

Research Application Summary

Distribution of maize lethal necrosis disease, its causal viruses and alternative hosts in north and central regions of Tanzania

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Abstract

Maize is an important income generating food crop in Tanzania. However, yields remain low due to several limiting factors including among others diseases caused by fungi and viruses. The threat caused by several biotic factors in the country was further worsened with an outbreak of maize lethal necrosis (MLN) disease in 2012 in Arusha and Mwanza regions of Tanzania. MLN disease is caused by the synergistic interaction between Maize chlorotic mottle virus (MCMV) and Sugarcane mosaic virus (SCMV). Of these two, MCMV is a new virus in Africa, first recognized in an MLN outbreak in Kenya, whereas SCMV is endemic to Africa. This study focused on understanding the extent of MCMV/MLN spread in north and central regions of Tanzania by conducting surveys between February to June, 2015. A total of 163 farmers' fields were sampled in 14 districts in Arusha, Dodoma and Manyara regions. Disease incidence and severity were estimated based on MLN symptoms such as chlorosis, mottling and necrosis. The reverse transcription-polymerase chain reaction (RT-PCR) method was used for the detection of MCMV and SCMV in field samples. Disease severity, assessed on a 1 to 5 rating scale, varied from 2 to 3.6, with an overall mean disease incidence of 16.1%. Thirty nine percent of the samples tested positive to MCMV, 22% for SCMV and 5.5% for both MCMV and SCMV. A total of 254 non-maize crops and weeds tested for MCMV and SCMV revealed SCMV in seven samples (2.8%) in sugarcane (*Saccharum officinarum*), finger millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*) and bristly foxtail (*Setaria verticillata*). The nucleotide sequence of the coat protein region of MCMV showed very high levels of homology (99%) between MCMV from Tanzania and those from Kenya and other countries. However, the SCMV nucleotide sequence of the coat protein region was divergent by up to 11%, compared to other isolates. This study demonstrated wide distribution of MCMV/MLN in the north and central regions of Tanzania and also showed that SCMV occurs in maize as well as other cereal hosts. Further studies on factors influencing disease distribution and occurrence is recommended as interventions for disease management are developed and deployed.

Key words: Disease surveys, maize, maize lethal necrosis, Tanzania, virus detection

Résumé

Le maïs est une importante culture vivrière génératrice de revenus en Tanzanie. Cependant, les rendements demeurent faibles en raison de plusieurs facteurs limitatifs, y compris parmi d'autres maladies causées par des champignons et des virus. La menace causée par plusieurs facteurs biotiques dans le pays a été encore aggravée avec une éclosion de la maladie de nécrose létale mortelle (MLN) en 2012 dans les régions d'Arusha et Mwanza en Tanzanie. La maladie MLN est causée par l'interaction synergique entre le virus de la marbrure chlorée de maïs (MCMV) et le virus de la mosaïque de la canne à sucre (SCMV). Parmi ces deux, MCMV est un nouveau virus en Afrique, d'abord reconnu dans une éclosion de MLN au Kenya, alors que le SCMV est endémique en Afrique. Cette étude a porté sur la compréhension de l'ampleur de la propagation de MCMV / MLN dans les régions du nord et du centre de la Tanzanie en menant des enquêtes entre février et juin 2015. Un total de 163 champs d'agriculteurs ont été échantillonnés dans 14 districts des régions d'Arusha, Dodoma et Manyara. L'incidence et la sévérité de la maladie ont été estimées sur base de symptômes de MLN tels que la chlorose, le marbrillage et la nécrose. La méthode de transcription inverse-réaction en chaîne par polymérase (RT-PCR) a été utilisée pour détecter le MCMV et le SCMV dans des échantillons du terrain. La gravité de la maladie, évaluée sur une échelle de 1 à 5, variait de 2 à 3,6, avec une incidence moyenne globale de la maladie de 16,1%. Trente-neuf pour cent des échantillons ont été testés positifs à MCMV, 22% pour SCMV et 5,5% pour MCMV et SCMV. Un total de 254 cultures différentes du maïs et les mauvaises herbes, testées pour le MCMV et le SCMV, ont révélé le SCMV dans sept échantillons (2,8%) dans la canne à sucre (*Saccharum officinarum*), le mil de mouton (*Eleusine coracana*), le sorgho (*Sorghum bicolor*) et la sétaire brune (*Setaria verticillata*). La séquence nucléotidique de la région de protéine d'enveloppe de MCMV a montré des taux de séquence d'homologie très élevés (99%) entre le MCMV de la Tanzanie et ceux du Kenya et d'autres pays. Cependant, la séquence de nucléotides de SCMV de la région de protéine d'enveloppe était divergente jusqu'à 11%, comparée à d'autres isolats. Cette étude a démontré une large distribution de MCMV / MLN dans les régions du nord et du centre de la Tanzanie et a également montré que SCMV se produit dans le maïs ainsi que d'autres hôtes céréales. Il est recommandé de poursuivre les études sur les facteurs qui influent sur la répartition et l'apparition des maladies, à mesure que les interventions pour la gestion des maladies sont développées et déployées.

Mots clés: Enquêtes sur les maladies, maïs, nécrose létale du maïs, Tanzanie, détection des virus

Introduction

Maize (*Zea mays* L.) is an important and widely grown cereal worldwide after wheat and rice (FAOSTAT, 2010). In East Africa, low maize yields are due to several factors, including diseases caused by fungi and viruses. More recently, maize lethal necrosis (MLN) caused by the synergistic interaction of two co-infecting viruses, Maize chlorotic mottle virus (MCMV) and Sugarcane mosaic virus (SCMV) is negatively

impacting maize production in East Africa (Wangai *et al.*, 2012).

The outbreak of MLN in East Africa was first reported in Kenya in 2011 where it caused up to 100% yield losses (Wangai *et al.*, 2012). By 2012, the disease had spread to Tanzania and Uganda (Makumbi and Wangai, 2013). In Tanzania, the first reports of the disease occurrence emerged from Mwanza and Arusha regions in the north-western and northern parts of the country. Analyses of samples from plants with typical MLN symptoms by CIMMYT and IITA in Mwanza and Arusha regions were serologically positive for MCMV and SCMV, confirming presence of the MLN disease (Makumbi and Wangai *et al.*, 2013). Reports of symptoms similar to MLN affecting maize in Mara, Shinyanga and Manyara in north-western and northern parts, and Singida and Dodoma regions in central Tanzania were also documented (MAFC, 2013). However, the disease causal agents have not been confirmed, nor the extent of their occurrence in Manyara and Dodoma regions. Similarly the incidence and distribution of their causal agents has not yet been determined. There is limited information on incidence and severity in the Arusha region. Further, little is known about alternative hosts to MCMV or SCMV that could potentially play an important role in off-season survival of these viruses. This study was, therefore, undertaken to determine the incidence and distribution of MLN causal viruses in the north and central regions of Tanzania.

Study Description

Diagnostic surveys were conducted during the short rains (February-March 2015) and the long rains (May-June 2015). The surveys covered 163 fields, in 14 districts within the regions of Manyara, Dodoma and Arusha. Surveyed districts occur in three major agro ecological zones. (i) High rainfall zone located at 1500 - 2200 masl, with annual rainfall approximated to be 1200 - 1500 mm. The zone is located in districts of Babati, Mbulu, Karatu and Hanang, (ii) Moderate rainfall zone, with elevated flat areas (900 - 1500 masl) and annual rainfall of 900 - 1100 mm. The zone is located in districts of Meru, Arusha, Kiteto, Kondoa, Simanjiro, Monduli, Kongwa and Chemba. (iii) Low rainfall zone characterized by flat areas and small valleys and annual rainfall of 500 - 800 mm; these areas are in the lowland plains below 900 masl (Nkonya *et al.*, 1998).

The surveys were conducted at 8 - 16 weeks after sowing, at 10 - 30 km intervals along the motorable roads. Disease incidence and severity were assessed visually for the presence or absence of typical MLN-like symptoms along a 'X' transect by counting 15 randomly selected plants at equal distance in each transect. Samples of maize, weeds and other cereals grown within the vicinity of maize for assessing MLN causal agents were obtained. Samples collected were analyzed using RT-PCR using standard procedures (Murray and Thompson, 1980); MCMV and SCMV isolated were selected for sequencing in order to determine the diversity of these viruses in Tanzania. The region used for sequence analysis corresponded to 711 bp for MCMV and 785 bp to 808 bp of the 3' end of the coat protein region for SCMV, which is commonly used for phylogenetic analysis (Mahuku *et al.*, 2015).

Incidence and severity of MLN disease. Results showed the presence of MLN in all three regions surveyed over the two maize growing seasons (Table 1). Dodoma had a low incidence (8.68%), compared to Manyara (28.26%) and Arusha (25.6%) regions. There was no significance difference observed in disease incidences between Arusha and Manyara. Disease severities were significantly different ($p < 0.05$) between Arusha and Dodoma; as well as between Dodoma and Manyara regions. Dodoma had a low severity of 2 compared to Manyara (2.3) and Arusha (2.5) regions.

Alternative hosts

Of the 254 samples of other monocot weeds and crops within and around maize crops, only seven (2.8%) tested positive for SCMV and none of them tested positive to MCMV. Among the tested samples, sugarcane (*Saccharum officinarum*), finger millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*) and bristly foxtails (*Setaria verticillata*) were affected by SCMV.

Table 1: Maize samples tested positive in PCR over two growing seasons in 2015

Region	District	Surveys	No. of Sample	Number positive		
				MCMV	SCMV	MCMV& SCMV
Manyara	Babati	Short rains	35	7(20)	4(11.4)	1(2.8)
	Mbulu		19	8(42)	-	-
Arusha	Meru		26	13(50)	-	-
Dodoma	Mpwapwa		16	4(25)	3(18.8)	-
	Kongwa		11	3(27.3)	-	-
			107	35(32.7)	7(6.5)	1(0.93)
Manyara	Babati	Long rains	21	17(80.9)	8(38.1)	8(38.1)
	Simanjiro		6	2(33.3)	-	-
	Kiteto		7	-	2(28.6)	-
	Hanang		7	5(71.4)	1(14.3)	1(14.3)
	Mbulu		6	4(66.7)	-	-
Arusha	Meru		10	4(40)	-	-
	Karatu		9	3(33.3)	-	-
	Monduli		5	3(60)	-	-
	Arusha_urban		2	1(50)	1(50)	1(50)
Dodoma	Konoda		8	3(37.5)	1(12.5)	-
	Kongwa		6	1(16.7)	3(50)	-
	Chamwino		4	-	-	-
	Mpwapwa		1	-	1(100)	-
	Chemba		1	-	-	-
			93	43(46.2)	15(16.1)	10(10.7)

Values in parenthesis are percentages

Phylogenetic analysis. A set of seven MCMV and twelve SCMV isolates were sequenced; these isolates represent a broad geographic diversity in the surveyed regions. Sequence analysis indicated very high levels of homology (99 to 100%) between Tanzania MCMV isolates and MCMV sequences that are available in the NCBI GenBank Database. It was observed that only two of the SCMV isolates were perfectly (100%) identical. Other isolates differed up to 11% as revealed by the pairwise percent homolog analysis and the divergence analysis.

Discussions

The MLN disease incidence and severity varied from region to region and from district to district, with high rate in the regions of Manyara in Babati districts and Arusha in Meru district and low rate observed in region of Dodoma in Mpwapwa districts. High incidence could be due to the cropping systems in these regions which contribute to vector population growth and virus spread. Farmers in these regions exercise continuous maize production throughout the year due to availability of water for irrigation. This makes early planted maize serve as reservoirs of both virus and vectors. In addition, a number of wild grasses could also serve as virus reservoirs (Shepherd *et al.*, 2010). The moderate level of disease severity observed in this study could be attributed to single infections of either MCMV or SCMV (Pita *et al.*, 2001).

This study has also uniquely identified SCMV as infecting sugarcane, finger millet, sorghum and bristly foxtail in Tanzania. Therefore, this information serves as justification for regular weed management in maize, as an Integrated Pest Management (IPM) option for sustainable control of MLN causing viruses in Tanzania (Nelson *et al.*, 2011). The high degree of homology in coat protein region also indicates that MCMV isolates spread in Africa have common origin. In contrast to MCMV, the SCMV isolates sequenced were divergent by up to 11%. They are also widely distributed compared to MCMV and seem to have wider host range. Greater SCMV diversity suggests presence of virus isolates in the region for much longer period.

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