



Transmission of Maize lethal necrosis disease causing viruses from crop debris and soil

R. K. NYAKUNDI, D. W. MIANO, D. KILALO and D. MUKUNYA

Department of Plant Science and Crop Protection, University of Nairobi, P. O.Box. 30197, GPO, Nairobi, Kenya

Corresponding author: roseknyakundi@yahoo.com

ABSTRACT

Maize lethal necrosis (MLN) disease, a result of synergistic interaction between Maize chlorotic mottle virus (MCMV) and Sugarcane mosaic virus (SCMV), is a serious threat to maize production in the eastern Africa region. The role of plant debris and contaminated soil in the epidemiology of the disease is important for its management. A greenhouse study was carried out to determine the transmission of the two viruses causing MLN from crop debris and soil to healthy plants. Treatments included Sugarcane mosaic virus (SCMV), Maize chlorotic mottle virus (MCMV), co-infections (SCMV+MCMV), inoculum obtained from MLN-infected plants and healthy plants. Maize varieties used were three hybrids (H614, H513, and Duma43), and two landraces (Kikamba and Kinyanya). The plants were inoculated at three leaf stage with the respective viruses, after two months, plant materials were chopped and incorporated into one set of planting bags while another set had the soil previously holding infected plants but without debris. In the third season, all plant debris were removed from the bags and replanted with same maize varieties to assess if the viruses were still present in the soil. Disease severity was scored on a scale of 1-5 and area under disease progress curve (AUDPC) determined. Viral presence was confirmed using DAS-Elisa. There was no significant difference in infection of plants by viruses either from the soil with debris or with contaminated soil alone, although treatments with combination of the two viruses had higher levels of infection. However, the landraces recorded high disease incidences, severity and AUDPC for most of the treatments in comparison to the hybrids. On the Elisa test results, 58.3% of the samples tested positive for MCMV while on the subsequent planting 28.3% were positive. None of the samples were positive for SCMV. This demonstrates that MCMV can be easily acquired from the soil with or without debris so long as there was infection before. Hence field hygiene and crop rotation will help in reducing the recurrence of the disease.

Key word: AUDPC, coinfections, Das-Elisa, maize lethal necrosis, MCMV, SCMV

RÉSUMÉ

La maladie de la nécrose létale du maïs (NLM), résultat d'une interaction synergique entre le virus de la marbrure chlorotique du maïs (VMCM) et le virus de la mosaïque de la canne à sucre (VMCS) constitue une menace sérieuse pour la production de maïs dans la région de l'Afrique de l'Est. Le rôle des débris végétaux et des sols contaminés dans l'épidémiologie de la maladie est important pour sa gestion. Une étude en serre a été réalisée pour déterminer la transmission des deux virus responsables du NLM à partir des débris de culture et du sol et aux plantes saines. Les traitements comprenaient le virus de la mosaïque de la canne à sucre (VMCS), le virus de la marbrure chlorotique du maïs (VMCM), les co-infections (VMCS +

VMCM), l'inoculum obtenu à partir de plantes infectées par le NLM et de plantes saines. Les variétés de maïs utilisées étaient trois hybrides (H614, H513 et Duma43) et deux variétés locales (Kikamba et Kinyanya). Les plantes ont été inoculées aux trois stades de feuilles avec les virus respectifs. Après deux mois, le matériel végétal a été haché et incorporé dans un ensemble de sacs de plantation tandis qu'un autre ensemble avait le sol contenant auparavant des plantes infectées mais sans débris. Au cours de la troisième saison, tous les débris végétaux ont été retirés des sacs et replantés avec les mêmes variétés de maïs pour évaluer si les virus étaient toujours présents dans le sol. La sévérité de la maladie a été notée sur une échelle de 1 à 5 et l'aire sous la courbe de progression de la maladie (CPM) a été déterminée. La présence virale a été confirmée à l'aide de DAS-Elisa. Il n'y avait pas de différence significative dans l'infection des plantes par des virus provenant du sol avec des débris ou avec un sol contaminé seul, bien que les traitements avec une combinaison des deux virus aient eu des niveaux d'infection plus élevés. Cependant, les variétés locales ont enregistré une incidence, une sévérité et un AUDPC élevés pour la plupart des traitements par rapport aux hybrides. Les résultats du test Elisa montrent que 58,3% des échantillons ont été testés positifs pour VMCM tandis que lors de la plantation suivante, 28,3% étaient positifs. Aucun des échantillons n'était positif pour VMCS. Cela démontre que le VMCM peut être facilement acquis du sol avec ou sans débris tant qu'il y a eu une infection antérieure. Par conséquent, les bonnes conditions d'hygiène des champs et la rotation des cultures aideront à réduire la récurrence de la maladie.

Mot clé: CPM, coinfections, Das-Elisa, nécrose létale du maïs, VMCM, VMCS

INTRODUCTION

Maize lethal necrosis (MLN) is a complex disease caused by synergistic interaction between Maize chlorotic mottle Machlomovirus (MCMV) and any of the maize infecting potyviruses (Scheets, 1998). The potyviruses involved include Wheat streak mosaic Rymovirus (WSMV), Maize dwarf mosaic Potyvirus (MDMV) and Sugarcane mosaic virus (SCMV). The disease is widespread all over the world including Peru, USA, Argentina, Mexico and China (Xie *et al.*, 2011). In Kenya, the disease was confirmed to be caused by the synergistic interaction between MCMV and SCMV (Wangai *et al.*, 2012).

In the USA, repeated outbreaks of MLN (also referred to as Corn lethal necrosis, CLN)) was attributed to MCMV surviving in the soil and hence causing disease outbreaks season after season (Uyemoto, 1983). The spread was enhanced by the presence of maize rootworm (Uyemoto, 1983). The virus can overwinter and

survive in ploughed-in maize stubble and maize residues in the absence of maize (Montenegro and Castillo, 1996). Experiments to demonstrate the efficiency of crop rotation confirmed that disease incidents were high in plots that had maize the previous year while those that had other crops remained free of the disease. This is a clear demonstration that the virus overwinters in the soil and plant debris (Uyemoto, 1983).

Sugarcane mosaic virus (SCMV) has been isolated from most parts of infected plants (Jiang *et al.*, 1992). These include leaf, stem, roots, cob, seed, sheath tissues, kernel, anther, husks and silk. The virus was also detected in immature kernel, root and terminal leaf tissues of dry eared plants (Jiang *et al.*, 1992). In studies done to confirm soil transmission of the virus, non inoculated sorghum plants became infected with the SCMV when grown in containers with infected plants, indicating the possibility of soil transmission (Bond and Pirone, 1970). The

scenario in Kenya since the outbreak of MLN disease is similar to that observed in the USA where there was an outbreak after every season of maize planting. For proper management of this disease, it is important to determine the role of the soil and plant debris in continuous cropping systems to the disease outbreak. This study aimed at unraveling the role of the debris and contaminated soil to the outbreaks experienced in Kenya.

MATERIALS AND METHODS

Source of plant materials, virus and inoculations. Maize crop was used for the experiments which ran from November, 2013 to September, 2016. All experiments were carried out in a screenhouse for two seasons at the University of Nairobi, Kabete Campus field station. Five maize genotypes H614, H513, Duma43, Kikamba and Kinyanya were used. These were grown in different Agro Ecological Zones (AEZ) with the first three representing hybrids while the last two were landraces. The maize genotypes were acquired from the University of Nairobi, Kabete Campus field station seed store. The seeds had been in the store before the MLN disease outbreak and were sown in soil: sand: organic manure mixture at a 2:1:1 ratio, respectively in a 5-litre bag at the rate of two seeds per bag and maintained in a screen-house treated prior to planting with an insecticide (Abamectin + Dynamec). Weekly insecticide spraying was done to control the insect vectors.

Two viruses, Sugarcane mosaic virus (SCMV) and Maize chlorotic mottle virus (MCMV) were used in this experiment either as single or co-infections. In total, there were five treatments; plants inoculated with SCMV alone, plants inoculated with MCMV alone, plants inoculated with a combination of SCMV and MCMV (abbreviated as SCMV+MCMV), and plants inoculated with both viruses from an MLN-infected plant (abbreviated as MLN) and a control (no virus). Each treatment had two bags

of a variety replicated four times. These were arranged in a completely randomized design (CRD).

The viruses were obtained from the Maize lethal necrosis lab at Kenya Agricultural and Livestock Research Organization (KALRO). Viral inoculations were done at 3-4 leaf stage, with only the three upper leaves being inoculated with the viral extract acquired by homogenizing 10g of plant material in 0.01M phosphate buffer with 0.2g of carborandum.

After two months, the experimental plants were chopped into pieces and the debris incorporated into the soil of half of the bags used earlier for planting while the other half remained with the soil alone. The bags were then planted with the same maize varieties planted earlier. Planting was done two weeks after debris incorporation. After six weeks of data collection, the crop was destroyed and the soil re-used for planting the same varieties for the third time. There was no debris incorporation this time round. This constituted the third planting on the same soil.

Disease assessment, data analysis and serological analysis. Disease severity was assessed using a scale of 1 to 5 adopted from KALRO/CIMMYT where 1= no symptoms observed, 2= fine chlorotic streaks on upper leaves, 3= chlorotic mottling throughout the plant, 4= excessive chlorotic mottling and dead heart and 5= complete plant necrosis. The average severity per treatment combination was determined. To further assess the severity of the treatments, the Area under disease progress curve (AUDPC) was determined using the formula $AUDPC = \sum [(0.5) (Y_{i+1} + Y_i) (T_{i+1} + T_i)]$ from Shaner and Finney (1977), where Y=Disease severity score and T=Time (Weeks) of the severity assessment. Percent disease incidence was assessed by using the formula $n/N \times 100$, where; N=Total No. of plants per treatment and n=Total no. of plants with disease symptoms.

All data collected were subjected to Analysis of Variance using Genstat statistical package (Version 12) to determine the effects of the different treatment; differences among the means were separated using the Fischer's Protected LSD test at 5% probability level ($P=0.05$).

The top most leaf samples were collected on the last day of viral scoring and taken through Das-Elisa using Agdia kit and following the procedure provided with the kit.

RESULTS

Initial inoculation of the maize genotypes. After mechanical inoculation, all the varieties recorded more than 90% disease incidence both for single and coinfection (Figure 1). Only the coinfections had significant differences between varieties at $P=0.05$. The coinfections also recorded high severity with the landraces having an average of 4.72 while the hybrids had an average of 4.2 (Figure 2). Conversely, the coinfections had a large AUDPC when compared with the single infections with the landraces having larger areas as compared to the hybrids (Table 1).

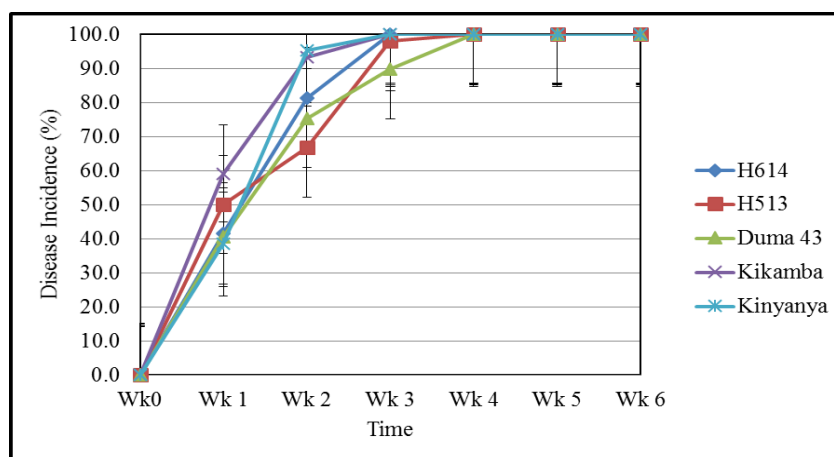


Figure 1. Disease incidence of different maize genotypes due to the infection by a combination of MCMV+SCMV. All the varieties had a 100% disease incidence by week four post inoculation. (Wk=Weeks after inoculation)

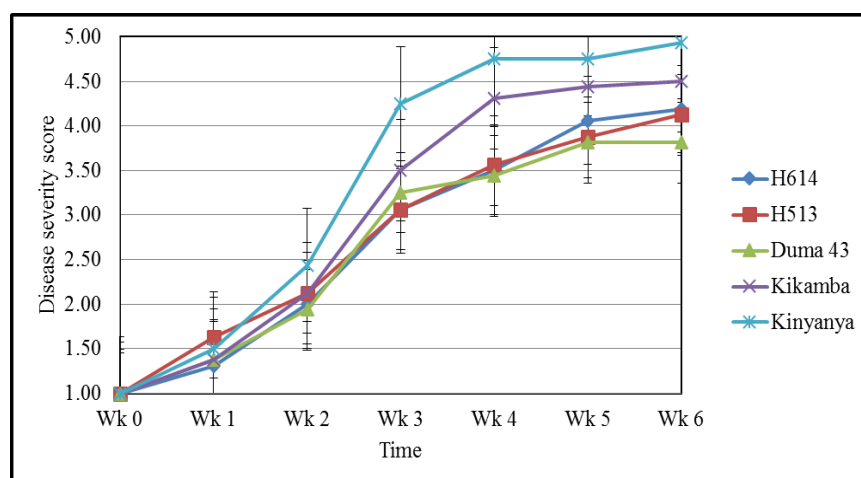


Figure 2. Disease severity on different maize varieties due to infection by a combination of MCMV+SCMV. (Wk=Weeks after inoculation)

Table 1. Area under disease progress curve for the initially inoculated crop, those with debris incorporated and contaminated soil and the third planting in contaminated soil alone

Inoculation	Variety method	SCMV	MCMV	SCMV+ MCMV	MLN	Control (-Ve)	LSD _{p=0.05}	Pvalue
Mechanical	H614	15.00	13.72	17.10	17.05	0.00	2.562	<.001
Mechanical	H513	14.59	13.35	15.59	17.47	0.00	1.632	<.001
Mechanical	Duma 43	14.78	14.22	17.56	17.06	0.00	2.908	<.001
Mechanical	Kikamba	14.84	13.94	21.90	15.28	0.00	2.427	<.001
Mechanical	Kinyanya	15.09	14.72	22.70	20.47	0.00	3.073	<.001
Plant Debris	H614	7.88	8.31	7.63	6.69	0.00	1.52	0.17
Plant Debris	H513	8.00	8.75	7.88	6.88	0.00	1.97	0.26
Plant Debris	Duma 43	7.50	7.50	7.50	6.69	0.00	0.19	<.001
Plant Debris	Kikamba	9.62	8.62	11.69	6.88	0.00	5.83	0.36
Plant Debris	Kinyanya	9.06	7.50	7.50	7.88	0.00	2.39	0.44
Soil (2nd planting)	H614	7.94	7.75	7.63	6.50	0.00	0.49	<.001
Soil (2nd planting)	H513	7.50	7.56	8.06	7.62	0.00	0.96	0.56
Soil (2nd planting)	Duma 43	7.50	7.50	7.81	6.50	0.00	0.50	0.00
Soil (2nd planting)	Kikamba	7.69	7.81	14.44	7.62	0.00	5.13	0.04
Soil (2nd planting)	Kinyanya	8.25	7.56	8.50	8.06	0.00	7.15	0.01
Soil (3rd planting)	H614	8.22	8.06	8.50	8.00	0.00	0.97	<.001
Soil (3rd planting)	H513	7.91	7.84	8.12	8.00	0.00	0.72	<.001
Soil (3rd planting)	Duma 43	8.69	8.28	8.41	8.00	0.00	1.34	<.001
Soil (3rd planting)	Kikamba	8.88	7.72	8.41	8.88	0.00	1.39	<.001
Soil (3rd planting)	Kinyanya	8.22	8.09	8.00	8.62	0.00	1.19	<.001

SCMV= Sugarcane mosaic virus; MCMV= Maize chlorotic mottle virus; MLN= Maize lethal necrosis

Infected plant remains incorporated in soil versus contaminated soil alone. For the SCMV, the landraces showed disease symptoms immediately on emergence while the hybrids exhibited symptoms later. Generally, they had a higher incidence and severity rate than the hybrids. The plants in contaminated soil plus debris generally had more incidence and higher severity than those in contaminated soil alone. However, no significant difference was noted between varieties.

For MCMV, plants in soil with debris showed symptoms earlier than those in soil alone. The symptoms observed included chlorosis and mottling of the leaves. Kikamba had high disease incidence and severity in the soil incorporated with debris. Generally, the maize varieties acquired the viruses almost equally from the soil and debris although Duma 43 showed

no symptomatic plants in soil with debris.

For the co-infection, the landraces showed symptoms immediately after emergence with or without debris. There was general chlorosis which led to necrosis and eventual death of some of the infected plants. Plants in soil with no debris were more diseased compared to those in the soil with debris albeit not significantly different. On disease severity, the same trend was observed where the plants in soil without debris were more affected. Kikamba was more affected than the other varieties and had some of its plants dying from the infection. There was however no significant difference among the varieties. For the MLN, Kikamba had more affected plants than the other varieties and it was significantly different from all the others through weeks 4-6 for the plants in soil without debris. On disease severity, there was no difference noted among

the varieties, however those in the soil without debris recorded more severity when compared to those in the soil with debris.

Area under disease progress curve. The AUDPC was high in plants coinfecting with both viruses whereby those in soil plus debris and without debris had the same area (Table 1). Plants in the SCMV infected soil and debris had the second highest symptoms (although they were negative on serological test) and closely followed by those in the MCMV infected soil and debris. Plants in soil and debris infected with MLN coinfection had the least area (Table 1).

Assessing the AUDPC for the five varieties used in the experiment, Kikamba without debris had a large area under disease followed closely by Kikamba with debris. Duma 43 had the least AUDPC. Differences were also recorded in Duma 43 with and without debris, H614 without debris and Kikamba without debris. In the first three scenarios, MLN was significantly different from the rest while in the last case, the combination was different from the rest of the treatments ($P=0.05$).

Confirmation of the Maize chlorotic mottle and Sugarcane mosaic viruses through serological tests. In each treatment, two leaf samples were collected for serological analysis, making a total of four samples per variety and 20 samples per each virus treatment. None of the samples for SCMV virus tested positive upon serological analysis while 35 of the 60 samples tested for MCMV were positive with 17 of them from the soil with debris while the other eighteen were from the soil without debris. Duma 43 had a sample from asymptomatic plants testing positive for MCMV (data not shown).

Third planting with no debris incorporated
Disease incidence and symptoms severity. For SCMV, Duma 43 and Kikamba showed symptomatic plants one week after planting. The symptoms included fine chlorotic streaks

on all the leaves. On incidence, Kikamba was significantly different from the rest of the varieties at $P=0.05$ with more affected plants. Generally, the severity on the affected plants was low with most of the plants showing only the fine chlorotic streaks. However, some few plants for Duma 43, Kikamba and Kinyanya recorded severe chlorosis and dead heart.

For MCMV, the varieties started exhibiting symptoms on emergence. By week three all the varieties had plants showing chlorotic mottling and stunting which is characteristic for the MCMV. The landraces recorded higher disease incidence while mild severity was recorded on the affected varieties. There was however no significant difference between the different maize varieties tested.

In the co-infection, few plants for Duma 43 and Kikamba showed disease symptoms on emergency. These included mild mottling and streaks on all the leaves. The plants that showed symptoms late had only their upper young leaves with symptoms while those that showed symptoms one week after planting had all the leaves symptomatic. On the disease severity, Kikamba recorded a higher score. Overall, no difference was noted among the varieties.

For the MLN, only the landraces had plants showing disease symptoms one week after planting. H614 only showed one symptomatic plant in the 5th week of data collection while H513 showed a symptomatic plant only in the last week of data collection. Some differences were noted in weeks 4 and 5 when the incidences for the landraces were significantly higher than those for the hybrids. The disease severity recorded was low for this treatment with Kikamba recording a higher severity score but no significant difference between the varieties.

Area under disease progress curve. The Sugarcane mosaic virus had a large AUDPC as compared to the rest of the treatments, followed

closely by the MLN and the combination respectively, while MCMV had the least area (Table 1). There was however no significant difference between the treatments. On varieties, Kikamba had a large area under disease progress curve followed closely by Duma 43. Kinyanya was third with H513 recording the least.

Confirmation of the presence of the maize chlorotic mottle and sugarcane mosaic viruses through serological tests. Four samples were collected per treatment for serological analysis. This made a total of 20 samples per viral treatment. All samples tested negative for SCMV. However for MCMV, 17 of the 60 samples analysed tested positive for the virus while the rest were negative.

DISCUSSION AND CONCLUSION

Recurrence of Maize lethal necrosis (MLN) disease season after season is a worrying trend threatening food security in Kenya and the African continent at large, as the highest population depends on maize. There is a clear indication that the viruses causing the disease survive either in the soil, the plant debris or in both and most of the varieties planted were susceptible. The rate at which the maize plants are able to acquire the viruses from the soil with or without infected debris formed the core of this work.

When infected plant debris were incorporated into the soil, the plants were able to acquire the viruses one week after planting. All varieties exhibited symptoms related to the two viruses, MCMV and SCMV. The landraces seemed to acquire the viruses more easily since they showed symptoms earlier than the hybrids. There was however no major difference among the varieties whether the soil had debris or not. Plants that were planted in soils previously containing plants coinfecting with the two viruses had the largest AUDPC while at variety level, the landrace Kikamba without debris had a

large AUDPC followed closely with that for the same variety but with debris. Duma 43 had the least area under disease progress curve. Albeit not significant, the addition of debris seem to enhance disease acquisition as was previously demonstrated for MCMV (Uyemoto, 1983). Plants that had acquired the viruses immediately on emergence developed severe chlorosis resulting in necrosis and eventually dead hearts. These plants were generally stunted. This is a clear demonstration that the disease can cause severe damage if it attacks early.

Irrespective of whether the soil is incorporated with the plant debris or not, the viruses can still be acquired from the soil that has not been given a rest to allow for the viruses to degrade. The small roots left behind after harvest and uprooting of the maize plants also play a significant role in the survival of the viruses. As earlier demonstrated (Jiang *et al.*, 1992), both viruses can be found in any part of the maize plant so long as the plant was infected. This is important in planning the disease management strategy. Plant residues have also been demonstrated to play a very crucial role in the survival of the MCMV when the maize plants are off season (Montenegro and Castillo, 1996). In earlier experiments the role of crop rotation was emphasised in managing the outbreaks (Uyemoto, 1983). In the third planting in the infected soil without any debris incorporated, few plants were able to acquire the viruses, an indication of viral load reduction with time.

On the serological analysis of the collected samples however, all the samples for the SCMV tested negative despite the fact that they had very clear symptoms on the plants during data collection. The probable reason could be that the antisera used for this test was of a different strain from the one used for this experiment. There are varied strains of SCMV in East Africa where the Kenyan strain has 87% identity to the Rwandan strain which is 95% related to the SCMV-DMB

strain (Adams *et al.*, 2014). Handling of the samples also during storage could have affected the results. More investigations need to be done to explain the scenario for the SCMV.

The acquired virus on the few plants act as focal points for further spread of the virus. These are the sources of inoculum which is spread to healthy plants mechanically or through the insect vectors. Earlier studies of SCMV in sorghum had shown that the virus can be easily acquired from infected soil or contaminated containers (Bond and Pirone, 1970).

From the above results, it is evident that infected soil and debris are crucial in the survival and spread of the viruses causing MLN disease. Their management is critical in addressing the spread and control of this disease. It is therefore important to put measures in place to ensure maize debris is properly managed and the farmers encouraged to carry out crop rotation to reduce the chances of picking the viruses from the infected soil. The role of few infected plants in the field should also be addressed to avert spread to other non infected plants by vectors and mechanically through the crop management processes. Asymptomatic plants also play a role in reoccurrence of the outbreak as demonstrated by the asymptomatic Duma 43 hence proper field sanitation should be encouraged during the crop production period to avoid unnecessary spread of the disease.

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STATEMENT OF NO-CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this paper.

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