ukiriguru was distributed to Mara region and Biharamulo and Ngara districts in Kagera region. Varieties distributed from Ukiriguru included: TMS 4(2)1425, TMS 81983, TMS 83/01762(5), TMS 30337 and TMS 30572(6). Material from Maruku (principally variety SS4 harvested from the open quarantine field). Number of cuttings and districts where the ratooned material was sent is presented in Annex 2. In all, a total of 230,160 cuttings were obtained from the ratooning exercise.

Indicator:	Ke. - 6 million stems; Tz. - 3 million stems; Ug – 2 million stems
Current Quarter's Measure:	Ug. – 127,800 stems; Ke. – 270,000 stems; Tz. – 230,160 stems
Cumulative Project Measure:	Ug. – 127,800 stems; Ke. – 270,000 stems; Tz. – 230,160 stems

Objective # 3: Germplasm Diversification and Exchange

Uganda

- The 6 MAP farmer participatory evaluation was conducted in October at the technology transfer centres (TTCs) established in each of the two districts of Rakai and Masaka. The materials were well established with most of them > 1m high. The material for demonstration is also being well maintained. Most of the plants have branched and all sites have been weeded at least 5 times. The material will be ready for final evaluation and harvest in the third quarter. Details of the participatory evaluation at the TTCs will be presented at a later date.
- 200 bags of a new variety I 92/00067 which is both CMD and CGM resistant was delivered to the districts of Rakai and Masaka for establishment of 5 ha around each site at the TTCs. This new variety is also a preferred host for *T. aripo*, the biological control agent for cassava green mite and is sweet to taste. The germination of this new material is good (90%) and the crop is healthy.

Kenya

- On-farm trials, for the evaluation of the 14 elite fast-track materials selected from the germplasm introduced from the EARRNET regional germplasm programme at Serere, Uganda, were maintained in Teso, Butere-Mumias, Siaya, Rachuonyo, Migori and Kuria districts. The farmers have weeded the crop and data collection continues on the clones being evaluated. Relatively little CMD has been recorded in these trials to date, although clones MH 95/0183 and MM 96/9362 were consistently recorded with highest incidences of CMD and most severe symptoms amongst the fast-track clones, although these performed better in virtually all cases compared with the local check varieties with respect to CMD. Hailstones, which seemed to encourage vector-borne secondary infection, damaged the trials at Mumias but most of the clones have recovered. On station trials at Alupe, Kakamega and ICIPE were also maintained.
- Germplasm at KARI-Alupe was maintained in this quarter and weeds were controlled by the use of round up and hand weeding.

Tanzania

- Materials planted at the Open Quarantine facility were harvested and evaluated for their yield potential, pest and disease reactions and ability to produce planting material. The 10 best clones were selected for multiplication. A further 80 clones were selected and planted for further evaluation. Also 420 remaining clones were planted in a germplasm collection for future use. The performance of the 10 best clones is presented in Annex 2.
- Evaluation of tissue culture materials from Nyakasanga continued at Bushasha (medium disease pressure), Gera (high disease pressure) and Maruku (low disease pressure). 38 clones were evaluated and the full list of these clones is provided in Annex 2. The clones have sprouted well and have already been weeded. The evaluation will look at CGM, CMD and CBB incidences and severity.

Indicator: Clones introduced: Tz > 100 Vars. evaluated: Tz 8; Ke 8; Ug 6
Vars to multiplication: Ke 4; Tz 4
OQ Tz - 10 clones to fast-track multiplication; 80 clones to prelim. eval.
PQS Ke – Virus indexing established; 10 vars indexed

Current Quarter's Measure: On-going var. evaluation: Tz > 40; Ug 9; Ke 14
Vars to multiplication: Tz 6; Ug 1
OQ Tz – 10 clones to fast-track; 80 to prelim. evaluation

Cumulative Project Measure: On-going var. evaluation: Tz > 40; Ug 9; Ke 14
Vars to multiplication: Tz 6; Ug 1
OQ Tz – 10 clones to fast-track; 80 to prelim. evaluation

Objective # 4: Training and Technology Transfer Centres

Uganda

- Management of the material planted at the TTCs continued during this quarter of the project. A second participatory evaluation of the materials for the technology transfer centres planted in April was also done. Farmer attendance was good because of the effective mobilization by IFCD. Farmers participated well in the evaluation after which they were given some follow up training on management of cassava pests and diseases.

Kenya

- Sites to host the technology transfer centres have been identified as Butere/Mumias, Kuria and Siaya districts. A tour for extension workers and farmers to Uganda is planned.
- Training demonstrations were conducted in all districts of Western and Nyanza provinces with a total of 810 and 804 farmers for Western and Nyanza provinces respectively being trained.
- A three-day training was organized for farmers and extension personnel in November at Muguga.

Tanzania

- A scientist from the Plant Protection Department, funded through the OFDA CMD Project, was invited to provide training on CMD and its management at a training of trainers workshop for extension workers to be responsible in Bukoba and Muleba districts for the implementation of Farmer Field Schools in the two districts. The training programme of the OFDA CMD Project will be channelled through this medium in August.
- Eight farmer groups were selected in Kagera, Mara and Mwanza region to participate as technology transfer centres. Four farmers were selected and were provided with 8 CMD resistant varieties from Ukiriguru for their evaluation.
- For Mara region, four farmers were also selected from each FRG group for variety evaluation. Four varieties TMS 81983, TMS 30337, TMS 42029, Lwakitangaza and one local one, Karingisi, are to be evaluated. Details are provided in Annex 2.

Indicator: Tech. Trans. Centres: Ug, Ke, Tz: 8 each
Farmers trained: Ug, Ke, Tz: > 160 each
Resistant variety multiplication on-going: TTCs Ug, Ke, Tz

Current Quarter's Measure: Tech. transfer sites: Ke – sites identified; Ug – 4 sites running; Tz - 8 farmer groups selected in Mara, Mwanza and Kagera for TTC activities
Farmer training: Ke > 1,500
TTC multiplication: Ug – 200 bags I92/00067 planted;
Tz – 3 TTCs with SS4 + > 50 FAO farmer field schools

Cumulative Project Measure: Tech transfer sites: Ug 4; Tz 8
Farmers trained: Ke > 1,500
TTC multiplication: Ug 4 sites; Tz > 50 sites

Objective # 5: Project Management, Monitoring and Impact Assessment

The second regional Stakeholders Meeting was convened in Kampala, Uganda during this quarter of the project. This time, there were participants from three additional countries: Rwanda, Democratic Republic of Congo and Burundi. The first part of the three-day meeting involved a series of presentations by representatives of each of the Project's major stakeholders. Presentations detailed progress achieved during the second phase of the Project (October 1999-September 2000). During the second part of the proceedings, stakeholders split into smaller working groups and detailed implementation plans were developed for the third phase of the Project (October 2000-September 2001). The meeting provided a very useful opportunity for Project participants to share views candidly on project progress, future plans and the manner of its implementation. Additionally, provisional plans were developed for the extension of CMD management activities to the three 'new' countries which are either already affected by the pandemic, or are likely to be affected within the near future. Full proceedings of the meeting have been published.

Uganda

- There has been continued collaboration with the IFCD, the major project partner now represented by CEDO in Rakai and Masaka, which has continued to implement project activities. IFCD also participated in assessing the germination and performance of the evaluation material at the TTCs. The increased responsibility given to IFCD in the local co-ordination and management of Project activities has significantly strengthened their implementation.
- About 230 bags of SS4 were sold to interested buyers from the districts of Masaka, Rakai and Mbarara.

Kenya

- A sub-sector meeting for cassava was conducted in Machakos in December and Mr. H. Obiero presented a paper on the progress of accelerated cassava multiplication in western Kenya
- A tour of Nyanza province by the Provincial Director of Livestock Nyanza, Director, RRC-Kakamega and Provincial Crops Officer Nyanza was conducted in November. The respective Provincial Director of Livestock and Extension, Director, RRC-Kakamega and Mr. H. Obiero also toured Western Province. The aim of the tour was to assess the progress of the cassava multiplication project.
- A Biometrics team headed by Professor Nokoe of IITA visited western Kenya. Other members of the team included Dr. Ssemakula of NARO, Mr. H. Ojulong and Mr. P. Ragama of IITA-ESARC.

Indicator:	Regional Stakeholders meeting SC meetings: Ug, Ke, Tz Impact reports: Ug, Ke, Tz
Current Quarter's Measure:	Regional Stakeholders meeting - Kampala Stakeholder implementation plans updated New country stakeholders identified: DRC, Rw, Bu
Cumulative Project Measure:	Regional Stakeholders meeting - Kampala Stakeholder implementation plans updated New country stakeholders identified: DRC, Rw, Bu

B. Programme Success Stories

Distribution of CMD resistant material to target population

To date a cumulative figure of 1,278 bags of CMD resistant material have been given out to 1,131 vulnerable households in the districts of Rakai and Masaka. The recipients of these materials have been trained in the management of the crop.

One new variety, I92/00067, which is both CMD and CGM resistant, was delivered to each of the TTCs in Masaka and Rakai districts for establishment of 5ha. This new variety is not only a preferred host for *T. aripo*, the biological control agent of CGM, but is also sweet to taste. This introduction of new material will further diversify the CMD resistant material in the area, giving farmers a wider range to choose from.

Incomes of farmers boosted through the sale of CMD resistant material in Uganda

Some of the multipliers of new CMD resistant varieties in Rakai and Masaka districts, Uganda, have succeeded in selling their 60% of the SS4 planting material to interested buyers from the districts of Rakai, Masaka and Mbarara. About 230 bags were sold, each bag being sold for 8,000-10,000 Uganda shillings (approximately 1,700 Uganda shillings equal 1 US\$) thereby improving the incomes of these farmers. This venture also implies that the project is sustainable and that multipliers can now generate their own funds for maintenance of the multiplication plots.

Kenya initiates tertiary multiplication of CMD resistant material

During this quarter of the project, tertiary multiplication was initiated in Western and Nyanza provinces in Kenya. In Western province, 107.2 ha were established while 19.8 ha were established in Nyanza province. By initiating tertiary multiplication, the project is now putting CMD resistant material into farmers' hands, an act that will translate directly into improved yields and benefits associated with the improved yields.

C. Problems in Achieving Programme Activities

Multiplication activities in Kenya were affected by the harsh weather that prevailed during this quarter of the project. Hailstones destroyed the crop at some of the established primary sites, damaging the terminal buds. As a result lateral buds emerged and the material had to be ratooned and distributed to farmers.

In some areas, there was persistent drought, which affected the growth of the crop. Mole rats are also a serious problem in some areas.

Theft of materials from some multiplication plots was also a major problem. At KARI-Alupe, 2.4 ha of SS4 were destroyed as a consequence of this problem. Security guards have been deployed to guard the fields against thefts.

D. Diagnostic survey results, western Kenya, August 2000

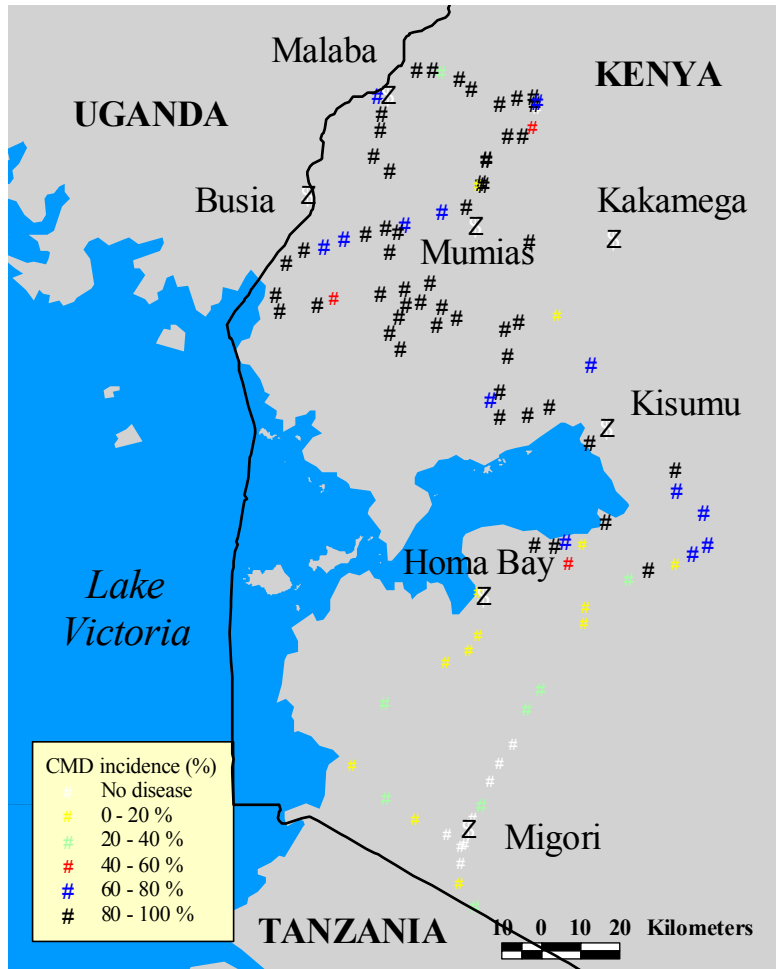


Fig. 1. CMD incidence, western Kenya, August 2000

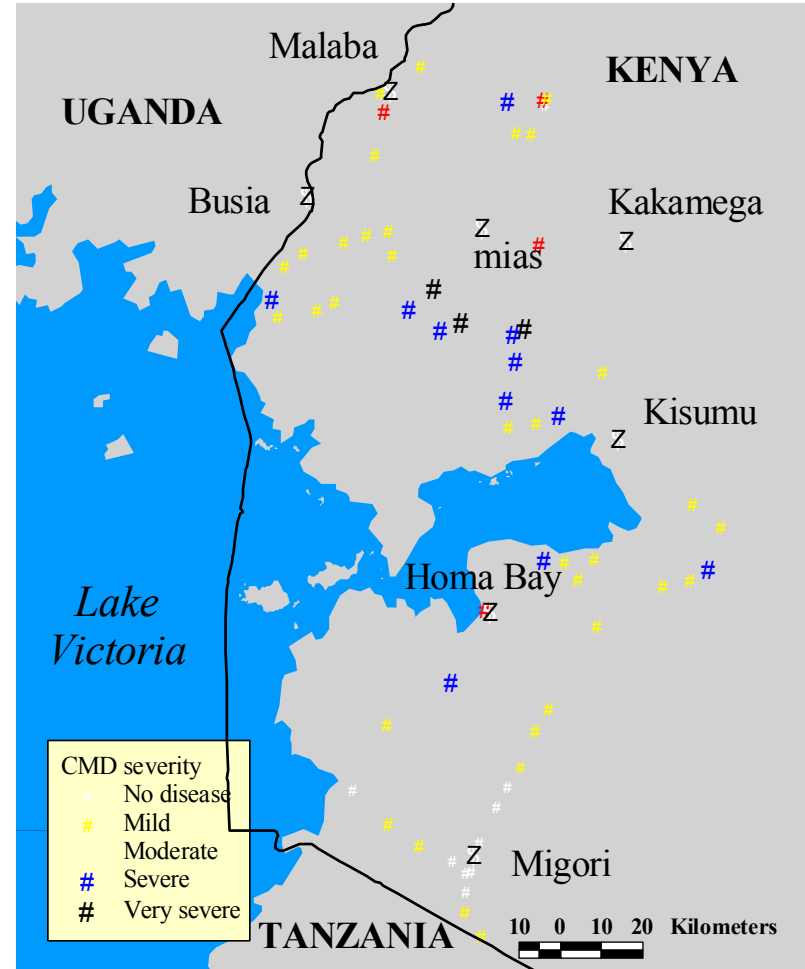


Fig. 2. CMD severity, western Kenya, August 2000

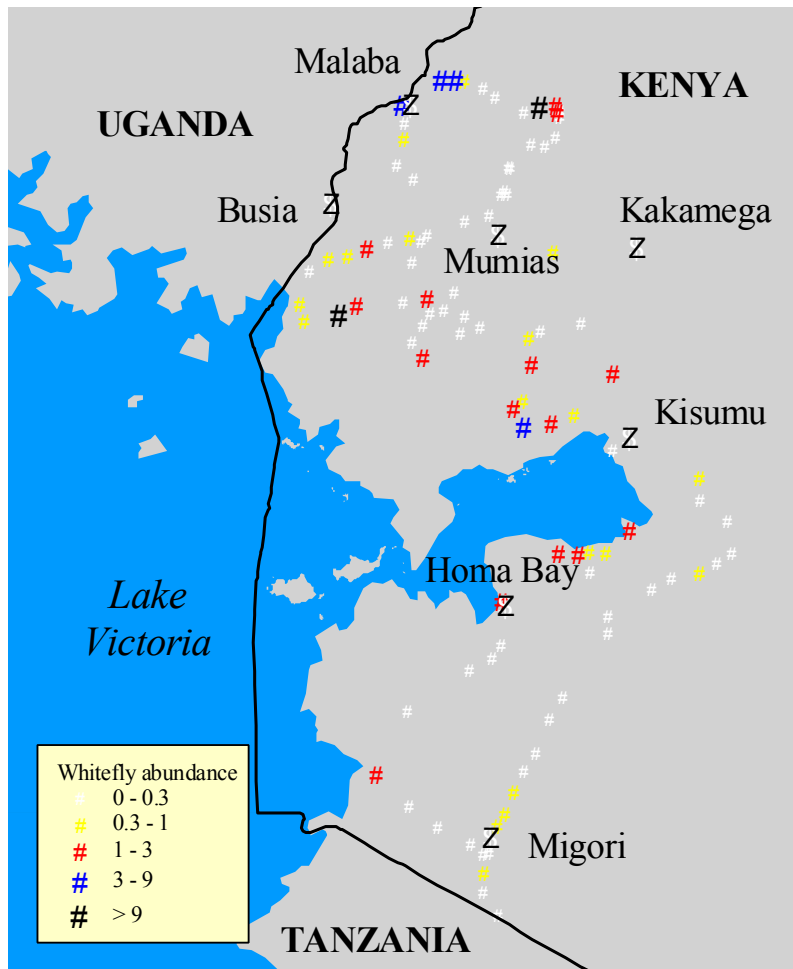


Fig. 3. Whitefly abundance, western Kenya, August 2000

ANNEX I

Multiplication activities in Uganda – Fourth quarter, 2000 Irish Foundation for Co-operative Development (IFCD)

Rosemary Mayiga

Location/Contact Persons

The locations and contact persons for the multiplication of mosaic resistant cassava remain the same as reported in the 6th quarterly report of December 1999-February 2000.

Cassava Status

Planted	Ratooned	Height	Status	Hectares
October 1998	*October 1999	1-2ft	Healthy	10.6
April 1999	April 2000	3-4ft	Healthy	19.4
October 1999	*Being ratooned	1-2ft	Healthy	39

*Being ratooned this season “B” 2000

Ratooning and distribution of SS4 cassava

The ratooning and distribution of SS4 cassava cuttings to the vulnerable households is in progress. Since the ratooning exercise started, up-to date a cumulative 1,278 bags have been ratooned and distributed to 1,131 vulnerable households. All households that received the cuttings were trained on the management of cassava. Other figures will be submitted in the next report since the personnel who were involved in the distribution exercise have not submitted other names of beneficiaries.

Some of the multipliers managed to sell their 60% of the SS4 cassava cuttings to interested buyers in the districts of Rakai, Masaka and Mbarara. Each bag of SS4 fetched between 8,000/= and 10,000/=. A total of 230 bags were sold by these multipliers thus improving their incomes.

The mosaic incidence still remains low.

Distribution of I92/00067 mosaic resistant cassava

IITA sent another new mosaic resistant cassava variety ‘I 92/00067’. This was distributed to farmers near the TTCs in Rakai and Masaka districts. These are farmers who actively participated in the maintenance of the TTCs. 200 bags were sent and each area in the vicinity of the TTC was allocated 50 bags to plant 5 ha. This was successfully done. The germination rate of this cassava was over 90% on average. The crop looks healthy and is 1 foot high above the ground.

Technology Transfer Centres (TTCs)

The farmer' participatory evaluation training at the four TTCs in Masaka and Rakai districts were conducted in October 2000 as planned by a collaboration between IITA/NARO/IFCD.

Where the moisture content is high and the fields well maintained the plants are generally healthy with an average height of 3-4 feet high. Most plants in the TTCs have already branched. All sites have at least had a fifth weeding.

Attendance of participants

Farmers were mobilized and sensitized by the IFCD. The attendance on average was 30% at all sites. (See lists already submitted to IITA personnel who conducted the training).

Financial Report.

The International Institute of Tropical Agriculture (IITA) disbursed the first percent of the funds for the Mosaic Resistant Cassava multiplication programme Phase III. The two districts, Masaka and Rakai received US \$ 3,000.00 in November 2000, which is equivalent to 5,555,550/=, (Five million five hundred fifty five thousand five hundred fifty). The funds were used for maintaining the multiplication sites and implementing the ratooning exercise.

Implementing Plan

During the next of quarter of December 2000-February 2001 the following activities will be carried out:

- Identification of vulnerable households and distribution of SS4 cuttings to them
- Conduct assessment training on each variety at the various TTCs
- Monitor the maintenance of multiplication sites and TTCs

ANNEX 2

OFDA CMD Project activities in Tanzania – Third Quarter 2000 Lake Zone Agricultural Research and Development Institute

S. C. Jeremiah and I. Ndyetabula

Multiplication and Distribution of Resistant Varieties

In November 2000, the plots of multiplication were ratooned at both Ukiriguru and Maruku. The cuttings were distributed to contact farmers/ FRGS/IPM/N groups and FFS. The number of cuttings and districts where the material were sent are as indicated below:

Location	Number of Cuttings
Muleba	31,200 of SS4
Bukoba	23,500 of SS4
Karagwe	31,400 of SS4
IPM/N and TTCs	9,072 of SS4
Musoma	17,464 of different varieties
Tarime	15,460 of different varieties
Biharamulo	80,744 of different varieties
Ngara	15,000 cuttings
Ukiriguru (On station)	6,320 of TMS 4 (2) 1425
Total	230,160

Germplasm Diversification

Open Quarantine materials

The clones which were planted at Open quarantine were harvested and evaluated on their yield potential, pest and disease reactions and ability to yield planting material. The ten best clones were selected for multiplication. The clones, their yield in kg/plant, number of roots per plant and number of harvested plants are presented below:

Table 1. Performance of ten best materials from > 500 clones introduced from the EARRNET germplasm collection, Serere, Uganda, and cultivated over 12 months at the Open Quarantine site, ARDI-Maruku, Bukoba, Tanzania

Variety	Yield (kg/plant)	No. of roots / plant	Taste	Plant harvest
MM 96/3075 B	4.8	10	Sweet	12
MM 96/4446	4.3	10.7	Bitter	3
MM 96/4619	4.0	7.5	Bitter	11
MM 96/8100	3.7	10.4	Bitter	11
MM 96/0876	3.7	6.8	Sweet	9
MM 96/4684	3.5	6.4	Bitter	9
MM 96/5373	3.4	8.8	Bitter	6
MM 96/2725	3.3	10.8	Bitter	12
MM 96/3462	3.3	10.1	Intermediate	11
MM 96/8233	3.2	8.8	Intermediate	20

Another set of 80 clones were selected and planted for further evaluation. The remaining clones (420) were planted in the germplasm for breeding purposes in the future.

Evaluation of IITA tissue culture material for resistance to UgV

Tissue culture materials for Nyakasanga (Ukiriguru) were evaluated at 3 sites in Kagera (Bushasha, Gera and Maruku). A list of these 38 clones is as below:

I 92/0600	I 93/0026	I 93/0658 (4x)
I 93/005	I 92/0042	I30572
I 91/00416	91B/00462	I 93/0170
TME 13	TME 6	I 93/0571 (3x)
TME 1	I 93/0584 (3x)	I 92/0429
I 93/0614 (3x)	I 93/0569(3x)	I 92/0455
I 93/0571 (3x)	I 93/0053	I 92/0019
I 93/0127	I 93/0161	I 92/034
I 93/0639 (3x)	I 92/0053	I 93/0560
I 92/0509	TME 12	I 92/0019
TME 4	I 92/097	

The plots have sprouted well and are already weeded. The characteristics of the sites area as follows: Gera (high disease pressure), Maruku (Low disease pressure) and Bushasha (Medium disease pressure). Parameters to be considered during the evaluations are CGM, CMD and CBB incidences and severity.

Establishment of TTCs/FRGs

Eight farmer groups were established in Kagera, Mara and Mwanza as follows:

Bukoba – 2 TTCs

Muleba – 2 TTCs

TRIME – 2 TTCs

Mwanza – 1 TTC

Musoma – 1 TTC

The TTCs were given varieties for multiplication and some for evaluation. The varieties for evaluation in Kagera were brought from Ukiriguru and these include:

Lwakitangaza

TMS 42029

TMS 30337

TMS 81983

TMS 83/01762 (6) and SS4

TMS 4 (2) 1425

TMS 30572

Four farmers were selected among the IPM/N members assuming that all IPM/N members will participate in the evaluation.

Mara region

The same procedures as for Kagera were applied. Four farmers from each FRG were selected for variety evaluation. The varieties under evaluation were:

TMS 81983

TMS 30337

TMS 42029

Lwakitangaza

Karingisi (local)

Distribution of TTCs AND FRGs within the zone

Region	District	Name of TTC/FRG
Mara	Musoma	Tegeruka
	Tarime	Gwitilyo Nyabisaga
Kagera	Bukoba	Nsunga Kilima
	Muleba	Kalembe Kanoni
Mwanza	Geita	Butundwe

Maintenance of Ratoon Crop

All multiplication plots at both Ukiriguru and Maruku were maintained and kept free of weeds. CMD infected plants were rogued.

Establishment of nursery from seeds collected from Open Quarantine

The seeds collected from OQ were planted in the nurseries at both Ukiriguru and Maruku. At Maruku a plot of 1648 square meters was established. The seeds planted were from SS4 and Migyera and a mixture of clones (510 clones). At Ukiriguru the seeds of three varieties were sown directly in the field. The area covered was 2.7 acres and 1.08 ha.

Future plans

- Conduct farmer training on multiplication (January 2001)
- Training on CMD aspects (Mid January 2001)
- Monitoring of trials – Continuous
- Disease monitoring and diagnostics
- Steering Committee – February and September

ANNEX 3

Cassava Multiplication Project in western Kenya Quarterly Report (Oct-Dec. 2000) Kenya Agricultural Research Institute

H.M. Obiero

Executive summary

Activities conducted during October- December 2000 included maintenance of primary sites, preparing planting materials for gapping, establishment of secondary multiplication sites in Nyanza and Western provinces and providing individual farmers, farmer groups, CBOs and NGOs with materials for tertiary multiplication. The quantity of materials provided by primary sites was 1351 bags. Area under secondary multiplication planted in Nyanza during the period is 24 ha while in Western province it is 14.8 ha. Farmers who undertook tertiary multiplication in Nyanza during the period were 205. The total acreage established by the farmers was 19.8 ha. In western 797 farmers established tertiary multiplication during the period and a total acreage of 107.2 ha was established.

On-farm trials and on station trials were maintained and three months data taken. One on-station trial at Homa Bay FTC was destroyed by persistent dry spell.

Monitoring and evaluation of cassava project was conducted by Provincial Directors of Agriculture, Livestock and Extension of Nyanza and Western provinces; Centre Director-RRC Kakamega, Provincial crop's officers and cassava co-ordinator of western region.

A Biometrics team headed by Professor Sagary Nokoe from IITA Ibadan visited all cassava on-farms established in the region.

Dr. DeVries of Rockefeller and Mr. Torano, a consultant for Rockefeller also visited western Kenya to acquaint themselves with the cassava activities in the region. Progress of accelerated cassava multiplication was also presented at several fora during the period Sept-Dec 2000.

Introduction

Cassava (*Manihot esculenta*) has until the recently been an important food security crop as well as income-generating crop in the lake basin region. The crop yields well per given unit area compared to basic staple cereals even under stress of poor soil fertility and low moisture regime. Western Kenya has in the past been recorded to cultivate and consume more that 60% of Kenya's cassava production.

Since 1995 when a severe form of African cassava mosaic infected cassava landraces in western Kenya, production and productivity of cassava in the region has declined drastically. By 1997 African cassava mosaic had become so serious that some farmers in affected districts such as Teso, Busia, and Siaya had given up growing cassava. During the same year Kenya Agricultural Research Institute technically collaborated with East African Root crops Research Network/International Institute of tropical Agriculture to combat the epidemic. Financial support to combat the epidemic has been provided by Gatsby Charitable foundation-UK, Office of foreign Disaster Assistance-USAID, and Rockefeller foundation-USA.

Eight hundred and forty five cassava clones have been introduced into open quarantine at KARI-Alupe since 1997 and are at various stages of evaluation (i.e. quarantine, PYT, AYT). Two cassava varieties SS4 and Migyera have been under multiplication since 1998. A three-tier multiplication system has been adopted (primary, secondary and tertiary).

Specific Objectives under review

1. Multiplication of SS4 and Migyera varieties
2. On-farm trials of fourteen promising clones
3. On-station trials of thirty second track promising clones
4. Monitoring and Evaluation progress of the project
5. Others e.g. training, meetings, and opening account in Kisumu

Description of Implemented Activities

Rapid multiplication of SS4 and Migyera

After September 2000, short rains continued favorably. Farmers' demand for improved cassava planting materials was still high. In October 2000, there were hailstones, which damaged terminal buds in almost all the primary sites. As a result lateral buds emerged. It was decided that all materials be cut back and farmers provided with planting materials through NGOs, CBOs, women/farmer groups as well as individual farmers. More secondary sites were established in the districts in both Western and Nyanza provinces during this quarter.

Two more primary sites were established in Nyanza during long rains and short rains.

Activities undertaken at various primary multiplication sites are as indicated below:

KARI-Alupe

Germplasm in the open quarantine blocks continue to be maintained. Control of weeds was by the use of round up herbicide and slashing around the field. PYT and AYT were hand weeded to control difficult weeds such as wondering jew (*Commelina spp*), which could otherwise not be controlled by the herbicide and also sprayed with round up.

Multiplication blocks of SS4 and Migyera were kept weed free by hand weeding and use of herbicides.

Due to heavy hail storms on 6th and 7th October 2000, which induced pre-sprouting of the ratoon crop, It was decided that planting materials be cut and given to the farmers. The fields were then top dressed with CAN to boost rejuvenation of the ratoon crop. There was serious theft of tubers at Alupe during this quarter resulting into loss of 2.4 ha of SS4. The area has been ploughed and replanted. Two security guards have been deployed to guard the fields against theft.

KARI-Kakamega

Kakamega site continued to provide planting materials (Migyera) to individual farmers, farmer groups and material for secondary sites establishment during the period of Oct-Dec 2000. Weed control has been through manual slashing and the use of round up. Due to the demand for SS4 by some of the farmers who visit the centre, SS4 variety was bulked on a plot of 0.5 ha and has been hand weeded three times during the period. The on-station trial at the centre was also hand weeded three times during the period and three months data was taken in October 2000.

KARI-Kibos

Planting materials were cut and provided to the farmers for a second time in the year between the months of Oct-Dec 2000. Some of the planting materials were used in establishment of new individual farmers' fields, secondary sites in both Nyanza Western provinces and gap filling. Weeds were controlled by spot weeding and use of round up herbicide. Thinning and pruning of the ratoon was in done December 2000.

Bukura FTC

Planting materials were cut and provided to individual farmers and also used for secondary multiplication in Kakamega, Lugari and Butere-Mumias-districts during the period of Oct-Dec 2000. Weeds have been controlled by manual slashing and use of round up.

Siaya FTC

Planting materials were provided to individual farmers in Siaya and Bondo districts. Weeds were controlled by round up and spot hand weeding. Because of theft of tubers, two guards have been deployed to guard the planting materials. Gapping was also done in both of Migyera and SS4 fields.

Bungoma FTC

SS4 was cut in October and given to farmers in Bungoma and Mt. Elgon districts. Control of weeds in the field of SS4 and Migyera was done using round up. Spot weeding was done on areas where weeds were not well controlled.

Busia

During the period Oct-Dec 2000, the FTC provided SS4 planting materials to Teso, Vihiga, Siaya and Busia districts. Weeds were controlled by herbicide and hand weeding during the period.

Homa Bay FTC

This primary site was established during the long rains of 2000 with 2 ha of SS4. The site has had a lot of problems amongst which are persistent drought, moles and also difficult weeds, some of which are difficult to control with round up herbicide. There will however be some planting materials ready during the long rains of 2001.

ICIPE

ICIPE has established a total of 20 ha of improved cassava varieties on its three farms; Nguku, Amoyo and Mbita Point. SS4 constitutes 7 ha while Migyera variety is 13 ha.

Termites reduced the plant population of Migyera at Amoyo farm and as a result heavy gapping was done during Oct-Dec 2000. At Nguku farm SS4 seriously suffered from die back that was suspected to be bacterial blight. This considerably reduced plant population. All fields were manually weeded during Oct-Dec. Earlier established blocks will provide planting materials during the month of March/April 2001.

Table 1: Summary of planting materials supplied by primary sites during Oct-Dec 2000

Site	Varieties (mini stem cuttings in large bags)		Total
	SS4	Migyera	
1. KARI-Alupe	360	72	432
2. KARI-Kakamega	-	450	450
3. KARI-Kibos	128	-	128
4. Busia FTC	116	-	116
5. Siaya FTC	-	40	40
6. Bungoma FTC	87	-	87
7. Bukura FTC	98	-	98
TOTAL	789	562	1351

Table 2: Summary of secondary multiplication sites established (Oct-Dec 2000) in Nyanza province

Districts	Varieties	Area in ha.	Status
1. Siaya	SS4	2.0	90% germination
2. Kisumu	Migyera	4.0	90% germination
	SS4	2.0	90% germination
3. Rachuonyo	Migyera	6.0	90% germination
4. Homa Bay	SS4	2.0	80% germination
5. Kuria	Migyera	4.0	80% germination
6. Migori	Migyera	4.0	100% germination
Total acreage	Total	24	
	SS4	6.0	
	Migyera	18.0	

■ Earlier established secondary multiplication were hand weeded at least two times.

Table 3: Summary of secondary multiplication (Oct-Dec) in Western Province

Districts	Variety	Area (ha)	Status
1. Vihiga	SS4	2.0	100% germination, weeded once
	Migyera	1.2	100 % germination, weeded once
2. Busia	SS4	6.0	90%germination, weeded once
	Migyera	2.0	90%germination, weeded once
3.Teso	Migyera	2.0	70% germination, weeded once
4. Mt. Elgon	Migyera	0.8	90% germination, weeded once
	SS4	0.8	100% germination, weeded once
Total acreage	Total	14.8	
	SS4	8.8	
	Migyera	6.0	

-All earlier established sites were hand weeded at least twice.

-All the recently established secondary sites have been weeded at least once.

Table 4:Summary of tertiary multiplication established in Nyanza province (Oct-Dec 2000)

Districts	Number of farmers who have planted	Area in ha
1. Siaya	73	6.0
2. Bondo	36	1.8
3. Kisumu	96	12.0
TOTAL	205	19.8

Table 5: Summary of tertiary multiplication established in Western province(Oct-Dec 2000)

Districts	Number of farmers who have planted	Area in ha
1. Butere-Mumias	48	12.2
2. Kakamega	210	16.4
3. Vihiga	127	11.2
4. Lugari	36	2.8
5. Bungoma	128	17.4
6. Busia	148	22.0
7. Teso	64	20.0
8. Mt. Elgon	36	5.2
TOTAL	797	107.2

On farm trials

Teso district (Amagoro)

The four on farm trials in Teso district continue to be maintained. Researchers and Extension personnel took three months data in October 2000. Preliminary data indicated that the clones MM96/5280, MM96/1871, MM96/7151, MM96/4466, TME 14, MM96/4052, MM96/9362, MM96/7688, MM96/3868/ SS4, Migyera and Unknown 3 were free of CMD at three months.

Butere-Mumias district (Matungu)

The four on-farm trials in Butere have been well maintained and weeded. Three months data was taken in November 2000. Preliminary data indicated that the clones CK2, Unknown 2, 55329 and MH95/0183 were already infected with CMD with severity range of 2-3. Hailstones, which damaged the trials in October, also induced secondary infections such as anthracnose disease. However, by December 2000 most of the clones had recovered.

Siaya district (Ukwala division)

The germination of the trial was 100%. The plots were weeded during Oct-Dec 2000. Three months data has not been taken.

Rachuonyo district (Oyugis)

All the four on farm trials are well maintained and have been weeded. Three months data was taken by researchers and extension personnel in Dec.2000. Preliminary data indicate that most of the test clones were free from CMD. Infected clones were identified as MH95/0183, MM96/9362, MM96/4884 and all the local varieties included in the trial.

Migori district (Muhuru Bay)

Three of the four farms were well maintained. The plots were kept free of weeds by hand weeding. One of the farms had very poor germination and the farmer had gap filled with other local materials. At the time of data collection in Dec. 2000, local varieties used in the trial, clones MH95/0183 and MM96/9362 were severely infected with CMD. At one trial, mealy bugs were observed on some of the clones.

Kuria district (Kehancha)

Three farmers in Kehancha maintained the trials well. The plots were well weeded. The fourth farmer's plot was neglected but if weeded in time good data is expected. Preliminary data indicated that all the test varieties were free of CMD except MH95/0183, MM96/9362, SS4 and the farmer's variety.

Kuria district (Subakuria)

Two farmers have maintained the on-farm trials well. One farm is not well weeded but the crop is promising. The fourth farmer ploughed down the entire test clones on the farm. Three months data taken on three farms indicate that clones MM96/4884, M96/9362, MH95/0183 and all the local varieties included in the trial expressed CMD symptoms.

On-station trials

The on-station trials at Alupe, Kakamega and ICIPE, were maintained by hand weeding. The ICIPE site was gap filled in November 2000. The on-station trial at Homa Bay took up well but due to persistent dry spell the trial dried up in October 2000 after weeding. The trial at Kakamega was weeded by hand thrice and the three months data was taken.

Monitoring and Evaluation

Monitoring and evaluation was conducted by five different teams between Oct-Dec 2000. The purpose of the exercise was to assess the activities on the ground and the progress of the project. The monitoring and evaluation tours conducted are as indicated below.

Tour in Nyanza Province on 6th to 10th Nov 2000.

The Provincial Director of Agriculture, Livestock and Extension Nyanza, Mr. Odock was accompanied by Regional Research Centre-Director, Dr. A. B. Orodho, Provincial Crops Officer- Nyanza, Mrs. Magut and Mr. M. Akhwale of KARI Kakamega, visited all districts implementing the project in Nyanza. They were able to see some primary and secondary sites, on-station trials, on-farm trials and tertiary multiplication. The overall impression was that good work was going on but there was need to for stakeholders to redouble their dedication for success since the crop can provide food security and alleviate poverty in the region.

Tour in Western Province on 21st-24th Nov 2000

The Provincial Director of Agriculture, Livestock Development and Extension, Western Province- Mr. Chepsaigut in company of the Centre Director, Dr. A.B Orodho and H. M. Obiero of KARI-Kakamega visited six districts (Vihiga, Butere-Mumias, Bungoma, Busia, Mt. Elgon, and Teso) to assess progress of the project. Primary sites, secondary sites, on-farm trials and on-station trials and tertiary bulking sites were visited. The impression of the team was similar to that of the Nyanza province tour.

Visit by Biometrics team on 10th - 15th November 2000

A biometrics team headed by Professor Nokoe of IITA visited the region from the 10th to 15th of November. With him were Dr. Ssemakula of NARO, Mr. Henry Ojoulong and Mr. P. Ragama both of IITA. The team was accompanied by Mr. H.M. Obiero of KARI-Kakamega and Mr. Orondo Ko'loo of KARI-Alupe. The team looked at all the 28 on farms in the region, four on station trials and some multiplication.

Visit by Dr. DeVries of Rockefeller Foundation, Nairobi office.

Dr. J. DeVries of Rockefeller foundation, Nairobi office visited Western Kenya on 4th & 5th of Dec 2000 to assess progress of the project. He visited on-station trial at RRC Kakamega and several cassava multiplication fields in Butere-Mumias, Bungoma, and Teso districts. During the visit some planting materials of Migyera was provided to some farmers.

Visit by Torino of Rockefeller foundation New York Office.

Mr. Torino, a consultant with Rockefeller foundation-New York office visited Western Kenya on 7th & 8th Dec 2000 to look at and document cassava activities in the region. He visited some tertiary multiplication sites in Butere-Mumias, Bungoma, and Teso districts.

Others

- A three day training was organized by Dr. Kariuki of Cassava Green Mite Project, Muguga for farmers and Extension personnel from 21st to 23rd of Nov. 2000. Mr. Michael Akhwale of KARI-Kakamega represented Regional Research Centre and presented a paper on the progress of Cassava project.
- A bank account for Western Kenya Cassava project was opened in Kisumu Barclays Bank branch with Ksh. 400,000/= on 10th Nov. 2000.
- A sub-sector meeting for cassava was conducted in Machakos on 11th Dec.2000. Mr. H.M. Obiero was invited and presented a paper on progress of accelerated cassava multiplication project in Western Kenya.

ANNEX 4

Virus diagnoses for CMD diseased cassava samples collected from western Kenya in August 2000

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Introduction

The western Kenya survey was carried out in 15 districts of the two provinces. One of the objectives of the survey was to gather CMD diseased leaf samples for laboratory diagnostics using PCR and RFLP to identify different viruses that affect cassava in these two provinces of western Kenya.

Materials and Methods

More than 100 farmers' cassava fields were sampled throughout Western and Nyanza provinces. An interval of about 5 km was used as the sampling distance between fields. In each farmer's field, samples of CMD infected leaves were collected from a plant randomly selected. One leaf from one plant showing mosaic symptoms of varying severities was collected from each sampled field. The samples were each put in micro-centrifuge tubes and transferred into a cool box containing ice blocks in order to preserve them. A total of 130 samples were collected.

DNA extraction

DNA was extracted from plant tissue using the IITA–ESARC, Uganda protocol based on the 'Dellaporta' method.

PCR amplification

ACMV specific (AL1/F & ARO/R), EACMV-Ug specific (UV-AL1/F1 & ACMV CP/R3) and universal primer pairs were used for the amplification in the reaction mixture as shown below;

Water	10.6µl
PCR buffer x10	2.5 µl
MgCl ₂ (25mM)	1.5 µl
Tween - 20(5%)	2.5 µl
DNTPs (2.5mM)	1.0 µl
Primer 1 (20µM)	0.2 µl
Primer 2 (20µM)	0.2 µl
DNA Template	3 µl
Taq polymerase	0.2 µl

Then two drops of mineral oil were layered on top of each tube containing reaction mix to stop evaporation. The target viral DNA was amplified in Techne and Hybaid thermocyclers using the conditions below.

Denaturation	94°C	1 min)	
Annealing	52°C C	1.30 min)	1 cycle
Extension	72°C C	2 min)	
Denaturation	94°C	1 min)	
Annealing	52°C	1.30 min)	30 cycles
Extension	72°C	2 min)	
Denaturation	94°C	1 min)	
Annealing	52°C	1.30 min)	1 cycle
Extension	72°C	10 min)	

The amplified DNA fragments were electrophoresed in a 1.2 % agarose gel stained with ethidium bromide and run at 100 volts for 40 minutes in TAE buffer at pH 8. DNA bands produced by PCR products were then visualized under UV light and photographed using a Polaroid camera.

Results

Of the 130 samples analysed, 74 gave positive and 56 gave negative results. 45 samples out of 74 contained EACMV-Ug, 11 had ACMV while 15 had both ACMV and EACMV-Ug and 3 gave reactions typical of EACMV.

Conclusion

From the analysis it was found that EACMV-Ug incidence is high in most parts of the Western province compared to other virus types, but EACMV-Ug is less common in Nyanza province. This suggests that there is little change from previous diagnostic surveys carried out in previous years, and confirms the slow-down in the rate of progress of the CMD pandemic through south Nyanza province.

Table 1: Occurrence of cassava mosaic geminiviruses in the surveyed districts of Western Kenya

District	Virus type				
	ACMV	EACMV	EACMV -Ug	ACMV+EACMV -Ug	TOTAL
Bungoma	2	0	7	4	13
Busia	1	0	5	4	10
Gucha	1	0	0	0	1
Homa Bay	1	0	0	0	1
Kakamega	0	0	2	0	2
Kisii	0	0	0	0	0
Kisumu	0	0	4	0	4
Kuria	0	0	0	0	0
Migori	0	3	1	0	4
Mumias	0	0	9	1	10
Nyando	0	0	2	1	3
Rachuonyo	5	0	3	2	10
Siaya	0	0	5	3	8
Teso	0	0	5	0	5
Vihiga	1	0	2	0	3
TOTAL	11	3	45	15	74
Percentage	14.9	4.1	60.8	20.2	100

ANNEX 5

Identity and Distribution of Whitefly Vector Variants Associated with Begomovirus Infections of Cassava in Eastern African Countries

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Project Goal and Approach. The goal of this study is to apply molecular methodologies to define the identity and distribution of the whitefly vector associated with cassava-infecting begomoviruses in Eastern Africa. The purpose is to better understand the identity, dynamics, relationships, and specific circumstances surrounding whitefly vector populations in cassava, in relation to the new begomovirus epidemic and previously studied ACMV/EACMV/UGV in cassava.

Objective: We employed a strategy developed in the Arizona laboratory by which *B. tabaci* variants can be differentiated and identified using a PCR-based approach in which the mitochondria cytochrome oxidase I gene fragment, approximately 800 bp in size, is amplified and the DNA sequence is obtained (Brown et al., 1999; Frohlich et al., 1999). This sequence has been found useful for discriminating between *B. tabaci* biological and genetic variants, and for identifying whitefly species and genera by comparative analysis with reference sequences in the AZ laboratory.

Progress to Date

Whitefly collections. Whitefly adults and nymphs were collected from infested plants and placed live in 70-95% alcohol. Samples were received from our collaborator at IITA, James Legg. The mitochondria cytochrome oxidase I gene (COI) was targeted as an informative molecular marker for predicting biogeographic characteristics of *B. tabaci* and related whitefly genera (Frohlich et al., 1999). Prior to PCR, total nucleic acids were extracted from individual female whiteflies as described (Frohlich et al., 1999). Extracts were incubated at 65C for 15 min and 95C for 10 min and centrifuged to pellet debris. The PCR primers used for amplification of the mt COI gene were: C1-J-2195 (5' ttg att ttt tgg tca tcc aga agt 3') and L2-N-3014 (5' tcc aat gca cta atc tgc cat att a 3') obtained from the UBC Insect Mitochondria DNA Primer Oligonucleotide Set, compiled by B. J. Crespi and C. Simon (Simon et al., 1994). PCR products were obtained and cloned into the TA-cloning plasmid pCR2.1 vector (Invitrogen, Carlsbad, CA 92008). The nucleotide sequence for each was obtained using an automated sequencer at the University of Arizona. Sequences were edited using FAKTORY and a master sequence was obtained for each. A phylogeny was reconstructed using Clustal to align nucleotide sequences of approximately 770-800 nucleotides.

Results & Discussion

As in previous analyses (see Progress Reports #1&2), pairwise distance analysis (Clustal, MegAlign, DNASTAR) of mt COI sequences resolved several groups of *B. tabaci* amongst collections from Africa and reference *B. tabaci* from representative hosts and locations, worldwide (total of 166 sequences to date).

In this analysis, New World *B. tabaci* and geographic groups of Old World *B. tabaci* cluster are delimited. Old World *B. tabaci* are resolved as several groups with a strong geographical base. These groups comprise: (Ia) a large subgroup containing non-B type, non-cassava associated *B. tabaci* from African-Mediterranean locations: Ivory Coast from okra, Morocco, Spain (SP), Sudan (SC)], Turkey (TC), and (Ib) a closely related group containing only the B biotype (from many sites throughout the world); (II) most cassava-associated *B. tabaci* from Africa; (III) Uganda from Sweet potato; (IV) Ivory coast from cassava (V) non-cassava associated *B. tabaci* from China, India, Malaysia, Pakistan, and Thailand; (VI) *B. tabaci* from India, Nepal, and Pakistan (cassava and non-cassava); (VII) two outgroup species *Bemisia berbericola* and an unidentified *Bemisia* species from *Jatropha* in India; and (VIII) an two outgroup genera *Trialeurodes vaporariorum* and a related but unidentified genus/species from pepper in Mexico.

B. tabaci collected for the project from cassava in Kenya, Tanzania, and Uganda were found to comprise two large clusters: (a) a putative local, less aggressive genotype, historically associated with typical or pre-epidemic cassava mosaic disease (all three sites), and (b) a putative invasive genotype associated with recent epidemics of severe cassava mosaic disease (Uganda and Kenya). Exemplars from cassava and okra in Ivory Coast, and sweet potato in Uganda (Namulonge) group separately from each other and from cassava-colonizing *B. tabaci* from Kenya, Tanzania, and Uganda. With the IC cassava population, we expect that East and West African *B. tabaci* have diverged due to geographical separation. Likewise, the okra population differs from its local cassava type and clusters with non-cassava colonizing *B. tabaci* from North Africa-Mediterranean group. Separation of the sweet potato population from Uganda from cassava-colonizers from the same locale is probably due to host-specialization, even though they occur in the same locale and might have been expected to have common ancestors.

Here, we have added sequences for two collections from the Congo and fourteen from Rwanda. Congo and Rwanda *B. tabaci* cluster entirely with the 'local' genotype from Kenya, Tanzania, and Uganda. No evidence of the invader' genotype was demonstrated amongst these collections. As noted before, two distinct populations termed the 'invader' and 'local' genotypes have been documented in cassava throughout Uganda, and more recently, in Kenya. Although severe disease symptoms are noted in all areas from which collections have been made, virus identification has not been completed, so it is not yet possible to determine if a tight relationship exists between the severe cassava disease and the two main cassava-colonizing genotypes, as we hypothesized in Uganda.

Conclusions are as follows:

1. Cassava-colonizing populations in Uganda from behind (BH), at the front (FR), or ahead of the front (AH), and those from Kenya and Tanzania (and African exemplars included for comparison) originated from the African continent.
2. Cassava-colonizing populations in Uganda from behind, at, or ahead of the front are not the notorious 'B biotype' of *B. tabaci*. To date, within Africa, the 'B' biotype has been found only in South Africa (ornamentals and tomato).
3. Most collections (*see 4 below) from cassava in Uganda and Kenya (except one) designated as 'BH' or 'FR' of the epidemics form one large, distinct group and is thought to constitute the invading population. This invasive type may have displaced the 'local' or 'indigenous' *B. tabaci* that were present prior to the epidemic. Several collections labeled AH, and expected to represent the 'local' or indigenous whiteflies, were also placed in this group. This probably indicates that 'invasion' had not yet been documented at the time the collection was made from the site: Bukoba, I Zone, or, may provide evidence that the local population was not entirely displaced at the time of the collection.
4. The majority of collections from Uganda from 'ahead of the front' (AH), and hypothesized to represent the "less-invasive, indigenous" population from cassava prior to the UGV epidemic (1994) are placed in a distinct group. All collections from epidemic Tanzania (20) collected in 1999-2000 and one from Kenya collected in 1999-2000 also fall into this group. It is not clear why the (putative) indigenous *B. tabaci* from Tanzania is the only vector genotype detected in epidemic-afflicted areas in 1999-2000. Members of this latter group share only ~80% sequence identity (or less) with the group containing BH and FR collections (invader), indicating the two groups are divergent.
5. Congo and Rwanda collections are most closely related to the 'indigenous' or local less-aggressive genotype of *B. tabaci*.
6. 'Non-cassava' collections (sister taxa) from Africa (Uganda and Ivory coast on sweet potato and one from a weed in Benin (West Africa), i.e. are more slightly more divergent from the two cassava colonizing populations (BH/FR and AH) than are the two cassava groups (putatively, *invader* and *indigenous*) examined to date.