Sub-theme:

Pest diagnostics in phytosanitary systems

Assessment and Detection of Maize Chlorotic Mottle Virus Stability in Maize Seeds in Kenya

Presented by:

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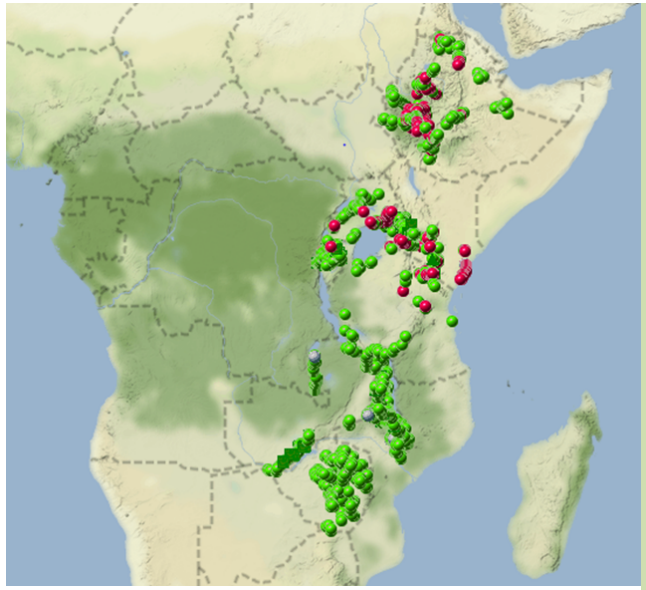
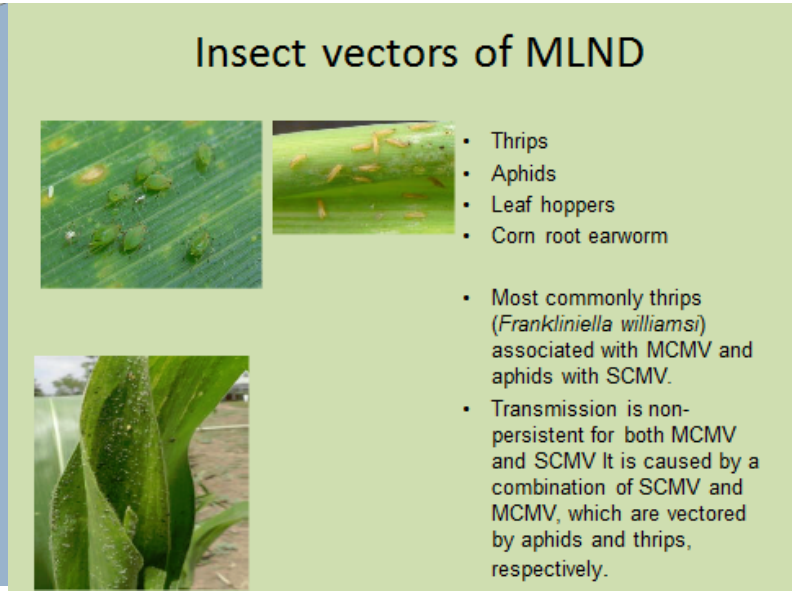
Introduction

- **Maize: Global Status**
- 184 million hectares worldwide; production 1016 MMT.
- >160 countries grow maize; ~125 of these in developing world.
- Maize as a food crop accounts for 73% and 64% of the total demand in ESA and WCA, respectively.
- Maize accounts for 15 to 56%of total calories intake of people in the developing countries; staple food to 900 million poor people who survive on <1-2 USD per day.

Introduction cont'

- **Maize lethal necrotic disease**
- Maize lethal necrosis disease (MLND) is an important constraint in maize production in sub-Sahara Africa that threatens food security and poses challenge in trade.
- It was first reported in Kenya in 2011 and has since spread to other neighbouring countries.





Problem Statement

- There has been concerted effort from different stakeholders to develop effective MLND management methods in Kenya.
- The challenge in provision of MLND free seeds has been the process of cleaning already affected seed lots.
- There exist a possibility of some seed lots being unfairly condemned basing on unreliable diagnostic methods;

Justification

- Maize seed production has become a challenge for merchants since other than the usual requirements, the number of field inspections has been increased by KEPHIS including mandatory lab testing.
- There is scanty information regarding effect of storage time and inactivation temperature for MCMV.
- Better management of MLND with a reduction in wastages due to MLND.

Objectives

- **General objective:** To contribute to better diagnosis of MCMV and reduce its spread through infected maize seeds as well as salvage already infected seed lots for improved nutrition and livelihood.

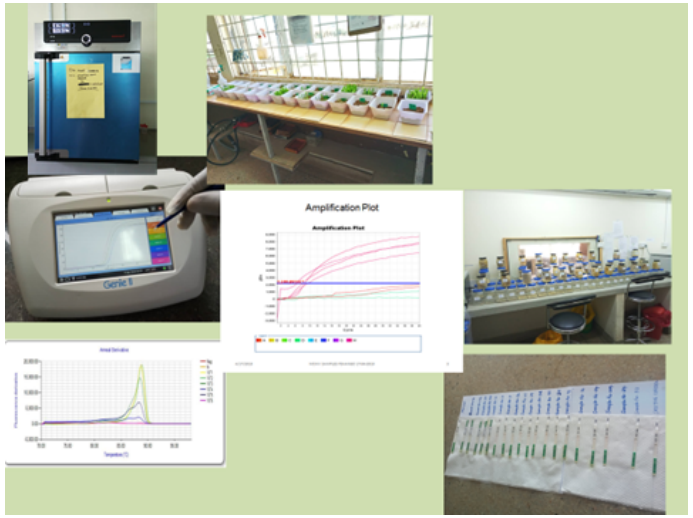
Specific objectives:

- To assess the longevity of MCMV virus in stored maize seeds.
- To determine the inactivation temperature for MCMV in infected maize seeds.

Methodology

Study area:

- This work was done at the KEPHIS Nakuru, Molecular testing Lab.
- All samples used were sourced from the submitted samples at the laboratory whose MCMV status had been established by RT-PCR.
- A total of 37 samples were selected from the remnant samples stored in the cold room at 20 ^{o}C.



Methodology cont'

Thermal inactivation of MCMV

- Infected maize seeds were subjected to different levels of heat in incubation chambers for virus inactivation.
- A working sample of 400 seeds comprising of 4 replicates of 100 seeds each were used.
- Each sample was replicated thrice where 400 seeds of each sample was incubated at 20, 30, and 40 ^{o}C for a period of one month.
- The seeds were then planted and after germination, RNA extraction was done followed by rtPCR.

Methodology cont’

- **Assessment of longevity of MCMV virus in stored seed**38 samples that had been tested and found positive previously were tested by real time PCR at different dates.
- These samples were kept at room 20 ^{o}C before being retrieved and planted for germination.
- Four hundred (400) seedlings per sample were harvested into a sampling bag before RNA was extracted by CTAB and real time PCR done on each sample.
- All the 400 leaves plucked on each seedling were mixed in one tube to make a composite sample which will be tested as one sample.

Methodology cont’

Inactivation of the virus by heat treatment.

- CQ/CT values were recorded for each real Time PCR test done on seeds incubated at different temperature. Germination results was recorded after evaluation of the germination test. Number of samples that tested positive using immunostrip kit were recorded.

Methodology cont’

Assessment of longevity of MCMV virus in stored seed

- CT Values readings was recorded at different intervals when the samples were tested.
- Readings was done after 12 month of sample storage and testing.

Results

Sources of variation	df	CT Threshold	CT Mean
Sample	37	203.66***	143.91***
Rep	1	15.64	0.19
Year	2	2697.64***	2740.73***
Error		45.63	35.23
CV		24.15	22.60
R2		0.67	0.71

Results cont': Means of Longevity of MCMV in infected maize seeds tested in different years

Year	Difference in Means	95% confidence Limits
2019 - 2018	12.880	15.228 ***
2019-2017	14.754	17.025***
2018-2019	-12.880	-10.533 ***
2018-2017	1.873	3.972
2017-2019	-14.754	-12.482 ***
2017-2018	-1.8773	- 0.226

Results cont': **Table 3: Inactivation temperature for MCMV in infected maize seeds(ANOVA)**

Sources of variation	df	CT Threshold	CT Mean
Sample	38	262.46***	259.39***
Temperature	2	2211.75***	2221.79***
Error		42.19	39.67
CV		23.59	22.97
R2		0.65	0.67

Results cont': Table 3: Means of thermoinactivation

Temp	CT Treshold Mean	Standard Error	CT Mean	Standard Error
20	22.73	8.67	22.72	8.50
30	27.73	9.78	27.21	9.55
40	33.22	8.60	33.21	8.56

The higher standard error is due to the sample range, the lowest being 0 to 40 as highest

Conclusion

- MCMV level in maize seed reduced with storage period albeit at slow rate.
- Temperature reduced the viral load in seed.

Recommendations

- More work to determine the viability of heat treated maize seeds.
- Assess the sensitivity of different viral detection technique LAMP, real time PCR and ELISA
- There is need for further research to be done to assess the relationship between the death of seed and the pathogenicity of MCMV
- The exact localization of the virus on the seed also needs to be established.
- There is need to understand the distribution of MCMV in maize seed and the potential for seed treatment to reduce virus transmission through seed.

Acknowledgements

Theme: *"Enhancing Phytosanitary Systems for Healthy Plants, Safe & Sustainable Trade"*

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