TRACKING THE SPREAD OF THE 'UGANDA VARIANT' EAST AFRICA CASSAVA MOSAIC VIRUS - UGANDA (EACMV-UG2) IN EAST AND CENTRAL AFRICA USING NUCLEIC ACID-BASED TECHNIQUES

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Cassava mosaic disease

Cassava mosaic disease (CMD) has been known on the African continent for more than a century although its impact on production of casssava is greater than ever. Following the outbreak of an epidemic associated with a novel recombinant virus designated *East Africa cassava mosaic virus*- Uganda (EACMV-UG2) in Uganda, EACMV-UG2 has spread to neighbouring countries. The spread of this virus poses a threat to cassava production in many countries. Urgent measures are required to address this regional threat and monitoring spread of the disease through extensive surveys plays a key role in this management strategy.

Sample collection and DNA Extraction

Fig 1. Youngest leaf showing CMD symptoms picked

Fig 2. Total DNA is extraction *in situ*

Step1. Fresh samples are collected from the top-most tender leaves showing CMD symptoms during regular surveys
Step 2. Fresh samples are put in tubes and stored in a cool box
Step 3. Geographical position where sample is collected noted using a GPS
Step 4. Total DNA is extracted using a portable kit using standard procedures (Fig. 2)

Findings

There is rapid spread of EACMV-UG2 into northwestern Tanzania (>400 km in 5 years) whilst spread is much slower through western Kenya (<100 km in 5 years). New spread of the Uganda variant into northeastern Burundi and eastern Gabon has been shown, with most EACMV-UG2 diagnoses associated with whitefly-borne infections. This represents an increase in the area covered by EACMV-UG2 and significant changes in the CMG situation.

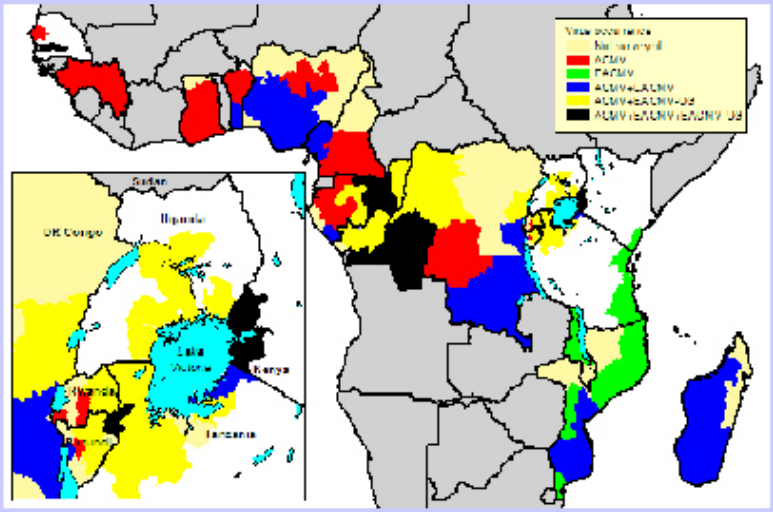

Fig. 3. Cassava mosaic geminivirus distribution-2003

CMG distribution maps

Virus diagnoses together with the corresponding GPS data are used to generate CMG distribution maps.

Conclusions

EACMV-UG2 has been detected in new areas in the cassava belt. Additionally, vital infirmation has been generated, which is important in aiding the targeting of control measures. This highlights a clear need to develop control strategies. This way, the impact of further spread of severe disease to important cassava-growing areas will be reduced.

Acknowlegments

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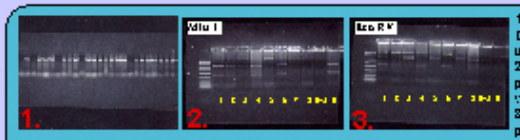
Restriction Fragment Length Polymorphism (RFLP)

Fig. 4. Nucleic acid-based diagnotics in the laboratory

1. Run PCR using Universal primers for cassava geminviruses detection.
2. Purify PCR product and dissolve in water to recover it
3. Restrict full length DNA A PCR product with endonucleases *Mlu*I and *Eco*RV
4. Digested DNA products are run on agarose electrophoresis gels
5. Stained fragments visualized with transilluminating UV light and gels recorded using a Polaroid camera
6. Interpretation of results based on fragment lengths

PCR-based Virus Diagnostics



1. Amplification of full length DNA 'A' of CMGs using universal primers
2. DNA 'A' restriction digest products following incubation with *Mlu*I
3. DNA 'A' *Eco*RV digestion products
ACMV: 6, 7, 8 EACMV-Ug: 4, 5 ACMV+EACMV-Ug: 1, 2, 3