

Maize lethal necrosis (MLN) disease in Kenya and Tanzania: Facts and actions

A serious new disease of maize appeared in the farmers' fields in eastern Africa in 2011. Called maize lethal necrosis (MLN; or corn lethal necrosis, CLN), it can devastate maize crops. The disease is difficult to control for two reasons:

1. It is caused by a combination of two viruses that are difficult to differentiate individually based on visual symptoms.
2. The insects that transmit the disease-causing viruses may be carried by wind over long distances.



National and global research and extension organizations, laboratories, and seed companies are working together to control the spread of the disease and to develop and deploy disease-resistant maize varieties for the farmers as soon as possible.

What causes MLN?

The disease was first identified in the USA in 1976 (Niblett and Claflin 1978). MLN is caused by the double infection of maize plants with Maize chlorotic mottle virus (MCMV) and any of the cereal viruses in the Potyviridae group, such as Sugarcane mosaic virus (SCMV), Maize dwarf mosaic virus (MDMV), or Wheat streak mosaic virus (WSMV). MCMV or SCMV typically produce milder symptoms when they infect maize alone; in combination, these two viruses rapidly produce a synergistic reaction that seriously damages or kills infected plants.

Where has MLN appeared?

Kenya: Initial reports of an unknown disease outbreak surfaced in September 2011 in the Bomet county in the South Rift Region; further reports appeared in early 2012 in Naivasha, Narok North, Narok South, Chepalungu, and Sotik, as well as parts of the Eastern Province (Embu and Meru) and the Central Province (Murang'a, Kirinyaga, and Nyeri). MLN has also been reported recently in Trans-Nzoia, Uasin Gishu, and Busia.

A scientific team from the Kenya Agricultural Research Institute (KARI) and the International Maize and Wheat Improvement Center (CIMMYT) sampled infected maize plants in Bomet and Naivasha in February-March 2012. The samples were tested by serology and molecular methods for the presence of MCMV and SCMV at USDA-ARS/Ohio State University (Peg Redinbaugh's Laboratory), Wooster, Ohio, USA, as well as at the Food and Environment Research Agency, Sand Hutton, York, using next-generation sequencing with results and clearly indicating the presence of MLN (Wangai et al. 2012, Adams et al. 2012).

Tanzania: In August 2012, reports of an unknown maize disease emerged from Mwanza, near Lake Victoria, and Arusha. CIMMYT was invited by the government of Tanzania to survey the affected regions and identify the disease. Infected plant samples from the Mwanza and Arusha regions were serologically positive for MCMV and SCMV, confirming the presence of MLN.

Is MLN a new disease?

Yes, although one of its component viruses, SCMV, was reported in Kenya many years ago (Louie 1980). MCMV is a new virus for Africa; it had not been reported previously in Kenya, but was first identified in Peru in 1973 (Castillo and Hebert 1974) and subsequently reported in the USA, parts of Latin America, and China (Niblett and Claflin 1978; Uyemoto 1983; Xie et al. 2011). Wangai et al. (2012) reported MCMV and MLN in Kenya for the first time.

What are the typical symptoms?

- Mild to severe mottling on the leaves, usually starting from the base of young leaves in the whorl and extending upwards toward the leaf tips.
- Stunting and premature aging of the plants.
- Dying (known as "necrosis") of the leaf margins that progresses to the mid-rib and eventually the entire leaf.
- Necrosis of young leaves in the whorl before expansion, leading to a symptom known as "dead heart" and eventually plant death.



How severe are farm-level crop losses?

Infection rates and damage can be very high, seriously affecting yields and sometimes causing complete loss of the crop (Wangai et al. 2012; Adams et al. 2012). Infected plants are frequently barren; ears formed may be small or deformed and set little or no seed.



When and how are maize plants infected?

Maize plants are susceptible to MLN at all stages in their growth, from seedling to maturity. As with all viral diseases in plants, a carrier—known as a “vector”—transmits the MLN-causing viruses from plant to plant and field to field. MCMV is carried by thrips and beetles (Nault et al. 1978; Jiang et al. 1992) and SCMV by aphids (Brandes 1920; Pemberton and Charpentier 1969). Transmission of MCMV via seed from infected plants is normally very low (0.04%; Jensen et al. 1991).



How can MLN be controlled?

Based on CLN/MLN management experiences in the USA, rigorous disease management practices in seed production plots, including use of resistant varieties, controlling weeds/alternate hosts, keeping unnecessary machines/people out of the field, controlling insect-vectors using appropriate insecticide (at weekly intervals), and having adequate isolation from MLN-infected fields, can prevent the spread of the disease. Because individual plants with MCMV or SCMV alone show milder symptoms, seed production fields must be carefully inspected and plants that appear infected removed immediately.

How can farmers prevent MLN in their fields?

- MLN does not occur on crops other than maize; so avoid growing maize after maize. Diversify your farm enterprise by planting different crops each season.
- Do not plant a new maize crop near an infected field. Wind-blown insect vectors can transmit the disease from the infected field to the new crop.
- Plant maize at the onset of the main rainy season, rather than during the short rain season; this creates a break between maize crops and interrupts the disease cycle.

- Weed fields regularly to eliminate alternate hosts for insect vectors.
- Use maize varieties that are resistant to MLN.

What should farmers do if they find MLN in their fields?

- Immediately remove diseased plants from your fields. You can feed the leaves to livestock.
- Do not allow humans or animals to eat infected ears or grains, which may contain secondary fungal infections and harmful mycotoxins. Burn infected ears and grains.

Can MLN resistant maize varieties be developed soon?

Preliminary data from one season of screening under natural disease pressure of 43 pre-commercial maize hybrids and 7 commercial hybrids at Bomet, Chepkitwal and Naivasha (Table 1), and of 200 elite inbred lines at Naivasha (Table 2) suggest that MLN-resistant maize germplasm can be identified and developed quickly. KARI, CIMMYT, and other partners will reconfirm the potential resistance of pre-commercial hybrids and inbreds that showed the least susceptibility to MLN and work urgently to develop resistant varieties.

Are there potential sources of SCMV resistance?

Because MLN requires simultaneous infection by two viruses, resistance against any one of the causal viruses could significantly reduce crop damage. Results of a trial of elite CIMMYT inbred lines under artificial SCMV inoculation showed several highly-resistant lines (Table 3).

Table 1. MLN incidence on selected pre-commercial CIMMYT hybrids with least susceptibility under natural disease pressure (Kenya 2012 trials).

Entry	Naivasha			Bomet			Chepkitwal		
	DS	% DPLT	Rating	DS	% DPLT	Rating	DS	% DPLT	Rating
CKH10767	2.0	0	MR	2.6	26.2	MR	2.3	0	MR
CKH114272	2.0	8.9	MR	2.3	19.5	MR	2.1	15.2	MR
CKH101509	2.5	16.0	MR	1.9	13.3	MR	2.5	17.8	MR
Mean of three most susceptible commercial hybrids (checks)	2.9	44.5		3.4	34.7		2.8	23.4	
Min (across trial)	1.5	0		1.1	9.3		2.1	0.0	
Max (across trial)	4.0	42.8		4.2	67.1		3.9	53.5	
LSD (0.05)	1.2	19.1		1.2	11.8		0.62	10.8	

The trials were undertaken under natural disease (MLN) pressure at all the locations, using an alpha-lattice design with two replications per location, following standard agronomic management. They received no insecticide application.

DS: Disease Severity score (on 1-5 scale, with 1 = no symptoms; 5 = highly diseased) at different stages; %DPLT: % Dead Plants; MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible.

Table 2. MLN incidence on CIMMYT elite inbred lines with least susceptibility under natural disease pressure (Naivasha, Kenya 2012 trial).

Entry	DS	% DI	Rating
[CML312/CML444//DTP2WC4H255-1-2-2-BB/LATA-F2-138-1-3-1-B]-1-3-2-3-B]-2-1-2-BB-B-B-B	1.3	6.0	R
CL-02510-B	1.9	3.9	R
La Posta Seq C7-F64-1-1-1-2-B-B-B-B-B	1.7	7.2	R
CKL05003	2.2	1.9	MR
La Posta Seq C7-F64-2-6-2-2-B-B-B-B	1.5	13.5	MR
(KU1403 x 1368)-7-2-1-1-B-B-B-B-B	1.8	10.2	MR
(La Posta Seq C7-F86-3-1-1-1-B-B-B/ CML495)DH1-B-B	2.4	9.3	MR
La Posta Seq C7-F64-1-1-1-1-B-B-B-B-B	1.8	10.6	MR
P502c2-185-3-4-2-3-B-B-B-B-B-B-B	1.9	13.6	MR
CKL05017	2.7	11.0	MR
DRB-F2-60-1-1-1-B*6-B	3.5	34.8	S
[CML444/CML395//DTPWC8F31-4-2-1-6]-3-1-2-1-1-B*4-B-B	3.3	32.9	S
INTA/INTB-B-41-B-7-1-B-B-B	3.2	56.2	S
(La Posta Seq C7-F64-2-6-2-2-B-B-B/CML495)DH29-B-B	4.2	59.8	S
(DTPWC9-F92-2-1-1-1-BB/[MSRXG9]C1F2-205-1(OSU23i)-5-3-X-X-1-BBB-1-B)DH3-B-B	4.1	69.9	S
CML503 (one of the highly susceptible entries in the trial)	3.4	97.2	S
Min (across trial)	1.0	1.9	
Max (across trial)	4.4	100.0	
LSD (0.05)	1.1	26.0	

The trials were undertaken under natural disease (MLN) pressure at all the locations, using an alpha-lattice design with two replications per location, following standard agronomic management. They received no insecticide application.

DS: Disease Severity score (on 1-5 scale, with 1 = no symptoms; 5 = highly diseased) at different stages; %DI: % Disease Incidence; R: Resistant; S: Susceptible.

Table 3. SCMV incidence on most resistant and susceptible CIMMYT elite inbred lines under artificial inoculation (Data courtesy of Monsanto, Waterman, Illinois, USA; 2012 trial).

Entries	% DI (1st rating)	% DI (2nd rating)	Response to SCMV
CML144	0%	0%	Highly Resistant
CML312	0%	0%	Highly Resistant
CML511	0%	0%	Highly Resistant
P100C6-200-1-1-B***	0%	0%	Highly Resistant
P300C5S1B-2-3-2-#-#-1-2-B-B-#	0%	0%	Highly Resistant
CML539	6%	6%	Resistant
CML395	14%	18%	Moderately Resistant
CML78	15%	18%	Moderately Resistant
CML159	16%	29%	Moderately Susceptible
La Posta Seq C7-F64-2-6-2-1-B-B-#	26%	33%	Susceptible
DTPWC9-F16-1-1-1-1-BB-#	47%	47%	Susceptible
La Posta Seq C7-F86-3-1-1-1-BB-#	20%	52%	Susceptible
CML445	50%	77%	Susceptible
CML204	47%	70%	Susceptible
CZL03007	57%	72%	Susceptible
CML202	64%	72%	Susceptible
DTPWC9-F115-1-4-1-1-B-B-#	65%	65%	Susceptible
CML488	68%	73%	Susceptible
CML489	71%	97%	Susceptible
CZL00003	77%	81%	Susceptible
DTPWC9-F104-5-4-1-1-B-B-#	79%	80%	Susceptible
La Posta Seq C7-F180-3-1-1-1-BB-#	81%	80%	Susceptible
CML444	83%	96%	Susceptible

Each entry was inoculated twice with SCMV, and the disease ratings were recorded twice – at the vegetative stage, and after flowering.

DI: Disease Incidence; Disease response based on following scale: 0-10% - Highly Resistant; >10-20% - Moderately Resistant; >20-30% - Moderately Susceptible; >30% - Susceptible.

What are the next steps to develop and deploy MLN-resistant varieties?

- CIMMYT and KARI will screen a large set of diverse pre-commercial hybrids from CIMMYT and other public and private institutions during the ongoing short rain season (November to March) at selected sites in Kenya where MLN has already been reported, to identify and validate MLN resistance. Resistant hybrids may be considered for quick release in Kenya.
- In collaboration with the Ministry of Agriculture in Tanzania, CIMMYT will screen the same pre-commercial hybrids and inbred lines at selected locations in Tanzania during 2012-13 under natural disease pressure, to identify potential sources of MLN resistance.
- CIMMYT will continue to search for MLN resistance in its vast germplasm base, focusing in particular on elite and diverse inbred lines.
- CIMMYT will immediately begin research to identify maize genomic regions associated with MLN, SCMV, and MCMV resistance, for DNA marker-assisted accelerated breeding for resistant germplasm.
- Partners in eastern and southern Africa will multiply seed of parental lines of resistant hybrids.
- MLN screening procedures, including generation of reliable phenotypic data through controlled inoculation, will be developed jointly by USDA, ARS/Ohio State University, KARI, and CIMMYT, with expert support from relevant public and private institutions.
- KARI and CIMMYT will explore possibly establishing a centralized MLN screening facility with artificial inoculation capacity at a disease hotspot in Kenya, to generate high-quality data on MLN resistance or susceptibility in maize breeding lines and varieties.
- An MLN disease manual will be developed, including standardized disease screening and artificial inoculation protocols.
- Investment will be sought for large-scale training of seed service, seed company, and national research and extension service personnel in MLN disease identification and preventive measures. A training workshop will be organized in the first quarter of 2013.

We seek your active support to contain this new threat to food security in Africa.

For further information, to provide assistance, or to report an MLN sighting:

- **Dan Makumbi**, Maize Breeder, CIMMYT-Nairobi, Kenya (d.makumbi@cgiar.org)
- **Anne Wangai**, Chief Research Officer, KARI (anne_wangai@yahoo.com)

References

- Adams IP et al. (2012) Use of next-generation sequencing for the identification and characterization of Maize chlorotic mottle virus and Sugarcane mosaic virus causing maize lethal necrosis in Kenya. *Plant Pathology* (Published Online: Doi: 10.1111/j.1365-3059.2012.02690.x).
- Brandes EW (1920) Artificial and insect transmission of sugarcane mosaic. *Journal of Agricultural Research* 19: 131-138.
- Castillo J, Hebert TT (1974) Nueva enfermedad virosa afectando al maiz en el Peru. *Fitopatologia* 9:79-84.
- Jiang XQ et al. (1992) Maize chlorotic mottle virus in Hawaiian-grown maize – vector relations, host range and associated viruses. *Crop Protection* 11:248-254.
- Nault LR et al. (1978) Transmission of maize chlorotic mottle virus by Chrysomelid beetles. *Phytopathology* 68: 1071-1074.
- Niblett CL, Claflin LE (1978) Corn lethal necrosis – a new virus disease of corn in Kansas. *Plant Disease Reporter* 62: 15-19.
- Jensen SG et al. (1991) Seed transmission of maize chlorotic mottle virus. *Plant Disease* 75: 497-498.
- Louie R (1980) Sugarcane mosaic virus in Kenya. *Plant Disease* 64: 944-947.
- Wangai A et al. (2012) First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis in Kenya. *Plant Disease* 96: 1582.
- Pemberton CE, Charpentier LJ (1969) Insect vectors of sugarcane virus diseases. In: *Pests of Sugarcane*. (Eds. Williams JR, Metcalfe JR, Mungomery RW, Mathers R), pp. 411-425.
- Uyemoto JK (1983) Biology and control of maize chlorotic mottle virus. *Plant Disease* 67:7-10.
- Xie L et al. (2011) Characterization of maize chlorotic mottle virus associated with maize lethal necrosis disease in China. *Journal of Phytopathology* 159: 191-193.