Maize Lethal Necrosis (MLN):

A Technical Manual for Disease Management

Editor

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CGIAR
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In collaboration with international and national research and development partners

Chapter 1

Maize Lethal Necrosis (MLN) in Africa: Incidence, Impact, Rapid Response, and Management

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1. Background

Maize (*Zea mays* L.) is the most important cereal crop in sub-Saharan Africa (SSA), covering over 40 million ha, largely in smallholder farming systems, with a production of over 70 million metric tonnes (MMT) of grain (FAOSTAT, 2021). The crop is critical for food security, incomes, and livelihoods of several million smallholders across SSA, especially in eastern and southern Africa where nearly 85% of the maize produced is used as food (Shiferaw et al., 2011). However, average maize yield in SSA (~2 t/ha) is far below the global average (~5 t/ha), due to various reasons, including frequent occurrence of drought, poor soil fertility, inadequate use of inputs (both improved seed and fertilizers), and challenges imposed by various pests and diseases (Prasanna et al., 2021).

The spread of transboundary pests and diseases has increased significantly in the recent years, affecting the food security and livelihoods of several million resource-constrained smallholders, especially in SSA, Asia, and Latin America. Globalization, trade, and climate change, as well as reduced resilience in production systems due to decades of agricultural intensification, have all played a part. One such major example is the emergence of maize lethal necrosis (MLN) in sub-Saharan Africa, which was first reported in the southern Rift valley area of Kenya in 2011 (Wangai et al., 2012), and then rapidly spread to several other eastern Africa countries during 2012 to 2014 (Mahuku et al., 2015; Redinbaugh and Stewart, 2019; Prasanna et al., 2020). MLN is a viral disease caused by combined infection of maize plants with Maize chlorotic mottle virus (MCMV) with any one of the members of family *Potyviridae*, such as sugarcane mosaic virus (SCMV), Maize dwarf mosaic virus (MDMV) or Wheat streak mosaic virus (WSMV) or Johnson grass mosaic virus (Stewart et al., 2017). MCMV was a recent introduction into eastern Africa, possibly in 2011, while SCMV has a worldwide distribution, including in SSA, over many decades. Therefore, the outbreak of MLN in Africa was primarily triggered by the introduction of MCMV.

2. Global Occurrence and Impact of MLN

MCMV was first reported in Peru in 1971 (Castillo and Hebertt 1974), and subsequently in the USA in the 1970s (Niblett and Claflin 1978). The disease later emerged in several countries across the Americas, Asia, and Africa, including Argentina (1982), Mexico (1987), Thailand (1983), Brazil (1983), Mexico (1987), China (2010), Kenya (2011), Tanzania (2012), Uganda (2012), Rwanda (2013), D.R. Congo (2014), Ethiopia (2014), Taiwan (2014), South Sudan (2015), Ecuador (2016), and Spain (2016) [reviewed in detail by Redinbaugh and Stewart, 2018; Prasanna et al., 2020].

MLN had a serious impact on maize production and grain yield in eastern Africa. During 2012-2013, the estimated maize yield losses in Kenya due to MLN were reported as 23-100% in the affected counties in the country (Prasanna et al., 2020). De Groote et al. (2016) estimated that the aggregate national loss of maize production due to MLN in Kenya alone was about 0.5 million tons with a value of US$180 million. An average yield reduction of 1.4 t/ha was reported in Uganda, estimated at US$ 332 per ha (ASARECA, 2014; Kagoda et al., 2016). In 2016, Isabirye and Rwomushana (2016) indicated that MLN may pose high potential yield losses in several countries in SSA, including Uganda (81.1%), Tanzania (65.9%), Ethiopia (59.8%), Malawi (53.8%) and Madagascar (45.1%). Annual economic impact associated with MLN on smallholder farmers in eastern Africa was estimated as about US$ 261 million (Marenya et al., 2018).

The adverse impact of MLN was not just limited to the maize crops and the livelihoods of the resource-poor farmers in the affected countries, but also on other actors in the maize seed value chain, especially small- and medium enterprise (SME) seed companies. Although not empirically quantified and published, rejection of MLN-contaminated commercial seed by the regulatory authorities and decreased demand for seed of commercial maize varieties in the years soon after the MLN outbreak in eastern Africa led to significant losses to SME seed companies.

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3. Tackling the MLN Challenge on Multiple Fronts

Effectively countering the incidence, spread and adverse impacts of MLN in Africa requires strong, coordinated, and synergistic efforts from multiple institutions as the challenge is complex and multi-faceted. Since 2012, a team including CIMMYT, Kenya Agricultural and Livestock Research Organization (KALRO), NPPOs and commercial seed companies across sub-Saharan Africa, International Institute of Tropical Agriculture (IITA), International Centre of Insect Physiology and Ecology (icipe), several advanced research institutions in the USA and Europe, and non-government organizations, such as Alliance for Green Revolution in Africa (AGRA), and African Agricultural Technology Foundation (AATF), has been intensively implementing a multi-disciplinary strategy for curbing the spread and impact of MLN in Africa. These initiatives are briefly highlighted in this Chapter, and the practical details were elaborated in subsequent Chapters in this Technical Manual.

3.1. Breeding and Deployment of Elite, MLN-Tolerant/Resistant Maize Hybrids in Africa

Studies during 2012-2013 confirmed that nearly all commercial maize varieties (approximately 98%) in Kenya were susceptible to MLN, both under natural and artificial infection (Marenya et al., 2018; Prasanna et al., 2020). Severe MLN infection in the farmers' fields can cause up to 100% yield loss in susceptible varieties (Mahuku et al., 2015). Development of MLN-tolerant/resistant maize varieties is, therefore, the most economically viable and environmentally sustainable approach. This requires intensive screening of germplasm, identification of resistant genotypes, and then incorporation of MLN resistance in combination with other relevant farmer-preferred traits into suitable genetic backgrounds. All this needs to be done in an accelerated manner so that improved varieties with MLN tolerance/resistance are released in the affected countries, and farmers can access the seed of such varieties.

As an important first step, in partnership with KALRO, CIMMYT established a dedicated and centralized MLN Screening Facility at KALRO Research Center, Naivasha, in September 2013. The 20-hectare facility, established with financial support from support from the Bill & Melinda Gates Foundation (BMGF) and Syngenta Foundation for Sustainable Agriculture (SFSA), includes 17ha for field screening under MLN artificial inoculation, an MLN diagnostics laboratory, nearly 2000sq.m. of greenhouses, 3500sq.m. of net houses (for screening separately for MCMV and SCMV under artificial inoculation.), etc. MLN phenotyping is carried out throughout the year (two times a year for field-based MLN screening, and thrice for MLN indexing). The facility provides MLN phenotyping service to both public and private sector partners across Africa under artificial inoculation, with uniform disease pressure across field trials and high-quality data on the responses of genotypes to MLN. During 2014 to 2021, CIMMYT has screened over 200,000 germplasm entries with more than 300,000 rows (3 m each) at the MLN Screening Facility in Naivasha under artificial inoculation. Of these, 61% were from CIMMYT, 17% were from NARS institutions, and 22% from private sector.

From less than 5 inbred lines with tolerance/resistance to MLN in 2013, today we have more than 50 elite and diverse CIMMYT lines with MLN resistance. Before the onslaught of Covid19 pandemic, annual field days at the MLN Screening Facility at Naivasha (Figure 1) provided public and private sector partners with the first-hand information on the performance of MLN-resistant inbred lines and hybrids (under artificial inoculation). Since 2015, an array of public and private sector institutions globally accessed the seed of CIMMYT's MLN-resistant inbred lines.

Figure 1. Field Day at the MLN Screening Facility, Naivasha, Kenya (2019).

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.

By 2020, a total of 19 MLN-tolerant/resistant hybrids were released in East Africa (Prasanna et al., 2020). On-farm trials conducted in eastern Africa (Kenya, Uganda, Tanzania, and Rwanda) also confirmed the superior performance of these hybrids for MLN tolerance as well as grain yield under high disease pressure, besides other agronomic traits, as compared to the popular commercial checks in the region. Two of the prominent examples of CIMMYT-derived, MLN-tolerant maize hybrids that are being commercialized in eastern Africa are “Bazooka” by NAECO in Uganda, Burundi, and D.R. Congo, and “H6506” by Kenya Seed Company in Kenya (Figure 2).

Figure 2. Bazooka (A) and H6506 (= H12ML1) (B), two of the CIMMYT-derived, elite, MLN-tolerant maize hybrids being commercialized by seed companies in eastern Africa.

Besides deriving MLN-tolerant/resistant inbred lines and hybrids through conventional breeding, molecular marker-based analyses undertaken by CIMMYT has helped in: a) understanding the genetic architecture of resistance to MLN and its causal pathogens; b) identifying the molecular markers associated with resistance, which could be used to improve the resistance or as potential diagnostic markers for early-generation identification of MLN resistant materials or as a part of forward breeding strategy to select lines for MLN resistance during early generations of breeding.

An MLN Quarantine Facility was established by CIMMYT, with funding support from USAID and CGIAR Research Program MAIZE, at the Plant Quarantine Institute (PQI) at Mazowe (near Harare), Zimbabwe. The facility, functional since April 2017, is enabling safe introduction and exchange of maize breeding materials from CIMMYT (including from the Kenya breeding hub) to partners in southern Africa. The committed NPPO in Zimbabwe is undertaking monitoring and surveillance during the cropping cycle at the MLN Quarantine Facility, and seed from MLN-free plants is multiplied under quarantine conditions. Seed is further tested at the Quarantine Laboratory in the PQI before declaring it virus-free and suitable for further distribution to partners across Africa. CIMMYT has also developed detailed operational guidelines for the management of MLN Quarantine Facilities (see Chapter 6).

3.2. An MLN Surveillance and Monitoring System in SSA

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 made surveillance and diagnosis a key activity in tracking the disease and minimizing its spread in the continent. The surveillance and disease tracking for MLN were modelled on another successful initiative i.e., for wheat stem rust (Ug99) (Hodson et al., 2012; Park et al., 2011). The MLN surveillance system was developed by CIMMYT in partnership with National Plant Protection Organizations (NPPOs) and Aarhus University, Denmark, under the USAID-funded MLN Diagnostics and Management Project. After its initial development in 2015, a regional MLN surveillance system was put in place in ESA.

MLN surveillance and diagnostic protocols (https://mln.cimmyt.org/mln-status/protocols-survey-forms/), as described in Chapters 4 and 5, was carried in eight sub-Saharan African countries, namely Kenya, Uganda, Tanzania, Rwanda, Malawi, Zambia and Zimbabwe during 2016-2019 (Prasanna et al., 2020). All the surveys conducted since 2016 used MCMV immunostrips to detect the presence of MCMV from a bulk sample of six young leaves per field, collected randomly using a staggered X pattern in each of the surveyed fields. Survey fields were selected at random every 10-20 km in maize growing areas. Fields sprayed with pesticide were not surveyed. All the surveyed fields were geo-referenced using GPS. Field survey data from 2017, 2018 and 2019 are depicted in Figure 3 (the results were explained by Prasanna et al., 2020). The surveys indicated that MCMV continues to prevail in eastern Africa. Kenya, Uganda, Rwanda, northern Tanzania, and Ethiopia all detected the presence of MCMV in farmers' fields. However, no further spread to new countries in SSA has been detected, and the current survey data indicates the continued absence of MCMV/MLN in southern and West Africa.

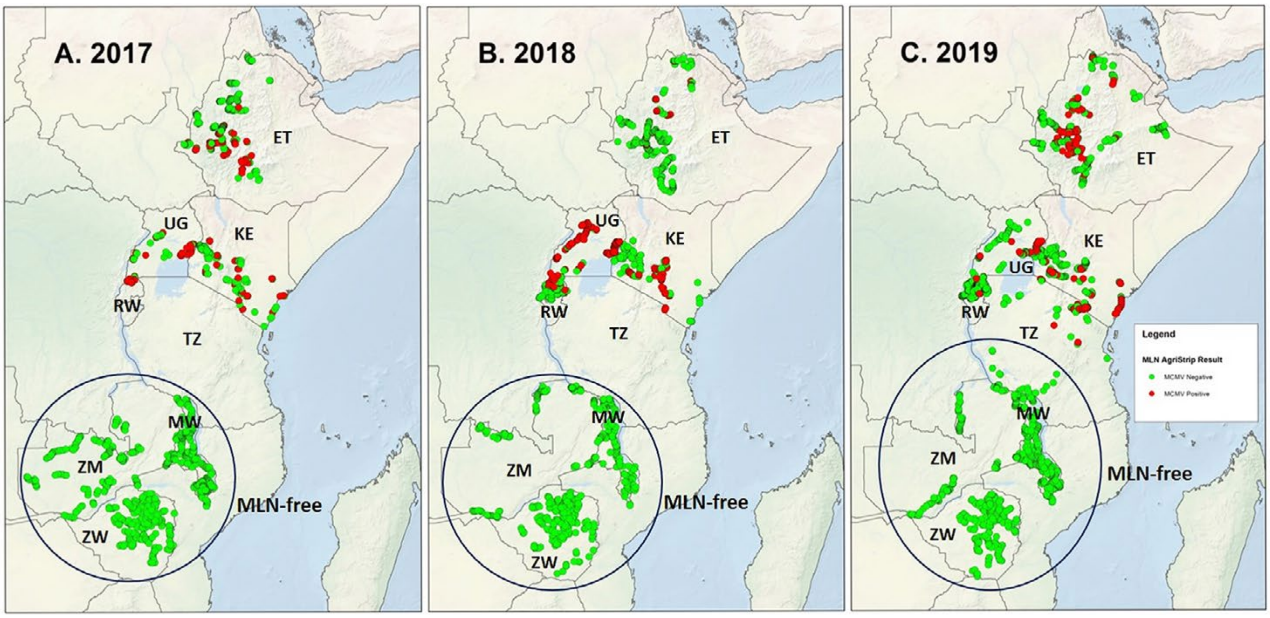

Figure 3. Results of MLN surveys (based on MCMV immunostrip data, coupled with evaluation for MLN symptoms) undertaken by the NPPOs in eight countries in eastern and southern Africa, in partnership with CIMMYT, in **(A)** 2017; **(B)** 2018; and **(C)** 2019. Source: Prasanna et al. (2020).

CIMMYT, in partnership with Aarhus University, Denmark, developed an MLN Surveillance Data Management Toolbox, an on-line data management system that supports field surveillance and seed surveys of MLN and other major maize diseases in SSA. The MLN toolbox enables centralized and secure management of standardized data for transboundary diseases at the continental level but with data managed, validated, and published at the country level. Features of the MLN toolbox include controlled access, secure storage in structured databases, data editing, data visualization through interactive maps and charts, and data export of country-specific data. Only when data has been checked and approved by authorized country managers does it enter in public domain data dissemination tools. The MLN toolbox represents an increasingly rich data resource on the status of MLN in SSA, with over 4500 field survey records from eight countries and more than 400 seed survey records currently in the database (Prasanna et al., 2020).

3.3. Production and Exchange of MLN Pathogen-free Commercial Maize Seed

As a rule, planting of healthy, certified, and treated seed is the first step for production of healthy crop that can in turn result in healthy seed. If MLN-causing viruses, especially MCMV, enter a new area through contaminated seed, and the infected plants are not diagnosed and rogued out in a timely manner, the control of the disease could become quite difficult. This is due to the possible presence of insect-vectors in the field that can potentially transmit the viruses within and across the fields.

In a recent study, Kimani et al. (2021) analyzed the seed contamination rates of MCMV in four seed lots; the results ranged from 4.9 to 15.9%. MCMV transmission frequency for 37,617 seedlings, tested in 820 pools of varying seed amounts, by Double Antibody Sandwich-Enzyme-linked Immunosorbent Assay (DAS-ELISA), was 0.17%, whereas a transmission frequency of 0.025% was obtained from 8,322 seedlings tested in 242 pools by real-time RT-PCR (Reverse Transcription-Polymerase Chain Reaction). Seeds from plants mechanically inoculated with MCMV had an overall seed transmission rate of 0.04% in 7,846 seedlings tested in 197 pools. 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. Nevertheless, even such low rates of transmission can be significant under field conditions where insect vectors can further spread the disease from infected seedlings, unless diseased plants are detected in time and properly managed.

From the phytosanitary perspective, it is critical to follow rigorous procedures to produce and exchange MLN pathogen-free commercial maize seed within and between countries to prevent the further spread and negative impact of the disease in Africa or in other continents where the disease is prevalent (see Chapter 8). In principle, the level of tolerance should be zero for acceptance or rejection of a seed lot where one of the two viruses causing MLN is detected. Ideally, seed produced from a plot/field that had MLN-infected plants must NOT be transferred to a known MLN-free location in the same country or outside the country. In practice, keeping a commercial seed production field completely free from the MLN-causing viruses in prevalent areas requires significant efforts and resources, but is important for protecting the food security, income, and livelihoods of the resource-poor smallholder farmers. Testing for MLN viruses in the seed is also important for NPPOs to ensure that the seed shipped to other countries, especially those which are free from MLN, is devoid of the pathogens.

During the early years of MLN outbreak in eastern Africa, most of the local/regional seed companies in the MLN-prevalent countries lacked necessary knowledge of the disease and its transmission, as well as access to protocols to produce and exchange MLN pathogen-free clean commercial seed. It was, therefore, critical to develop and implement protocols for MLN-free commercial seed production and exchange, especially from the affected to non-affected areas, and make these protocols widely accessible to the regulatory agencies and commercial seed companies. Several strategies have been put in place to achieve this objective, including development of comprehensive checklists and standard operating procedures (SOPs) for MLN-free seed production at various points along the seed value chain (see Chapter 8). Three consultative meetings, one each in Kenya, Tanzania, and Ethiopia, organized jointly by CIMMYT, AATF and AGRA, with active participation of researchers, seed companies and extension agents, helped in harmonizing the MLN-free seed production checklist and SOPs. Several training workshops were organized, targeting seed companies, seed trade associations, contract growers, NARS institutions, regulatory and extension agencies for disseminating SOPs and MLN management checklist. Various communication materials on MLN diagnostics and management were developed and disseminated to relevant seed stakeholders in MLN-prevalent as well as MLN-free countries in Africa and Latin America (see Chapter 8).

3.4. MLN Information Portal

As part of the strategy to ensure wider dissemination of information and increase awareness among relevant stakeholders, CIMMYT and partners in Africa established an MLN Information Portal (https://mln.cimmyt.org/). This portal is indeed a single source for updated information on MLN in sub-Saharan Africa, and provides access to research information (e.g., the availability of new MLN-tolerant/resistant germplasm), MLN screening facility updates, MLN surveillance status, communication products, and training course materials (Figure 4). The MLN Toolbox (data management system) is connected directly to the MLN Information Portal, enabling database-driven interactive maps and charts of surveillance data to be displayed automatically. User statistics for the MLN Information Portal indicate an increasing number of visitors with a near global distribution.

Figure 4. MLN Information Portal (https://mln.cimmyt.org).

3.5. MLN Phytosanitary Community of Practice

MLN mitigation strategies requires well-coordinated institutional efforts that effectively leverage expertise across multiple institutions. To achieve this, the MLN Phytosanitary Community of Practice (CoP) was established by CIMMYT in Africa in 2016, bringing together diverse partners, including phytosanitary and regulatory organizations, seed trade associations, NARES scientists, regional bodies etc. The objectives of the CoP are: a) to identify, gather, and seek agreement on the phytosanitary community requirements, especially for effective control of MLN in SSA; b) to provide a forum/platform for cooperation on activities where the CoP adds value to the existing initiatives; c) to share learning across borders on key aspects, such as standardized MLN diagnostics procedure(s), providing training on MLN diagnostics, expediting adoption of appropriate phytosanitary and diagnostic procedures, identifying/validating and deploying novel and low-cost MLN diagnostic protocols, etc.; d) to identify linkages and opportunities for collaborative strategic and technical projects related to MLN phytosanitation and diagnostics in SSA; e) to report on progress and provide updates of the projects and programs that have phytosanitary and diagnostics components related to MLN; and f) to provide information for the review of maize seed certification and import/export procedures in relation to MLN for formulation of appropriate SOPs (Prasanna et al., 2020).

4. Conclusions

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, including maize-dependent smallholder farmers, researchers, national plant protection authorities, commercial seed sector, etc. across the African continent. Rapid response and Intensive multi-disciplinary and multi-institutional efforts by an array of national and international institutions resulted in co-development and deployment of an array of tools/technologies to effectively tackle the MLN challenge (Prasanna et al., 2020). The fact that no maize-growing country in southern Africa or West Africa has reported further outbreak of MLN is a testimony to the huge and successful initiative to collectively manage the deadly disease. Nevertheless, there is no room for complacency! 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 still looms. It is, therefore, imperative to sustain MLN disease management, as outlined in various Chapters of this Technical Manual, not only in Africa but also globally through proactive and synergistic efforts.

5. References

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

FAOSTAT (2021) http://www.fao.org/faostat/en/#home

Hodson DP, Grønbech-Hansen J, Lassen P, Alemayehu Y, Arista J, Sonder K, Kosina P, Moncada P, Nazari K, Park RF, Pretorius ZA, Szabo LJ, Fetch T, Jin Y (2012) Tracking the wheat rust pathogens. In: McIntosh RA (ed) Proc. BGRI Technical Workshop, Beijing, China, 1-4 September 2012, pp. 11-22. Available online at: http://www.globalrust.org/sites/default/files/BGRI-2012-plenary.pdf.

Isabirye BE, Rwomushana I (2016). Current and future potential distribution of maize chlorotic mottle virus and risk of maize lethal necrosis disease in Africa. Journal of Crop Protection 5: 215-228.

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 https://doi.org/10.1094/PHP-02-21-0018-RS

Mahuku G, Lockhart BE, Wanjala B, Jones MW, Kimunye JN, Stewart LR, Cassone BJ, Sevgan S, Johnson N, Kusia E, Lava Kumar P, Niblett CL, Wangai A, Kiggundu A, Asea G, Pappu H, Prasanna BM, Redinbaugh MG (2015) Maize lethal necrosis (MLN), an emerging threat to maize-based food security in sub-Saharan Africa. Phytopathology 105: 956-965. https://doi.org/10.1094/phyto-12-14-0367-fi

Marenya PP, 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. https://doi.org/10.1016/j.agsy.2018.01.016

Mezzalama M, Das B, Prasanna BM (2015) MLN Pathogen Diagnosis, MLN-free Seed Production and Safe Exchange to Non-endemic Countries. CIMMYT, Mexico DF.

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

Park R, Fetch T, Hodson D, Jin Y, Nazari K, Prashar M, Pretorius Z (2011) International surveillance of wheat rust pathogens: progress and challenges. Euphytica 179: 109-117. https://dx.doi.org/10.1007/s10681-011-0375-4

Prasanna BM, Suresh LM, Mwatuni F, Beyene Y, Makumbi D, Gowda M, Olsen M, Hodson D, Worku M, Mezzalama M, Molnar T, Dhugga K, Wangai A, Gichuru L, Angwenyi S, Alemayehu Y, Grønbech-Hansen J, Lassen P (2020) Maize lethal necrosis (MLN): Containing the spread and impact of a devastating transboundary disease in sub-Saharan Africa. Virus Research 282: 197943. https://doi.org/10.1016/j.virusres.2020.197943

Prasanna BM, Cairns JE, Zaidi PH, Beyene Y, Makumbi D, Gowda M, Magorokosho C, Zaman-Allah M, Olsen M, Das A, Worku M, Gethi J, Vivek BS, Nair SK, Rashid Z, Vinayan MT, Issa AB, Vicente FS, Dhliwayo T, Zhang X (2021) Beat the stress: Breeding for climate resilience in maize for the tropical rainfed environments. Theoretical and Applied Genetics 134: 1729-1752. https://doi.org/10.1007/s00122-021-03773-7

Redinbaugh, M.G., Stewart, L.R., 2018. Maize lethal necrosis: an emerging, synergistic viral disease. Annual Review of Virology 5: 301-322. https://doi.org/10.1146/annurev-virology-092917-043413

Shiferaw B, Prasanna B, Hellin J, Banziger M (2011) Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. Food Security 3: 307-327. https://doi.org/10.1007/s12571-011-0140-5

Stewart LR, Willie K, Wijeratne S, Redinbaugh MG, Massawe D, Niblett CL, Kiggundu A, Asiimwe T (2017) *Johnsongrass mosaic virus* contributes to maize lethal necrosis in East Africa. Plant Disease 101: 1455-1462. https://doi.org/10.1094/PDIS-01-17-0136-RE