Seed certification as a means of curbing emerging diseases: A case study of Maize Lethal Necrosis (MLN) in Kenya

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Abstract: Maize lethal necrosis (MLN) 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 countries in the region. In Kenya, the disease is caused by a combination of Sugarcane mosaic virus (SCMV) and Maize chlorotic mottle virus (MCMV), which are vectored by aphids and thrips, respectively. Maize is the main staple food in Kenya and therefore, with the advent of the MLN, there was need to establish mechanisms aimed at combating the spread of the disease. This resulted in the amendment of seed certification protocol which included testing of seed. In consultation with seed stakeholders, KEPHIS incorporated guidelines for MLN inspection in maize seed certification program where all maize seed crops should be inspected four times including preliminary, first, second and third inspections. A seed sample was taken before seed dressing for laboratory test to ensure the lot is free from MLN. Imported seeds were also tested for MLN before being accepted into the country. Furthermore, there have been concerted efforts by breeders and researchers to develop and screen maize lines for resistance to MLN. Other strategies that have been put in place include control of vectors and use of certified seed which are free from MLN. Amendments in seed certification program that were implemented in Kenya have led to drastic reduction of MLN. It is further hoped that resistant maize lines from the breeding program will further support the effort to manage the disease.

Keywords: Maize, MLN, Resistance, Screening, Seed certification, seed testing

Introduction

Maize lethal necrosis (MLN) is an important constraint in maize production in sub-Sahara Africa that threatens food security and poses challenge in trade. This viral disease is caused by a synergistic interaction of Maize Chlorotic Mottle Virus (MCMV) and Sugarcane Mosaic Virus (SCMV) or other cereal viruses like Maize Dwarf Mosaic Virus (MDMV) or Wheat Streak Mosaic Virus (WSMV). In Kenya, the disease has been reported to be caused by a combination of MCMV and SCMV which are vectored by aphids and thrips, rootworms and leaf beetles (Brandes, *et al.*, 1920). Sugarcane Mosaic Virus is endemic in Kenya but the entry of MCMV in 2011 led to MLND. This new disease has since led to significant reduction in maize yield and in some cases, total loss. It is from this threat that a concerted effort and research aimed at development of diagnostic protocols and revision of certification standards focusing on MCMV was initiated.

Maize chlorotic virus (MCMV) is the only established member of the genus

Machlomovirus in the family *Tombusviridae* (Fauquet *et al.*; 2005) which was first detected in maize from Peru (Castillo and Hebert, 1974).

The disease is more prevalent in places where maize is being grown continuously. All the MLN causing viruses are transmitted individually in the field by their respective vectors, from infected maize plants or other host plants of MCMV and SCMV. MCMV has been reported to survive in maize residues in the field for long (Stanley, *et al.*, 1991). MCMV has also been reported to be either seed-borne or seed-transmitted. There has been no quantitative assessment of seed transmission of MCMV that has been published recently but evidence in Eastern Africa so far indicate that these virus among others can be carried through seed more so where seed production fields had high incidence of MCMV. A small source of inoculum of the virus in the field either from the seed surface contamination or seed-borne would have a severe effect in the entire field when the vectors take up the virus and spread it in wider areas especially where no proper phytosanitary measures have been established to control the vectors. Whether the virus occurs on the outer surface of the maize seed or inside the maize seed, chances of the virus being transmitted to the new generation of crop in the field are high (Stanley *et al.*, 1991). This paper reviews measures that have been developed and implemented to reduce the impact of MLND in maize production in Kenya.

Strategies adopted to mitigate spread of MLN

MLN free seeds production

In Kenya, a concerted effort was initiated by the government through various institutions including both governmental and nongovernmental bodies. Their goal was to find ways of curbing the MLN menace that was threatening maize production. The team included higher learning institutions, research organizations, Ministry of Agriculture and KEPHIS,a regulatory body mandated to protect Kenya's agriculture. Disease free seed production was identified as one of the most viable target to ensure the disease cycle is stopped. This led to amendments in maize seed field inspections guidelines. MLN was given a weight priority in scoring during inspection with a zero per cent tolerance level being adopted for field inspection and laboratory analysis. Seed dressing with different systemic chemicals was also recommended for management of vectors. Seed dressing was strategically scheduled after final laboratory results have been released to minimize loss where the seed lot is tested and found positive as such lots could be used for consumption. On the other hand, rejection during field inspection would enable the seed company to make a decision including using the seed crop for other uses thus reducing losses.

Site selection for maize seed production

It has been difficult to find an MLN free production areas in Kenya though surveillance has not been comprehensively done for the entire country. The idea of disease exclusion has been an effective approach in disease management and this option was applied for MLN. KEPHIS has been training seed growers for site selection to ensure maize seed crop is only planted on farms that do not have history of the disease. Closed seasons for maize growing has also been taken up seriously by many seed merchants. An isolation distance for commercial seed crops has always been considered and this ensures that vectors are not able to spread the disease to the seed crop. The inspectors have to ensure that the neighbouring crops do not harbour the disease. Continuous production of maize mainly in irrigated areas has been observed

to be a source of MLND inoculum hence measures should be taken to encourage closed season. Use of MLND free production areas has been established as a key strategy of ensuring MLND free seeds.

Proper field management practices for curbing MLND

Crop rotation has been an important aspect in site selection for commercial maize seed production. Usually, alternating of maize crop with a non-host species. Legumes are the most important crops that can act as alternative crops for such fields. It is difficult to achieve crop rotation where maize takes long on the farm on vast farms each year like in the North Rift. KEPHIS has been pushing for a maize free period of at least three month during each calendar year per geographical region. This strategy has been successful in areas where it has been accepted. Timely planting at the onset of growing season has also been used to reduce incidences of the disease. Farm machineries where possible should be sterilized since MCSV can adhere to them and persist for long.

MLND seed inspection procedure

Field inspection procedure for maize crop in seed certification protocol was amended to include inspection of the crop four times with MLN being given weight in all inspection stages. Usually, the inspection is done by counting a number of plants depending on the number of hectares under production. Random inspecting of 3000 plants is done (2000 females and 1000 males) in a production area that are between 1-2.5 ha.

Major aspects under consideration during this inspection include off types, diseases and any other parameters. For MLND, a different approach is applied to achieve a zero per cent tolerance at the 3rd and final inspection. No MLND infected plant is accepted in any field lot during final inspection.

First inspection is done one month after planting, where a tolerance of not more than 1% is accepted but with recommendations for rouging infected plants. Second inspection is done six weeks after planting or two weeks after first inspection. At this stage, the crop is almost flowering and de-tasseling and off type rouging underway. MLN infection of more than 0.9% is an automatic rejection. Rouging is recommended at this stage. Second inspection rouging is allowed for 0.9% infection and below.

For 3rd and final inspection MLN tolerance is curbed at one per cent although the tolerance percentage is expected to be lowered to zero per cent at this stage. The 3rd and final inspection is done normally one week after 2nd inspection. During this inspection, whenever MLN is spotted in the field under inspection, an outright rejection is done. The merchant has a right to appeal the outcome of the 3rd and final inspection results. Such an appeal if made should be followed by an inspection by a combined team of experienced inspectors within 48hrs of the appeal. If the results of the appeal are contested again, a seed tribunal or high court is sort to give direction which is a long process. Re-inspection after appeal can lift the first rejection. The crop rejected in the field depending on the age of the crop is not harvested until other seed crops have been harvested. If the crop was rejected at a stage that can be used by the farmer not as seed, it is harvested last after all other neighboring seeds have been harvested and removed from the field. If the crop is still young and leafy, it can be harvested for animal feeds.

Sampling process for Laboratory confirmation for MLND.

Each seed crop after harvesting and shelling is sampled for MLN laboratory confirmatory test. For imported maize seeds, a sample is taken at the port of entry where the seeds are held while awaiting the outcome of the laboratory results.

For locally produced seeds, immediately after shelling and before transportation, KEPHIS inspector takes a sample. A composite sample is collected randomly from the entire crop from which one kilogram of seed is submitted to the laboratory. Labelling of the sample is done by the inspector filling all the sample details in the official KEPHIS SR9 forms before the sample is dispatched to any of the KEPHIS labs for testing.

Laboratory diagnosis of MLND

KEPHIS has equipped three molecular testing laboratories that carry out MLND diagnosis. These are the Molecular laboratory at Plant quarantine and Biosafety Station Muguga, Plant health laboratory at KEPHIS Headquarters and Molecular Testing Laboratory at KEPHIS Nakuru. Plant Quarantine and Biosafety Station molecular laboratory was the first to start the diagnostics of MLN and is the only laboratory testing all imported maize seeds for MLN in Kenya.

Official samples are received in the laboratory accompanied by KEPHIS SR9 forms which contain all the details of the sample. The information on the SR9 is captured in the database and the sample bag coded with an identity number for use in the laboratory. Upon receiving the sample, it is dispatched to the testing area where testing commences immediately. Usually, 400 seeds per sample are planted on sterile media, usually sand. The sample is then incubated at 20 to 30 ^{0}C for at least six days until the sample is at two leaf stage. The sample is then harvested by cutting the tips of each seedling of the sample into a labelled sample bag for crushing. RNA is extracted from the leaf using the modified CTAB technique. Real time PCR is done using primers specific to MCMV. Positive control, blank (EB), and water are used as control in the process of real time PCR. Analysis is done using real-time PCR and results collected based on the CT values of each sample which correspond to viral load in each sample. The laboratory results are communicated back to the merchant and the sampler on the status of the sample.

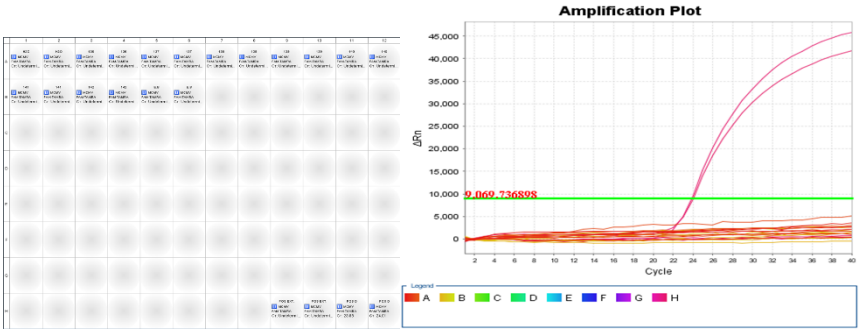

Figure 2: Plate layout and Sample Real time PCR Results for MLND.

Impact of MLND management strategies

Since the emergence of MLND in Kenya and subsequent amendment of Maize seed crop

field inspection guidelines, a total of 569.1Ha has been rejected at field level by KEPHIS inspectors. This rejection ultimately terminated the process of seed production for the affected fields when the crop was still in the field. A total of 408 Ha were rejected due to MLN by KEPHIS inspectors in Central Rift Valley in Kenya alone since 2013 (statement not clear). Most of the seed crops were rejected in areas around Marigat. Rejection was done across many varieties that were planted in this region, many of the lots being Certified 1st Generation class. A total of 154.7 Ha of maize seed crops has so far been rejected in the field by KEPHIS inspectors in The North Rift area in Kenya since 2013. This is the region where major maize production is done in Kenya and has not been affected much compared to the Central Rift.

In Embu region, 4.5Ha has since been rejected in the field due to MLN between 2013 and 2016. Nairobi region, 1.9Ha of maize seed crop were rejected at field level by KEPHIS inspectors between 2013 and 2016.In Kisumu and Mombasa regions, there has been no rejection done on the ground of MLN so far since 2013. In Mombasa region, seed maize is produced mainly in Bura and Taveta areas (Table 1).

Table 1: Rejection data per year per region

Number	**Region**	**Area rejected in Ha.**
1	North Rift(Kitale Region)	154.7
2	Central Rift (Nakuru Region)	408
3	Embu	4.5
4	Nairobi	1.9
5	Kisumu	0.0
6	Mombasa(Bura % Taveta)	0.0
Total		**569.1**

The status of samples tested for MLND since 2014

Maize samples have continuously been tested in KEPHIS laboratories for detection of MCMV and SCMV. Some of the samples have tested positive. (Table 2).

Table 2: Real Time PCR official laboratory results since 2013

No.	Period	No. of samples received	Negative samples	Positive samples	% of positive samples
1	2014	1444	1406	38	2.6
2	2015	2088	2040	48	2.3
3	2016	1588	1580	8	0.5
4	2017	1877	1869	8	0.4
Total		**6997**	**6895**	**102**	**1.5**

Discussion

The results above include samples that were from both local and imported consignments sampled during the period. From the above results, 1.5% of total samples that were tested during the period between 2013 and 2017 tested positive for MLN. Each sample represents a seed crop harvested from a field of not more than 2.5Ha. On average, a seed crop would range between 5000kgs to 40,000kg of maize. An average of 1.5million Kilograms of MLN infected maize was rejected. This amount of seeds plus more others rejected during field inspection together would have provided a huge amount of inoculum for spreading MLN.

Volume of loses to seed merchants and the Kenyan economy at large.

On average, one Hectare of maize crop seed can yield up to 2,940Kgs. From the field rejections above, a total of 569Ha of seed crop have so far been rejected. These translate to 1,672, 860Kgs of maize seed crop that was rejected in the field. Normally, for small scale farmers, the seed companies package their maize seed in 2kg packet. A two kilogram packet of maize seed retails at an average price of US $ 3.6. Working with the above figures projects to seed companies having lost up to US $ 3,011,148 from the field rejections alone. Calculations from the rejections done at the laboratory level, the 1.5M Kg of seeds that were rejected could have fetched up to US Dollars 2,700 000. In total on average, seed companies have lost more than US Dollars 5,711,148 excluding losses in production costs.

Conclusion

Embracing the idea of disease exclusion has been and is still the best approach in disease management. Significant success has been made in management of MLN in Kenya through the amendment of seed certification guidelines. Laboratory testing plays an important role in management of disease especially where many seed lots that were passed at field level failed at the laboratory stage. The overall multiplier effect of a single infected plant in a seed crop field is generally large when not controlled. Striving to ensure high quality of agricultural input, if well implemented can ultimately help in ensuring food security globally. MLN is still an important disease in Maize that still requires a keen eye especially in irrigated field where there are no closed seasons for maize.

Recommendations

Evaluations for new maize varieties for resistance or tolerance to MLN should be augmented as this will go a long way in management of MLN. Seed certification measures that have led to the reduction of the disease should be entrenched in the seed laws and implemented fully. Irrigation schemes and other seed growers should be guided to implement closed maize season to reduce disease inoculum. Phytosanitary measures should be observed strictly even for seed material to exclude MLN from countries where it is not known to occur. Cross border trade can also be monitored especially for MLN to ensure the disease is managed. National laboratories should be equipped to produce more efficient and accurate results.

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