Detection and Field Management of Plant Viruses and their Vectors (2025): 1-22
Editors: Selvarajan R, Balasubramanian V, Mohanasundaram A, Nagendran K and Gaur RK
Today & Tomorrow's Printers and Publishers-New Delhi (India)

Chapter 1

MAIZE LETHAL NECROSIS (MLN) AND ITS MANAGEMENT

Prasanna B.M.* and Suresh L.M.

CIMMYT (International Maize and Wheat Improvement Center), Nairobi, Kenya.

**Corresponding author: b.m.prasanna@cgiar.org*

ABSTRACT

Maize lethal necrosis (MLN) is a major viral disease caused by a synergistic interaction of Maize Chlorotic Mottle Virus (MCMV) and Sugarcane Mosaic Virus (SCMV) or other potyviruses. The first outbreak of MLN in Kenya in 2011, followed by its rapid spread to several countries in eastern Africa within a span of 3-4 years, caused huge concern to stakeholders across the African continent. Rapid response and intensive multi-disciplinary and multi-institutional efforts by the International Maize and Wheat Improvement Center (CIMMYT) in partnership with an array of national and international institutions resulted in development and deployment of an array of tools/ technologies to effectively tackle the MLN challenge. MLN is still prevalent in eastern Africa and has not been eradicated. The threat of the disease spreading to other regions in sub-Saharan Africa (e.g., southern Africa or West Africa) still looms. Therefore, it is important to continue implementing an integrated disease management approach for sustainable management of the disease in the MLN-prevalent countries whether in Africa, Americas or Asia, and continued efforts on MLN disease monitoring and surveillance globally. This Chapter provides an update on the disease and its management, including the causal viruses, especially MCMV and SCMV, their host range, symptoms, and conditions for development; modes of transmission of MLN-causing viruses; MLN diagnostics and surveillance; and approaches for sustainable management, particularly host plant resistance, MLN-free clean seed production and exchange, and agronomic management.

Keywords: Maize diseases; viral disease; Potyviruses; Disease Management; Host Plant Resistance; Disease Surveillance; Clean Seed Production

1. INTRODUCTION

Maize (*Zea mays* L.) is the most important staple crop in Sub-Saharan Africa (SSA), where more than 85% of the population depends on the crop as a source of food, income, and livelihoods (Prasanna et al. 2020). Maize in SSA covers approximately 40 million hectares, with a production of around 75 million metric tons (FAOStat 2021). However, the average maize yield in SSA (~2 tons/ hectare) is far below the global average maize yield (~5 tons/hectare) due to several reasons, including frequent droughts, poor soil fertility, inadequate input management, and various biotic stresses like pests and diseases (Prasanna et al. 2021).

In recent years, the spread of transboundary pests and diseases has increased, particularly in SSA, due to the changing climates and warming global temperature, affecting food security and the livelihood of several million small-holder farmers in SSA, Asia, and Latin America (Prasanna et al. 2022). Among several transboundary diseases, maize lethal necrosis (MLN) is one of the major examples in eastern Africa. The disease was first reported in the southern Rift Valley of Kenya in 2011 (Wangai et al. 2012) and rapidly spread to several other eastern Africa countries during 2012-2014 (Wangai et al. 2012; Adams et al. 2014; Mahuku et al. 2015a; 2015b; Redinbaugh and Stewart 2018). MLN is a viral disease caused by the co-infection of maize plants with maize chlorotic mottle virus (MCMV; genus Machlomovirus, family Tombusviridae), and any one of several viruses from the family Potyviridae, such as sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV) or wheat streak mosaic virus (WSMV).

MCMV was reported for the first time in eastern Africa in 2012, while SCMV has been prevalent worldwide and for several decades in Africa. Thus, the MLN outbreak in SSA is primarily due to the introduction of MCMV possibly through contaminated seeds (Prasanna et al. 2020; Wangai et al. 2012; Mahuku et al. 2015a; 2015b). In addition to SCMV, a distinct isolate of Johnsongrass mosaic virus (JGMV) was found to occur in the region and could be associated with MLN (Stewart et al. 2017). The epidemiological aspects of MLN, the various modes of transmission, including insect vectors of potyviruses and MCMV, and contaminated seeds as a source of infection (Bernardo et al. 2023; Jensen et al. 1991), and transmission through soil (Redinbaugh and Stewart 2018) could have all contributed to the MLN disease incidence in eastern Africa.

MCMV is the main emerging transboundary virus, which drives the spread of MLN in various geographies. MCMV was first identified in Peru in 1971 (Castillo and Hebert 1974), and subsequently reported in Kansas, USA (Nibblet and Claflin 1978) in 1978. Later, MCMV was reported in several countries across different parts of the world like in Argentina (1982), Mexico (1987), Thailand (1983), Brazil (1983), Mexico (1987), China (2010), Kenya (2011), Kenya (2011), Tanzania (2012), Uganda (2012), Rwanda (2013), D.R. Congo (2014), Ethiopia (2014), Taiwan (2014), South Sudan (2015), Ecuador (2016), and Spain (2016) (Mahuku et al. 2015a; Redinbaugh and Stewart 2018; Prasanna et al. 2020; 2021].

During 2012-2013, the estimated maize yield loss in Kenya due to MLN varied from 23 to 100% (Prasanna et al. 2020). In Kenya, a study conducted by De Groot et al. (2016) revealed that the estimated loss of maize production due to MLN was about 0.5 million tons, with an estimated value of US$180 million in 2013. In Uganda, it was reported that the average yield reduction was 1.4 tons/hectare, estimated at US$332 per hectare (ASARECA 2014; Kagoda et al. 2016). Isabirey and Rwomushana (2016) also indicated high potential yield losses due to MLN in several countries in SSA, including Uganda (81.1%), Tanzania (65.9%), Ethiopia (59.8%), Malawi (53.8%) and Madagascar (45.1%). In eastern Africa, the annual economic impact associated with MLN on smallholders was estimated at about US$ 261 million (Marenya et al. 2018).

The negative impact of MLN was serious, particularly for the smallholder farming communities in eastern Africa. Even seed producers, particularly small- and medium-enterprise companies whose main source of income is maize seed production, were also severely affected, and many have even closed their operations due to huge financial losses. The stringent measures taken by regulatory authorities to reject the MLN-affected seed production fields with zero tolerance made the seed companies to depend on importing the commercial seed produced in non-endemic countries; this not only increased the cost of seeds for the smallholder farmers but also lesser opportunity for the local seed growers.

In 2011, immediately after the first outbreak of MLN in Kenya, its rapid spread to several countries in eastern Africa within 3 to 4 years caused huge concern to stakeholders, including maize-dependent smallholder farmers, researchers, national plant protection authorities, commercial seed sector, etc. across the African continent. As a result of the MLN outbreak in East African countries, a rapid response and multi-pronged approach

with intensive multi-disciplinary and multi-institutional efforts with the cooperation of national and international institutions resulted in the co-development and deployment of various MLN management tools and methods to tackle MLN challenges in SSA effectively (Prasanna et al. 2020; Prasanna 2021a). Until now, MLN has not been reported in maize-growing countries in southern or West Africa. Stopping any further MLN outbreak is an excellent testimony to the various efforts and successful initiatives undertaken during the last ten years to manage the menace of deadly maize disease effectively.

Recent MLN surveillance activities implemented by National Plant Protection Organizations (NPPOs) in eastern and southern Africa (ESA) under the One CGIAR Plant Health initiative showed MLN incidence in eastern Africa, but at a lesser level than earlier. The risk of the disease spreading to other non-endemic countries in SSA still persists. Therefore, sustainable management of MLN is still important in eastern Africa.

2. MAIZE LETHAL NECROSIS: CAUSAL VIRUSES AND DISEASE SYMPTOMS

Different potyviruses including SCMV are more commonly observed in Africa, however, their effect on yield loss is minimal due to improved germplasm with SCMV resistance. Whenever MCMV is prevalent in an area where potyviruses are already present, their combined infection leads to maize lethal necrosis or MLN (Wangai et al. 2012; Adams et al. 2014).

The initial symptoms of MLN start with the development of fine chlorotic specks or mottling of young growing leaves. Further, these chlorotic specs coalesce to produce chlorotic stripes. After 15 days post-infection, the leaves gradually start showing some mottling. Eventually, the whole leaf becomes necrotic (Fig. 1.1). Depending upon the genotype and stage of virus infection, plant growth is stunted in MLN-affected plants. Plants infected at earlier growth stages generally develop more severe (chlorotic/necrotic) symptoms, which can lead to plant death. Death of young leaves in the whorl results in “dead heart”. Plants also develop smaller ears which start drying. Other symptoms include distortion of the male inflorescence, with hard panicles, a short rachis, and few spikelets, and reduced numbers and length of malformed and partially filled ears (Fig. 1.1) (Wangai and Suresh 2021; Prasanna 2021a).

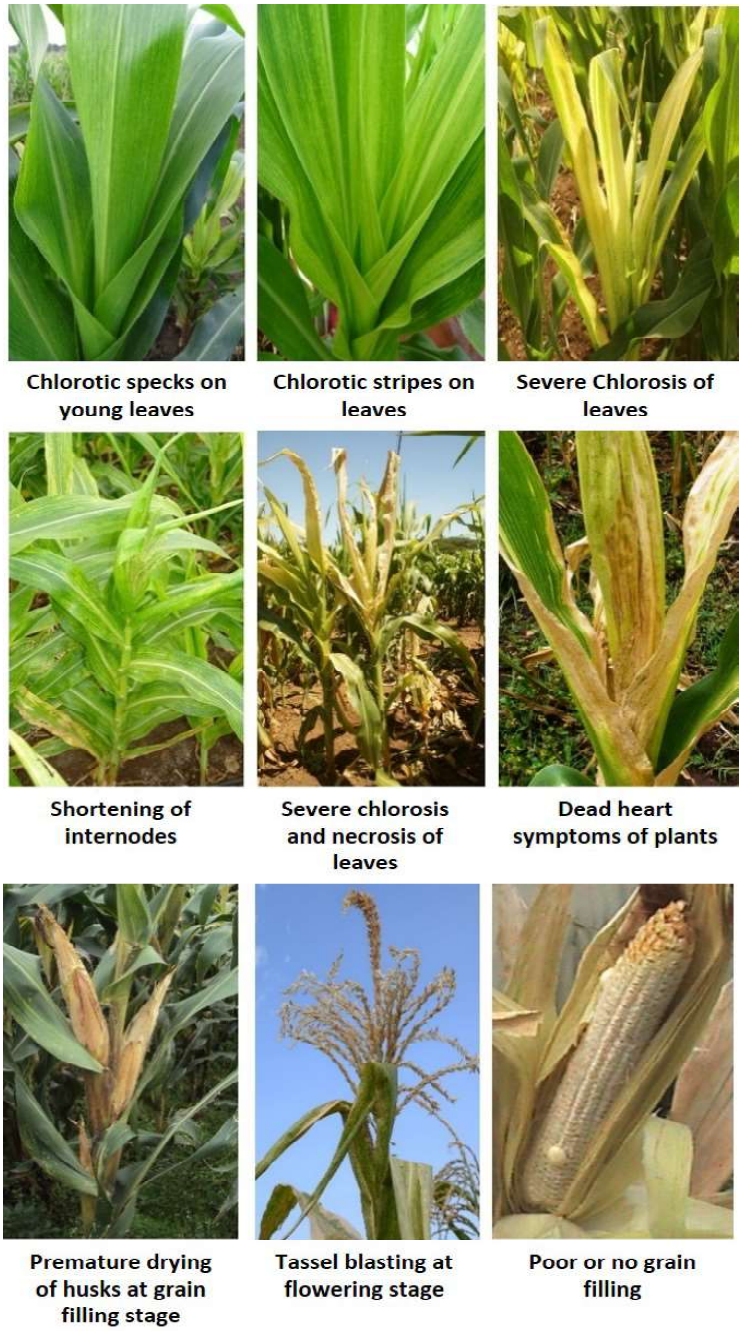

Fig. 1.1 Symptoms of MLN at different stages of maize crop growth

2.1. MAIZE CHLOROTIC MOTTLE VIRUS (MCMV)

2.1.1 Host range

In general pathogens infect several crop species, among them some serve as the main hosts which the pathogen always prefers to infect (causing economic loss), whereas in other hosts, called the "alternate hosts", the pathogen stays for survival particularly in the off-season when the preferred main host species is not available in the field. These alternative hosts serve as a "reservoir" of the pathogen for its survival. For MCMV, crops, weeds, soil, and seed may all serve as reservoirs. Virus reservoirs and vector populations are critical for viral disease to establish and perpetuate. Several factors, mainly crop practices, heavily influence to breakdown this reservoir of viruses. Developing effective disease control measures will require understanding of the relative importance of these reservoirs in disease initiation (Redinbaugh and Stewart 2018).

MCMV affects many host species but is restricted to the grass family (Poaceae). These include wild grasses (e.g., *Digitaria abyssinica*, *Cynodon dactylon*, *Pennisetum clandestinum*, *Digitaria velutina, Cyperus rotundus*, *Brachiaria brizantha*, teosinte *Zea diploperennis*), cereals [*Sorghum bicolor* (sorghum), *Eleusine coracana* (finger millet), *Saccharum officinarum* (sugarcane), *Zea mays* (maize), *Triticum spp*. (wheat), *Pennisetum purpureum* (Pearl millet), *Sorghum halepense* (Johnson grass)], and other grasses (*Andropogon, Bromus*, *Digitaria, Eragrostis*, *Panicum, Setaria*, *Spartina* spp. etc.) (Mahuku et al. 2015a; Kusia et al. 2015). The virus is not known to infect dicotyledonous species (Castillo and Hebert 1974; Niblett and Claflin 1978). Maize is an important natural host of MCMV (Bockelman et al. 1982), but maize genotypes range from highly susceptible to resistant. Experimental host range and alternative host species reported are also restricted to the Poaceae (Castillo and Hebert 1974).

2.1.2 Symptoms

The initial MCMV symptoms start with chlorotic specs and form chlorotic stripes developing from the base of the youngest leaves, progressing upward towards the leaf tips. Later, these stripes coalesce to form irregular patches with chlorotic mottle that finally turn necrotic. In severe infections of particularly susceptible lines, leaf necrosis can result in plant death (Castillo and Hebert 1974). Male inflorescences have hard panicles, short rachis, and few spikelets. In severe infections, fewer ears and ear malformation can also occur (Castillo 1976).

2.1.3 Conditions for disease development

The MCMV symptom expression is more pronounced during warm weather than in cooler or temperate climates. The warm weather is much more favorable to insect vectors, responsible for virus transmission. MCMV is transmitted by various modes, including mechanical, insect vectors such as thrips and chrysomelid beetles and through contaminated seeds at a low rate. All parts of the infected maize plants, including leaf, stem, cob, husk, silk, kernel, seed, anther, sheath tissues, and root, confirm the presence of MCMV during the serological detection. MLN symptoms are much more severe at the early crop growth stage due to synergistic interactions of MCMV with any potyviruses than the symptoms expressed due to MCMV or the potyvirus alone. The severe systemic necrosis, followed by rapid plant death, occurs during the coinfection of MCMV and a maize-infecting potyvirus. Invariably, wherever both MCMV and a maize-infecting potyvirus are prevalent in the host, MLN occurs (Wangai and Suresh 2021). Early stage infected (3-7 leaf stage) maize plants are adversely affected with severe symptoms, including stunted plants, premature death, and bearing small, deformed, partially filled, or no ears. Maize plants infected at later crop stage (14-leaf stage) showed normal green foliage compared to the previously mentioned early inoculated plants but exhibited prematurely yellowed and necrotic ears with shriveled kernels (Uyemoto 1983).

2.2 SUGARCANE MOSAIC VIRUS (SCMV)

SCMV is the most widespread viral disease affecting maize, sugarcane, and a few other crops. The SCMV complex (Family Potyviridae) consists of four distinct potyviruses and includes strains of Johnsongrass mosaic virus (JGMV), maize dwarf mosaic virus (MDMV), sorghum mosaic virus (SrMV), and SCMV (Shulka et al. 1994). Several potyviruses, including SCMV, MDMV, JGMV, and WSMV in the genus *Tritimovirus* (Niblet and Claflin 1978), Stewart et al. 2017; Uyemoto 1983) have been reported to cause MLN in coinfections with MCMV. SCMV was described as early as in 1924 on maize and Sugarcane in South Africa (Storey 1924). SCMV was reported in East Africa in 1935 in sugarcane (Hansford 1935), whereas it was later identified as a pathogen in maize in 1973 (Kulkarni 1972). As many as 21 different strains of SCMV were found in the USA (Yang and Mirkov 1997). Yield losses due to SCMV complex were reported to be as high as 21% in the USA (Grisham 2000) and up to 42% in South Africa (Balarabe et al. 2014).

2.2.1. Host range

SCMV causes mosaic diseases in sugarcane (Koike and Gillaspie 1989), but different strains of SCMV usually infects various members of the crop and wild species of the Poaceae family. Some hosts that have been identified are Sorghum bicolor, *Zea mays*, *Brachiaria piligera* (Sabi grass), *Sorghum verticilliflorum* (wild sorghum), *Urochloa mosambicensis*, *Dinebra retroflexa*, *Eragrostis cilianensis*, *Pennisetum glaucum* (pearl millet) and *Digitaria didactyla* (Teakle and Grylls, 1973; Persley and Greber, 1977). The SCMV strain formerly known as *maize dwarf mosaic virus* (MDMV) strain B infects maize and may infect other wild Poaceae grasses (Wangai and Suresh 2021).

2.2.2. Symptoms

The plants affected by SCMV show typical mosaic symptoms with contrasting shades of green on a background of paler green to yellow chlorotic areas. Sometimes, yellow stripes and/or necrosis also occur. The symptoms also vary depending on the virus strain, the host cultivar, and environmental conditions, particularly temperature. Infected plants develop a distinct mosaic, and irregularities in the distribution of normal green color, on the youngest leaf bases. Sometimes the mosaic appearance is enhanced by narrow chlorotic streaks extending parallel to the veins (Wangai and Suresh 2021). Later, the youngest leaves show a general chlorosis, and streaks are larger and more abundant. As plants approach maturity, the foliage can turn purple or purplish red. Depending on the time of infection, there may be severe stunting of the plant. Plants infected early may become totally barren.

2.2.3. Conditions for disease development

SCMV infection occurs at the seedling or other vegetative growing stages, but maximum concentration of the viral particles is found in young leaves and minimum in the roots of older infected plants. Seed transmission of SCMV was also reported (Li et al. 2007; 2011). The main source of primary infection is the vegetative parts used for propagation in sugarcane. The virus overwinters in infected sugarcane or in appropriate perennial hosts of the specific strain. The virus is transmitted in a non-persistent manner by several species of aphids including *Rhopalosiphum maidis*, *R. padi*, *Myzus persicae*, *Schizaphis graminum*, and *Aphis craccivora* (Noone et al. 1994). The virus is easily sap-transmissible. Infected plants begin to show symptoms at about 4-6 weeks after planting. Crops of maize and sorghum are good hosts of SCMV vectors such as *R. maidis* and should not

be grown near infected sugarcane crops. Altering planting and harvesting times so they do not coincide with high aphid vector populations can also reduce losses (Bailey and Fox 1980).

2.3. Modes of transmission of MLN-causing viruses

Unlike other pathogens, plant viruses need effective biological carrier agents for movement from plant to plant and from location to location. Several mechanisms for initial infection, transmission, and spread among the host are needed for effective plant virus introduction and perpetuation in the ecosystem. For the survival and propagation of plant viruses, insect vectors play a key role (Wangai et al. 2021). The presence of virus reservoirs and vector populations is essential for the continued disease spread, followed by cropping practices. For disease to be perpetuated, there must be virus reservoirs and vector populations capable of sustaining diseases. These two factors, in turn, are heavily influenced by cropping practices (Redinbaugh and Stewart 2018).

The epidemiology of plant viruses is determined by insect vector dynamics and their long- and short-range dispersal, host selection, and feeding behaviours (Eigenbrode and Bosque-Perez 2016). In the case of transmission and spread of MLN-causing viruses, Wangai et al. (2021) highlighted the possible roles played by a) insect vectors; b) seed contamination and transmission; c) transmission through soil; and d) mechanical transmission.

2.3.1 Insect Vectors of MLN-causing Viruses

Plant virus transmission through insect vectors is categorized into four types like non-persistent; semi- persistent; persistent-circulative, and persistent-propagative (Ng and Falk 2006). MCMV is transmitted in a semi-persistent manner (Cabanas et al. 2013) by thrips, especially corn (maize) thrips, *Frankliniella williamsi* Hood (Nault et al. 1978; 1981; Cabanas et al. 2013), other associated thrips species in transmitting MCMV are Common blossom thrips *(Frankliniella schultzei*) (Gikonyo et al. 2017; Moritz et al. 2013 Nyasani et al. 2015), Western flower thrips (*Frankliniella occidentalis*) (Moritz et al. 2013), Onion thrips (*Thrips tabaci*) (Mortiz et al. 2013). Maize thrips do not transmit SCMV even when exposed to plants with mixed infections (Nyasani et al. 2015). Transmission of MCMV by maize thrips is estimated at 78% (Nyasani et al. 2015). In addition to thrips, other insect vectors associated with MCMV transmission include Chrysomelid leaf beetles, belonging to the genera *Diabrotica*, *Chaetocnema*, *Systena* and *Oulema* (Nault et al. 1978). *Chaetocnema pulicaria* identified

as a vector of MCMV in the US (Nault et al. 1978, 1981) is also occasionally observed on maize in East Africa. However, its ability to transmit MCMV in Africa needs to be confirmed. Another insect vector associated with MCMV transmission is maize weevil (*Sitophilus zeamais*, Order: Coleoptera; Family: Curculionidae) (Nyasani et al. 2014). Apart from flea beetles and maize weevils, maize in east Africa is also infested by other occasional beetles such as *Epilachna* sp. (Coccinellidae: Coleoptera) and Nitidulid corn-sap beetle, *Carpophilus* sp. which can also transmit MCMV (Nyasani et al. 2014). SCMV and other potyviruses are transmitted in a non-persistent manner by various aphid species infesting cereals, especially belonging to genus, *Aphis*, *Rhopalosiphum*, *Sitobion* and *Macrosiphum* (Adams et al. 2014; Brault et al. 2010; CABI 2019; Wangai et al. 2021).

Management of cropping systems and crop habitats is critical for effective management of key insect vectors such as thrips and aphids. For instance, avoiding intercropping or mixed cropping of maize with cruciferous vegetables (cabbage, kale) and Alliaceae crops (onion, garlic) is critical to reduce infestation of thrips such as *Thrips tabaci*. Since most of the cereal aphids and corn thrips prefer graminaceous hosts, effective management of grass weeds in the maize farms can reduce early onset of thrips and aphid population. High population of thrips and aphids, especially maize thrips and green corn aphids, at the early stages of crop growth can be detrimental. Coating maize seeds with systemic insecticides can ensure early-stage protection of seedlings against thrips and aphids, and there by MCMV and SCMV. Both thrips and aphids can be controlled naturally by a wide array of natural enemies, such as ladybird beetles, lacewing bugs, pirate bugs, syrphid flies, braconid and eulophid parasitoids, and predatory mites. Outbreaks of thrips and aphids often occur with extensive use of organophosphates and synthetic pyrethroids for the control of other major pests, such as stem borers and Fall Armyworm. These pesticides kill the natural enemies of aphids and thrips, resulting in their resurgence. Hence it is critical to effectively monitor aphids and thrips population with yellow sticky traps for timely and need-based management interventions, preferably with biorational pesticides (Wangai et al. 2021).

Application of biopesticides based on entomopathogenic fungi, *Metarhizium anisopliae* can provide early season protection against thrips and aphids. For sustainable management of MLN, the control strategies for insect vectors should be well integrated with other MLN management efforts, such as clean seeds, resistant cultivars, closed season planting, and maize-legume crop rotation (Wangai et al. 2021).

2.3.2 Seed Contamination versus Seed Transmission

"Seed contamination" refers to the presence of a pathogen within or on the seed surface. "Seed transmission" refers to the passage of a pathogen from the seed to the seedling and further to the whole plant (Sastry 2013). Any pathogen that may be either inside or attached to the outside surface of a seed that can affect the plant germination or affect an emerging seedling causing the disease symptoms may, in broad sense, be referred to as "seed-borne". It is well established that plant viruses are effectively introduced into new countries and continents through contaminated or infected seed (Maule and Wang 1996; Wangai et al. 2021).

Bernardo et al. (2018) conducted a study to understand the mechanisms underlying MCMV transmission through seed. The results indicated that there is significantly low level of the virus in the endosperm, and no virus was detected in embryos that were washed after dissection. MCMV was localized to pericarp and pedicel. MCMV virions are limited to maternal tissues in the seed, and seed treatments may reduce seed contamination and transmission of MCMV by seed.

2.3.2.1 Seed Transmission of MCMV

Seed movement across the border or continent is critical in global seed business. The concerning aspect of MLN from endemic to non-endemic areas is the seed transmission nature of MCMV. Jensen et al. (1991), through an earlier study, indicated that seed transmission rates of MCMV in maize seed from MCMV-infected plants range from 0 to 0.33%. Kimani et al. (2021) analyzed four commercial seed lots for the seed contamination rates, and indicated that the contamination rates ranged from 4.9 to 15.9%. MCMV transmission frequency through Double Antibody Sandwich-Enzyme-linked Immunosorbent Assay (DAS-ELISA) was recorded as 0.17%, whereas a transmission frequency of 0.025% by RT-PCR, whereas the seed transmission rate was 0.04% during the mechanical transmission. The study showed that even with substantial contamination of maize seed with MCMV, the transmission of the virus from the seed to seedlings was low.

Kinyungu et al. (2021) conducted a study on MLN infection, during grow-out tests with maize seeds obtained from plants with varied levels of MLN infection; the results revealed high levels of MLN incidence in the seedlings in both the laboratory (55–100%) and in the field (10.9–36.5%). MLN transmission was not observed with certified seeds obtained from plants with no incidence of MLN. However, considering the stable and aggressive nature of MCMV, even a small fraction of MCMV transmitted

to the non-endemic area through seed is enough to cause an epidemic in quick time.

2.3.2.2 Seed Transmission of SCMV

SCMV has not been reported to be transmitted by seed in sugarcane. However, seed cane (stalk pieces or setts), used to propagate sugarcane vegetatively commonly transmits SCMV and other viruses from one crop to the next. In the case of maize, SCMV-MB (Maize dwarf mosaic virus strain B) has been detected in the pericarp, but rarely in the endosperm or embryo of seeds 21 days after pollination. In mature seeds, it was occasionally detected in the pericarp and endosperm, but not in the embryo (Mikel et al. 1984). Li et al. (2011) revealed that seed transmission rate of SCMV was between 2.3% and 3.9% in two groups of maize seed tested. SCMV was reported to be mechanically, and seed transmitted but not pollen transmitted (Brunt et al. 1996).

2.3.2.3 Transmission through Soil

By definition, a virus is soil-borne if it holds the capacity to survive in the soil debris or other living organisms and infect the plants growing in that soil. To be soil-borne, a virus should have an existence in soil outside of its natural host (Hiruki and Teakle 1987). So far, no published reports have conclusive evidence on the specific mode(s) of soil transmission of MLN-causing viruses like MCMV. However, soil-based vectors that have been associated with transmission of viruses in the family Tombusviridae (to which MCMV belongs) are fungi in the genus *Olpidium*, and at least five genera of nematodes (*Longidorus* spp., *Paralongidorus maximus*, *Xiphinema* spp., *Trichodorus* spp., and *Paratrichodorus* spp. (Andika et al. 2016).

2.3.3 Mechanical Transmission

MCMV and all the members of the family Tombusviridae are known to transmit through mechanical means. Rochon (1999) reported that tombusviruses are readily sap-transmissible experimentally, and infected leaf extracts may retain infectivity after freezing for several years. All the farm machinery/equipment and vehicles, farm tractors entering the farmers' maize fields, or seed production fields should be properly cleaned using disinfectants both before and after use. As a good phytosanitary precautionary measure, it is advised not to feed the farm animals with MLN-infected maize plants or other plants infected by MLN-causing viruses (Wangai et al. 2021).

3. DIAGNOSIS AND SURVEILLANCE OF MLN-CAUSING VIRUSES

MLN is one of the successful examples where a surveillance and diagnostic system was rapidly developed and deployed by a CGIAR center (CIMMYT) together with an array of national and international partners. The complexity of transmission of MLN in the field through insect-vectors, contaminated seed lots, and mechanical means have makes diagnostics and surveillance a key activity in tracking the disease and minimizing its spread within and across continents (Prasanna 2021b).

Early and accurate detection of plant viruses is important in surveillance, seed multiplication, and seed exchange. MCMV infection may be difficult to diagnose based on symptoms alone as some of them (stunting, chlorosis) resemble those caused by nutrient deficiencies, moisture stressor other maize-infecting viruses like maize mosaic virus, maize streak virus and maize stripe virus. There are several diagnostic tools that are available for detection of MCMV and SCMV. The most reliable methods for detecting MCMV in host tissues include ELISA (enzyme-linked immunosorbent assay), immunostrips, and polymerase chain reaction (PCR). These protocols, including their sensitivity and utility for various purposes, were described in detail by Mezzalama et al. (2021).

Continuous surveillance for MLN causing viruses is required to monitor the disease incidence in farmers' maizefields and seed production fields. Surveillance informs decisions on deployment of management practices to limit the effect of the disease at the farm-, country- and regional levels. Effective diagnostics and surveillance of the possible incidence of MLN in the seed production fields is essential for producing and exchanging MLN-free seed. Hodson et al. (2021) described in detail the MLN surveillance protocols developed by CIMMYT, including leaf and seed sampling.

MLN disease surveillance is done with simple, sensitive, cost-effective diagnostic methods such as immunostrips for diagnosis of leaf samples in the field and ELISA for seed samples from the agro-dealers. The diagnosis method along with an electronic version of the MLN surveillance forms are being used together with an ODK software. The survey data collected using these tools are stored by CIMMYT on a secure server in the MLN Toolbox Data Management System developed in partnership with Aarhus University, Denmark. Survey data is not released in the public domain prior to approval of a country's authorized official

(i.e., the country's designated national plant protection officer) (Hodson et al. 2021).

4. MLN DISEASE MANAGEMENT

4.1 Safe Production and Exchange of MLN Virus-free Germplasm: CIMMYT Protocol

As MCMV can contaminate maize seeds, proper precautions need to be taken to produce "clean" seeds and then exchange with other countries, including endemic and non-endemic. CIMMYT has put in place a four-step strategy for MLN-free clean seed production and exchange of breeding materials from Kenya (an MLN-endemic country) to institutions in other countries, including non-endemic:

1. Seed production of breeding materials in Kenya is centralized at only one field station i.e., Kiboko. At the Kiboko station, during the crop season, the team implements careful measures to produce seed with periodic scouting to identify and rogue out any MLN-infected plants and incinerated. Ears from only the disease-free plants are harvested.
2. Sampling of seed from the production plots in Kiboko is undertaken at the CIMMYT Maize Seed Health Unit in Nairobi, Kenta, to check for presence of any latent infection by MCMV; this is done using commercial ELISA kits.
3. If the seed is to be exported to any other country, due phytosanitary protocol is followed. This means, only that seed which is found negative for MLN-causing viruses are sent from CIMMYT to KEPHIS for phytosanitary analysis, and issue of phytosanitary certificate,
4. Once the seeds are safely introduced in any non-endemic country (e.g., Zimbabwe, Mexico), the seed will be grown in a quarantine facility with stringent quarantine procedures. Again, the crop needs to be scouted at all critical growing stages and tested for MCMV and SCMV. Once the crop is found to be free from the MLN-causing viruses at the quarantine facility, the seed is certified by the phytosanitary authority for further distribution to any institution within the country.

Sengwe et al. (2021) provided detailed phytosanitary guidelines for effectively managing MLN quarantine facilities, and a rigorous multi-stage testing process followed by CIMMYT to ensure that there is no escape

of any MLN-infected seed, besides the guidelines stipulated by the NPPOs of the germplasm exporting and importing countries. We urge every institution (public/private) to follow this protocol for safe exchange of MLN-free maize seed.

4.2 MLN-free Commercial Seed Production

MCMV-contaminated seed movement locally and across borders through formal and informal channels must be rigorously avoided for sustainable management of MLN. Most of the local/regional seed companies in the MLN-prevalent countries in Africa have been trained by CIMMYT over the last 7-8 years on MLN disease, its mode of transmission, and standard operating procedures (SOPs) to produce MLN pathogen-free clean seed. Gichuru et al. (2021) provided detailed guidelines on the SOPs for MLN virus-free commercial seed production, including harmonized checklists.

4.3 Breeding and Deployment of MLN-resistant Maize Varieties

As a rapid response to tackle the challenges of the outbreak of MLN in 2011 in Kenya, breeding and deploying MLN tolerant/resistant hybrids was given high priority by CIMMYT together with other disease management strategies. Studies conducted during 2012-2013 confirmed that nearly all commercially grown varieties in Kenya were susceptible to MLN, both under natural and artificial inoculation (Marenya et al. 2018; Prasanna et al. 2020). To develop and deploy MLN-tolerant/resistant hybrids, CIMMYT undertook intensive screening of germplasm, identification of resistant genotypes, and then incorporation of MLN resistance in combination with other relevant farmer-preferred traits in suitable genetic backgrounds.

As an important step, in September 2013, CIMMYT in partnership with KALRO, established a dedicated and centralized MLN Screening Facility (Fig. 1.2) at KALRO Research Center at Naivasha, Kenya, with financial assistance from the Bill & Melinda Gates Foundation (BMGF) and Syngenta Foundation for Sustainable Agriculture (SFSA). The 20-hactare MLN Screening Facility includes 17 ha for field screening under MLN artificial inoculation, an MLN diagnostics laboratory, nearly 2000 sq.m. of greenhouses, 3500 sq.m. of net-houses (for screening separately for MCMV and SCMV under artificial inoculation.), etc. The facility provides MLN phenotyping services to both public and private sector partners across Africa under artificial inoculation, with uniform disease pressure across field trials and high-quality data. During 2014 to 2023,

CIMMYT has screened over 234,000 germplasm entries with more than 330,000 rows (3m each) at the MLN Screening Facility in Naivasha under artificial inoculation. Of these, 60% were from CIMMYT, 16% were from NARS institutions, and 20% were from the private sector.

Fig. 1.2. MLN Screening Facility operated by CIMMYT at the KALRO Research Center at Naivasha, Kenya.

Significant progress has been made in breeding for MLN resistance over the last 10 years (Prasanna 2021b). From less than five inbred lines with resistance to MLN in 2013, today we have more than 100 elite and diverse CIMMYT lines with MLN resistance (Fig. 1.3) in different genetic backgrounds. An array of public and private sector partners globally received CIMMYT-derived MLN resistant lines over the last decade.

Fig. 1.3. An MLN resistant line (left) developed by CIMMYT, adjacent to an MLN-susceptible line (right), after artificial inoculation at the MLN Screening Facility at Naivasha, Kenya.

CIMMYT team in Africa has also identified molecular markers for MLN resistance, which in turn accelerated the development of MLN resistance lines through marker-assisted backcrossing (MABC) and marker-assisted forward breeding. Over 60 elite inbred lines that were MLN-susceptible have been converted into MLN resistant versions using MABC.

MLN resistance linked markers are routinely used at the early stage of breeding pipelines in eastern Africa to discard lines with unfavorable alleles (Muruthi et al. 2021). Thus, breeding for MLN resistance is now an integral component of maize breeding pipelines at CIMMYT, especially in the ESA product profiles. This includes routine screening of breeding materials in various breeding stages under MLN artificial inoculation at the Naivasha facility; identification of resistance sources from diverse germplasm; accelerated breeding using doubled haploids (DH) technology and molecular markers; stage-gate product advancement, and varietal release and deployment of elite MLN resistant hybrids through public and private sector partners. Over 20 CIMMYT-derived MLN-tolerant/ resistant maize hybrids have been released in East Africa.

4.4 Agronomic Management Practices

Besides improved genetics, agronomic management practices do play an important role in sustainable control of MLN. These include:

a) Use of disease-free commercial seeds for raising a healthy crop

b) Keeping the maize fields free from alternative hosts of MCMV or any other potyviruses

c) Use of clean tools and equipment

d) Regular scouting to detect any suspected MLN-infected plants, with prompt roguing

e) A maize-free period of at least two months to break the virus cycle

f) Maize crop rotation with non-cereals, especially grain legumes

CONCLUSION

Tackling MLN in Sub-Saharan Africa is a complex challenge. Nevertheless, through extensive partnerships, research and development institutions have been able to respond rapidly to this serious threat to the food security, income and livelihoods of millions of small holder farmers and their families in SSA. MLN management has been effectively addressed through several simultaneously-implemented strategies, including a) development and deployment of elite MLN tolerant/resistant varieties adapted to Africa; b) strong engagement of the NARES and NPPOs on MLN surveillance; c) synergistic multi-disciplinary efforts of various national and international institutions; d) intensive awareness creation among stakeholders, and capacity building of relevant public and private sector institutions on MLN diagnostics and management; e) codeveloping

with national partners, and implementing harmonized checklists and SOPs for MLN-free commercial seed production and exchange, etc. (Prasanna et al. 2020; 2021).

While significant progress has been made on curbing the spread and impact of MLN in Africa (Prasanna et al. 2020; Prasanna 2021a), it is important to continue implementing an integrated disease management approach for sustainable management of the disease in the MLN-prevalent countries whether in Africa, Americas or Asia, and continued efforts on MLN disease monitoring and surveillance globally. Elite maize hybrids with climate resilience and tolerance/resistance to major diseases and insect-pests must be deployed at scale. Good agronomic practices (e.g., maize-free window for at least 2-3 months in areas where monocropping is being practiced; crop rotation with legumes, etc.) are critical to break the cycle of MLN-causing viruses like MCMV.

ACKNOWLEDGEMENTS

The work presented in this article was supported by various projects, especially: a) Breeding for MLN Resistance Project, funded by the Bill & Melinda Gates Foundation (BMGF), and the Syngenta Foundation for Sustainable Agriculture; b) MLN Diagnostics and Management Project, funded by the USAID East Africa Office; c) MLN Epidemiology Project, funded by the BMGF; d) the Stress Tolerant Maize for Africa (STMA), Water Efficient Maize for Africa (WEMA), and Accelerated Genetic Gains (AGG) Projects, funded by the BMGF, and co-funded by USAID; f) the CGIAR Research Program on Maize (MAIZE); and g) the One CGIAR Initiative on Plant Health. We are grateful to the national partners in Africa, especially KALRO for outstanding partnership in tackling the MLN challenge, particularly enabling CIMMYT to establish and operate the MLN screening facility at KALRO-Naivasha, Kenya.

References

Adams IP, Harju VA, Hodges T, Hany U, Skelton A, Rai S (2014). First report of maize lethal necrosis disease in Rwanda. New Disease Reports 29:22.

Andika IB, Kondo H, Sun L (2016). Interplays between soil-borne plant viruses and RNA silencing-mediated antiviral defense in roots. Frontiers in Microbiology 7:1458.

ASARECA (2014). Maize lethal necrosis disease in Africa: Past, present and planned initiatives. ASARECA 17:1-8.

Bailey RA, Fox PH (1980). The susceptibility of varieties of mosaic and the effect of planting date on mosaic incidence in South Africa. Proc. South African Sugar Technology Association 54:161-167.

Balarabe DD, Adama Y, Khan Azmat UU, Aisha ZM (2014). Identification of virus isolates inducing mosaic of sugarcane in Makarfi Local Government Area of Kaduna State, Nigeria. African Journal of Biotechnology 13:1351-1357.

Bernardo P, Redinbaugh MG, Barriball K (2018). Understanding Maize chlorotic mottle virus transmission through seed: localization and infectivity. Presented in ICPP: Plant Health in a Global Economy (July 28-August 3, 2018), Boston, USA.

Bockelman DL, Claflin LE, Uyemoto JK (1982). Host range and seed transmission studies of Maize chlorotic mottle virus in grasses and corn. Plant Disease 66:216-218.

Brault V, Uzest M, Monsion B, Jacquot E, Blanc S (2010). Aphids as transport devices for plant viruses. Comptes Rendus Biologies 333:524-538.

Brunt A, Crabtree K, Dallwitz M, Gibbs A, Watson L (1996). Sugarcane mosaic potyvirus. In: Viruses of Plants. Descriptions and Lists from VIDE Database. CAB International, University Press, Cambridge.

Cabanas DW, Higashi CHVS, Bressan A (2013). Dissecting the mode of Maize Chlorotic Mottle Virus transmission (Tombusviridae: Machlomovirus) by *Frankliniella williamsi* (Thysanoptera: Thripidae). Journal of Economic Entomology 106:16-24.

CABI (2019). https://www.cabi.org/isc/datasheet/49801#tovectors and intermediate hosts.

Castillo J, Hebert TT (1974). Nueva enfermedad virosa afectando al maíz en el Perú. Fitopatología 9:79-84.

Castillo JC (1976). Maize virus and virus-like diseases in Peru. In: Williams LE, Gordon DT, Nault LR (eds). Proceedings of the International Maize Virus Disease Colloquium and Workshop, 16-19 August 1976, The Ohio State University, Wooster, Ohio Agricultural Research and Development Center, USA, pp.40-44.

Gordon DT, Nault LR (eds). Proceedings of the International Maize Virus Disease Colloquium and Workshop,16-19 August 1976, The Ohio State University, Wooster, Ohio Agricultural Research and Development Center, USA, pp.40-44.

De Groote H, Oloo F, Tongruksawattana S, Das B (2016). Community-survey based assessment of the geographic distribution and impact of maize lethal necrosis (MLN) disease in Kenya. Crop Protection 82:30-35.

Eigenbrode SD, Bosque-Pérez NA (2016). Chemical ecology of aphid-vectored plant viruses. In: Vector-mediated Transmission of Plant Pathogens. JK Brown (ed), American Phytopathological Society Press, pp.3-20.

FAOStat (2021). FAO Stat. FAO, Rome. http://www.fao.org/faostat.

Gichuru L, Angwenyi S, Mwatuni F, Suresh LM, Prasanna BM (2021). MLN pathogen-free commercial seed production: standard operating procedures. In: Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management. BM Prasanna (ed). CDMX: CIMMYT, pp.56-66.

Gikonyo MW, Niassy S, Moritz GB, Khamis FM, Magiri E, Subramanian S (2017). Resolving the taxonomic status of *Frankliniella schultzei* (Thysanoptera: Thripidae) colour forms in Kenya – a morphological-, biological-, molecular- and ecological-

based approach. International Journal of Tropical Insect Science 37:57-70.

Grisham MP (2000). Mosaic. In: A Guide to Sugarcane Diseases. PR Rott et al. (eds). CIRAD/ISSCT, La Librairie du Cirad, Montpellier, France, pp.249-254.

Hansford CG (1935). Sugarcane disease in Uganda. East African Agricultural Journal 1:25-28.

Hiruki C, Teakle D (1987). Soil-borne viruses of plants. In: Current Topics in Vector Research Vol. 3. Harris KF (ed). Springer-Verlag.

Hodson D, Mezzalama M, Suresh LM, Mwatuni F (2021). MLN surveillance, leaf and seed sampling protocols. In: Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management. BM Prasanna (ed). CDMX: CIMMYT, pp.20-30.

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

Kagoda F, Gidoi R, Isabirye BE (2016). Status of maize lethal necrosis in eastern Uganda. African Journal of Agricultural Research 11:652-660.

Kimani EN, Kiarie SM, Micheni C, Muriki LG, Miano DW, Macharia I, Munkvold GP, Muiru WM, Prasanna BM, Wangai A (2021). Maize seed contamination and seed transmission of Maize Chlorotic Mottle Virus in Kenya. Plant Health Progress 22:496-502.

Kinyungu TN, Muthomi JW, Subramanian S, Miano DW, Olubayo FM (2021). Transmission of viruses causing maize lethal necrosis disease through seed in commercial hybrids and effect on growth in the subsequent crop. Archives of Phytopathology and Plant Protection 54(3-4):191-207.

Koike H, Gillaspie AG Jr (1989). Mosaic. In: Diseases of Sugarcane. C Ricaud et al. (eds). Elsevier, New York, USA, pp.301-322.

Kulkarni H (1972). Survey of viruses affecting East Africa major food crops. PhD Thesis, University of Nairobi, Kenya.

Kusia ES, Subramanian S, Nyasani JO, Khamis F, Villinger J, Ateka EM, Pappu HR (2015). First report of lethal necrosis disease associated with co-infection of finger millet with Maize chlorotic mottle virus and Sugarcane mosaic virus in Kenya. Plant Disease 99(6):899-900.

Li L, Wang X, Zhou G (2007). Analyses of maize embryo invasion by Sugarcane mosaic virus. *Plant Science* 172:131-138.

Li L, Wang X F, Zhou G H (2011). Effects of seed quality on the proportion of seed transmission for Sugarcane mosaic virus in maize. *Cereal Research Communications* 39:257-266.

Mahuku G, Lockhart B E, Wanjala B, Jones M W, Kimunye J N, Stewart L R, Cassone B J, Sevgan S, Johnson N, Kusia E, Lava Kumar P, Niblett C L, Wangai A, Kiggundu A, Asea G, Pappu H, Prasanna B M, Redinbaugh M G (2015a). Maize lethal necrosis (MLN), an emerging threat to maize-based food security in sub-Saharan Africa. *Phytopathology* 105:956-965.

Mahuku G, Wangai A, Sadessa K, Teklewold A, Wegary D, Ayalneh D, Adams I, Smith J,

Bottomley E, Bryce S, Braidwood L, Feyissa B, Regassa B, Wanjala B, Kimunye J N, Mugambi C, Monjero K, Prasanna B M (2015b). First report of *Maize chlorotic mottle virus* and *Maize Lethal Necrosis* on maize in Ethiopia. *Plant Disease* 99:1870.

Marenya P P, Erenstein O, Prasanna B, Makumbi D, Jumbo M, Beyene Y (2018). Maize lethal necrosis disease: Evaluating agronomic and genetic control strategies for Ethiopia and Kenya. *Agricultural Systems* 162:220-228.

Maule A J, Wang D (1996). Seed transmission of plant viruses: a lesson in biological complexity. *Trends in Microbiology* 4:153-158.

Mezzalama M, Redinbaugh M, Wangai A W, Suresh L M (2021). Diagnostic Protocols for *MCMV* and *SCMV*. In: Prasanna B M (ed), *Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management.* CDMX: CIMMYT, pp. 31-41.

Mikel M A, D'Arcy C J, Ford R E (1984). Seed transmission of Maize Dwarf Mosaic Virus in sweet corn. *Journal of Phytopathology* 110:185-191.

Moritz G, Brandt S, Triapitsyn S, Subramanian S (2013). Identification and information tools for pest thrips in East Africa. *QAAFI Biological Information Technology (QBIT)*, The University of Queensland, Brisbane, Australia.

Nault L R, Negi L S, Niblett C L, Gordon D T, Styer W E, Coffey M E (1978). Transmission of *Maize chlorotic mottle virus* by chrysomelid beetles. *Phytopathology* 68:1071-1074.

Nault L R, Gordon D T, Loayza J C (1981). Maize virus and mycoplasma diseases in Peru. *International Journal of Pest Management* 27:363-369.

Ng J C, Falk B W (2006). Virus-vector interactions mediating non-persistent and semi-persistent transmission of plant viruses. *Annual Review of Phytopathology* 44:183-212.

Niblett C L, Claflin L E (1978). *Corn lethal necrosis* – a new virus disease of corn in Kansas. *Plant Disease Reporter* 62:15-19.

Noone D F, Srinsk S, Teakle D S, Allsopp P G, Taylor P W J (1994). Ability to transmit *Sugarcane mosaic virus* and seasonal phenology of some aphid species (Hemiptera: Aphididae) in the Isis and Bundaberg districts of Queensland. *Journal of Australian Entomological Society* 33:27-30.

Nyasani J, Kusia E, Subramanian S (2014). Role of vectors and their host plants in the epidemiology of maize lethal necrosis in eastern Africa. In: *Entomological Society of America Annual Meeting*, Portland, November 16–19, 2014.

Nyasani J, Kusia E, Subramanian S (2015). Thrips as pests and vectors of *Maize chlorotic mottle virus* in maize. In: *Proc. Xth International Symposium on Thysanoptera and Tospoviruses*, Asilomar Conference Grounds, May 16–20, 2015.

Persley D M, Greber R S (1977). Additional field hosts of *Sugarcane mosaic virus* in Queensland. *APPS Newsletter* 6(4):54.

Prasanna B M (ed) (2021a). *Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management*. CDMX: CIMMYT.

Prasanna B M (2021b). Maize Lethal Necrosis (MLN) in Africa: Incidence, impact, rapid response, and management. In: Prasanna B M (ed), *Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management*. CDMX: CIMMYT, pp. 1-7.

Prasanna B M, Cairns J E, Zaidi P H, Beyene Y, et al. (2021). Beat the stress: Breeding for climate resilience in maize for the tropical rainfed environments. *Theoretical and Applied Genetics* 134:1729-1752.

Prasanna B M, Suresh L M, Mwatuni F, Beyene Y, et al. (2020). Maize lethal necrosis (MLN): Containing the spread and impact of a devastating transboundary disease in sub-Saharan Africa. *Virus Research* 282:197943.

Redinbaugh M G, Stewart L R (2018). Maize lethal necrosis: an emerging, synergistic viral disease. *Annual Review of Virology* 5:301-322.

Rochon D (1999). *Tombusviruses*. In: Granoff A, Webster R G (eds), *Encyclopedia of Virology, 2nd Edition.* Academic Press.

Sastry K S (2013). *Seed-borne Plant Virus Diseases.* Springer, India.

Sengwe T, Wangui A, Mezzalama M, Suresh L M, Prasanna B M (2021). Managing MLN quarantine facilities: Phytosanitary guidelines. In: Prasanna B M (ed), *Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management.* CDMX: CIMMYT, pp. 42-49.

Shulka D D, Ward C W, Brunt A A (1994). The *Sugarcane mosaic virus* subgroup. *The Potyviridae.* CAB International, Wallingford, UK, pp. 360–371.

Stewart L R, Willie K, Wijeratne S, Redinbaugh M G, Massawe D, Niblett C L, Kiggundu A, Asiimwe T (2017). *Johnsongrass mosaic virus* contributes to maize lethal necrosis in East Africa. *Plant Disease* 101:1455-1462.

Storey H H (1924). Diseases of sugarcane of the mosaic type in South Africa-Part 1. *South Africa Journal of the Department of Agriculture* 9:108-117.

Teakle D S, Grylls N E (1973). Four strains of *Sugarcane mosaic virus* infecting cereals and other grasses in Australia. *Journal of Agricultural Research* 21:549-556.

Uyemoto J K (1983). Biology and control of *Maize chlorotic mottle virus. Plant Disease* 67:7-10.

Wangai A, Bruce A, Nyasani J, Subramanian S, Prasanna B M (2021). Modes of transmission of MLN-causing viruses. In: Prasanna B M (ed), *Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management.* CDMX: CIMMYT, pp. 12-19.

Wangai A, Suresh L M (2021). MLN-causing viruses in Africa and their symptoms. In: Prasanna B M (ed), *Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management.* CDMX: CIMMYT, pp. 8-12.

Yang Z N, Mirkov T E (1997). Sequence and relationships of *Sugarcane mosaic virus* and *Sorghum mosaic virus* strains and development of RT-PCR-based RFLPs for strain discrimination. *Phytopathology* 87:932–939.