Seed Certification as a Means of Curbing Emerging Diseases: A Case Study of Maize Lethal Necrosis Disease in Kenya

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ABSTRACT

Aim: To evaluate effectiveness of the measures that was adopted by KEPHIS through seed certification process and establishment of volumes as well as percentage of rejections at different levels in the certification chain.

Materials and Methods: A combination of Sugarcane mosaic virus (SCMV) and Maize chlorotic mottle virus (MCMV), which are vectored by aphids and thrips, respectively. Seed samples were taken before seed dressing for laboratory test to ensure the lot is free from MLND. Imported seeds were also tested for MLND 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 MLND.

Results: Maize is the main staple food in Kenya and it resulted in the amendment of seed certification protocol which included testing of seed. In consultation with seed stakeholders, KEPHIS incorporated guidelines for MLND inspection in maize seed certification program. Other strategies that have been put in place include control of vectors and use of certified seed which are free from MLND.

Conclusion: Amendments in seed certification program that were implemented in Kenya have led to drastic reduction of MLND. It is further hoped that resistant maize lines from the breeding program will further support the effort to manage the disease.

Keywords: Maize, MLND, Resistance, Screening, Seed certification, Seed testing.

Introduction

Maize is the most important cereal crop in both Sub-Saharan Africa and Latin America. It is an important staple food for more than 1.2 billion people in Sub Sahara Africa. All parts of the plant can be used for food and non-food products. In industrialized countries, maize is largely used as livestock feed and as a raw material for industrial products. It had been estimated that 158 million hectares of maize are harvested worldwide of which 29 million hectares are harvested in Africa, with Nigeria, the largest producer in SSA, harvesting 3%, followed by Tanzania. Maize is widely grown in Kenya and ranks highly in food security, meeting dietary preferences of many communities [1].

Although many production constraints have been reported on Maize, MLND has emerged as an important constraint in maize production in sub-Sahara Africa that threatens food security and poses challenge in trade [2]. The 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) [3]. In Kenya, the disease has been reported to be caused by a combination of MCMV and SCMV although the two viruses independently are capable of causing a significant damage. Sugarcane Mosaic Virus has been in Kenya for a long time thus has not been a major threat in maize production. The entry of MCMV in 2011 has been the source of major threat to maize farming [4]. Maize chlorotic virus (MCMV) is the only established member of the genus Machlomo virus in the family Tombusviridae [1] which was first established in maize from Peru [5].

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Mlnd Transmission

MLND which is a combination of Sugarcane mosaic virus (SCMV) and Maize chlorotic mottle virus (MCMV), are vectored by aphids and thrips, rootworms and leaf beetles, respectively. Severe infection has been reported in areas where maize is grown continuously due to continuous availability of inoculum. The build-up of vector that transmits the virus is also evident during continuous cropping. MCMV has been reported to survive in maize residues in the field for long [6]. MCMV has also been reported to be either seed-borne where seed produced from infected plant may carry the virus or seed-transmitted. Although a seed transmission rate of 0.3% has been reported [6,7]. The effect is severely amplified when the small inoculum is taken up by vectors in the field. 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 will have a severe effect in the entire field when the vectors takes up the virus and spread the virus in wider areas especially where no proper phytosanitary measures has 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 stand high [8].

Mlnd Free Seeds Production

In Kenya, a concerted effort was initiated by the government through various institutions including both governmental and non-governmental bodies. Their target was to find ways of curbing the MLND menace that was threatening maize production. The team involved included higher learning institutions, research organizations, Ministry of Agriculture and KEPHIS. Among many strategies that were adopted, disease free seed production was taken up as one of the most viable target to ensure the disease cycle interrupted. This led to amendments in maize seed field inspections guidelines. MLND was given a higher weight and priority in scoring during inspection with a 0% tolerance level being adopted for field and laboratory stages [9]. Seed dressing with appropriate systemic insecticides was also recommended for the control of vectors. Seed dressing was strategically scheduled to be done after final laboratory results have been released so as to minimize loss where the seed lot is tested and found positive. Such lots were recommended for milling to reduce the losses incurred by the seed companies. Rejection during field inspection would enable the seed company to make a decision on whether the seed crop could be converted to commercial crop or even the maize could be sold for consumption while still green in the field.

KEPHIS has been utilising these strategies to ensure MLND free seeds are availed to the market for both imported and locally produced seeds. Since as a general rule, the quality of your input ultimately determines the final yield, planting healthy, certified and treated seeds is generally the first step in production of healthy crop [10]. When MCMV virus is introduced through seed to an area where it is has not been previously known to occur, the insect vectors immediately picks up the small inoculum and spread the virus rapidly if the virus is not diagnosed and infected crops rogued in time. In the long run, the virus will establish itself and management at later stages becomes difficult to achieve. In order to curb this menace, KEPHIS has from its mission of ensuring quality agricultural input strived to ensure that all maize seeds passes through rigorous tests to ensure the final quality that will reach the farmers is of high quality and disease free [8]. The major objective of this work was to ascertain whether the measures that were adopted by KEPHIS through seed certification process are effective. The volumes as well as percentage of rejections at different levels in the certification chain were also established.

Materials and Methods

This work has mainly relied on the general operations carried out in KEPHIS especially Nakuru Regional office to curb the spread of MLND. Activities were ranging from site selection, seed treatment, field inspections and laboratory testing. Most of the field activities observed during this study were in Central Rift Valley Kenya. Laboratory samples were taken from both field samples and also from import consignments at the port of entry.

Mlnd Seed Sampling Procedure

Field inspection of maize crop after amendments of seed certification protocol is done three times. For 1 to 2.5 Hectares, 2000 maize plants are counted;. At final level inspection, MLND infected plant is not supposed to be present in the field during 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, de-tasseling and off type rouging underway. MLND 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 MLND 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 MLND is spotted in the field under inspection, an outright rejection is done, tolerance is pegged at zero percent.

Sampling Process for Laboratory Confirmation for Mlnd

Each seed crop after harvesting and shelling was sampled for MLND laboratory confirmatory test. For imported maize seeds, a sample was 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, an inspector took a sample using a sampling probe. Labelling of the sample was done by the inspector filling all the sample details in the official KEPHIS SR9 forms before the sample being dispatched to the KEPHIS Molecular laboratory 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, KEPHIS headquarter molecular laboratory and Molecular Testing Laboratory at KEPHIS Nakuru. Official samples are received accompanied by KEPHIS FormSR9 forms which contain all the details of the sample. The information on the Form SR9 was captured in the database and the sample bags coded with an identity number for use in the laboratory. Four hundred seeds per sample were planted in sterile media (Current ISTA Rules 2018) and incubated at 20^0C to 30 ^{0}C for seven days until the sample was at two leaf stage. The sample was then harvested by cutting the tips of each seedling of the sample into a labelled sample bag for crushing. RNA was then extracted from the leaf using modified CTAB method. Positive control, blank (EB), and water were used as control in the process of real time PCR. The primers indicated in Table (1) were used.

Table 1. Primers for MCMV and SCMV

MCMV-F	5' – CCG GTC TAC CCG AGG TAG AAA - 3'
MCMV-R	5' – TGG CTC GAA TAG CTC TGGB ATT T- 3'
MCMV-P	5'– CAG CGC GGA CGT AGC GTG GA- 3'
SCMV-F	5' – CCA GGC CAA CTT GTA ACA AAG C- 3'
SCMV-R	5' –CAT CAT GTG TGG ATA AAT ACA GTT GAA- 3'
SCMV-P	5' -– TGT CGT TAA AGG CCC ATG TCC GCA '

The results were collected basing on the CT Values of each sample which corresponded to the viral load in each sample. The laboratory results were communicated back to the merchant and the sampler advising whether to process or reject the seed lots. The crop rejected in the field depending on the age of the crop was not harvested until other seed crops have been harvested.

Results

Field Rejections Between 2013 and 2016 in Kenya were evaluated and illustrated in Table 2.

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 and Taveta)	0.0
Total		569.1

Real-time PCR laboratory results since the year 2013 were evaluated and illustrated in Table 3.

No.	Period	No. of MLND samples received	No. of MLND samples Tested Negative	No. of MLND samples Tested Positive
1	2014	1444	1406	38 (2.6%)
2	2015	2088	2040	48(2.3%)
3	2016	784	776	8 (1.02%)
Total		4316	4222	91 (2.1%)

Discussion

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, 860 Kgs 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 shows that 1.5M Kg of seeds that were rejected could otherwise have fetched up to US Dollars 2,700 000. In total on average, seed companies lost more than US Dollars 5,711,148this amount is excluding losses incurred from other cost of production that were already invested by this seed companies during the process of this seed production to the levels at which rejections were done [10].

On combating MLND, the results above include samples that were from both local and imported consignments sampled during the period. From the above results, 2.3%of total samples that were tested during the period between 2013 and 2015 tested positive for MLND. 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 MLND infected maize seed were prevented from reaching the farmers in Kenya during that period. This amount of seeds plus more others rejected during field inspection together would have provided a huge amount of inoculums for spreading MLND [11]. This would have been a big blow to the fight against MLND especially where the seeds would have been purchased and planted in many parts of the country.

It had been had no positive samples properly due to their geographical isolation from maize growing areas in Kenya [12]. On the other hand, the central Rift has had the highest rejections considering that this is the area where the disease was first reported in Kenya. Furthermore, cultural practice for maize farmers in this region could have immensely contributed to this since small scale farmers had maize on their farms throughout the year. The climate in the central and south Rift could also favour the disease since it is almost evergreen throughout the year. Trend analysis of the above results indicates that the disease severity has been on the decline since 2012 [13].

Conclusion

Embracing the idea of disease exclusion has been and is still the best ever approach in disease management MLND not being an exception. From the data above, a long stride was made in management of MLND 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 approved 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 at ensuring high quality of agricultural input, if well implemented can ultimately help in ensuring food security globally. MLND is still a disease in Maize that still requires monitoring in irrigated field where there is no observation of closed seasons for maize.

Recommendation

Evaluations for new maize varieties for resistance or tolerance to MLND should be augmented in management of MLND. 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 observe closed maize season to reduce disease inoculum. Phytosanitary measures should be observed strictly even for seed material to exclude MLND from countries where it is not known to occur. A full proof of laboratory test for MLND should be demanded at all border points to ensure the disease is managed and spread to disease free countries is controlled. National laboratories

should be equipped to produce more efficient and accurate results.

List of abbreviations

Form SR9: Seed Regulation 9
ISTA: International Seed Testing Association
KEPHIS: Kenya Plant Health Inspectorate Service
MCMV: Maize chlorotic mottle virus
MLN: Maize Lethal Necrosis
MLND: Maize lethal necrosis disease
PCR: Polymerase Chain Reaction
SCMV: Sugarcane mosaic virus

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References

[1] Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA (2005). Virus taxonomy; classification and nomenclature of viruses. Elsevier/Academic Press, Amsterdam

[2] Goldberg KB and Brakke MK (1987). Concentration of maize chlorotic mottle virus in mixed infections with maize dwarf mosaic virus, strain B. Phytopathology 77, 162-167

[3] De Groote H, Doss C, Lyimo SD and Mwangi W (2002). Adoption of Maize Technologies in East Africa – What Happened to Africa's Emerging Maize Revolution? Paper presented at the FASID Forum V, "Green Revolution in Asia and its Transferability to Africa", Tokyo, December 8-10, 2002

[4] Adams IP, Miano DW, Kinyua ZM, Wangai A, Kimani E, Phiri N, Reeder R, Harju V, Glover R, Hany U, Souza-Richards R, Deb Nath P, Nixon T, Fox A, Barnes A, Smith J, Skelton A, Thwaites R, Mumford R and Boonham N (2013). Use of next-generation sequencing for the identification and characterization of Maize chlorotic mottle virus and Sugarcane mosaic virus causing maize lethal necrosis in Kenya. Plant Pathology 62, 741-749.

[5] Elizabeth Nambiro, Hugo De Groote and Willis Oluoch K'osura (2001). Market structure and conduct of the hybrid maize seed industry, a case study of the trans nzoia district in western kenya. Seventh Eastern and Southern Africa Regional Maize Conference11 – 15th February. pp. 474-479.

[6] ISTA Rules 2017; Current version; CH-8303 Bassersdorf, Switzerland

[7] Subramanya Sastry K (2013). Seed-borne Plant Virus Diseases.pp 307-08.

[8] Sastry KS (2013). Methods of combating seed-Transmitted Virus Disease Pg 186.

[9] Kumari S and Makkouk K (1996). Inactivation of broad bean stain comovirus in lentil seeds by dry heat treatment. Phytopathologia Mediterranea, 35(2), 124-126.

[10] Morales FJ, Arroyave JA, Catillo J and Leon CD (1999). Cytopathology of Maize chlorotic mottle virus in Zea mays L. Maydica 44, 231-235Scot Nelson, James Brewbaker, and John Hu; 2011; Maize Chlorotic Mottle. Journal paper 79 of College of Tropical AGRICULTURE AND Human Resources, University of Hawai'.

[11] Redinbaugh MG and Stewart LR (2018). Maize Lethal Necrosis: An Emerging, Synergistic Viral Disease. 5:301-322

[12] Jensen SG, Wysong DS, Ball EM and Higley PM (1991). Seed transmission of maize chlorotic mottle virus. Plant Disease. 75(5) :497-498.

[13] Wangai AW, Redinbaugh MG, Kinyua ZM, Miano DW, Leley PK, Kasina M, Mahuku G, Scheets K and Jeffers D (2012). First report of maize chlorotic mottle virus and maize lethal necrosis in Kenya. Plant Disease. 96(10): 1582.

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