Maize viral diseases: MLN and MSV

Global Maize Program

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Maize Pathology – Sub Saharan Africa

CIMMYT- Kenya

Workshop title - Maize foliar fungal diseases: field scoring to molecular diagnostics

Maseno University, Kisumu, Kenya

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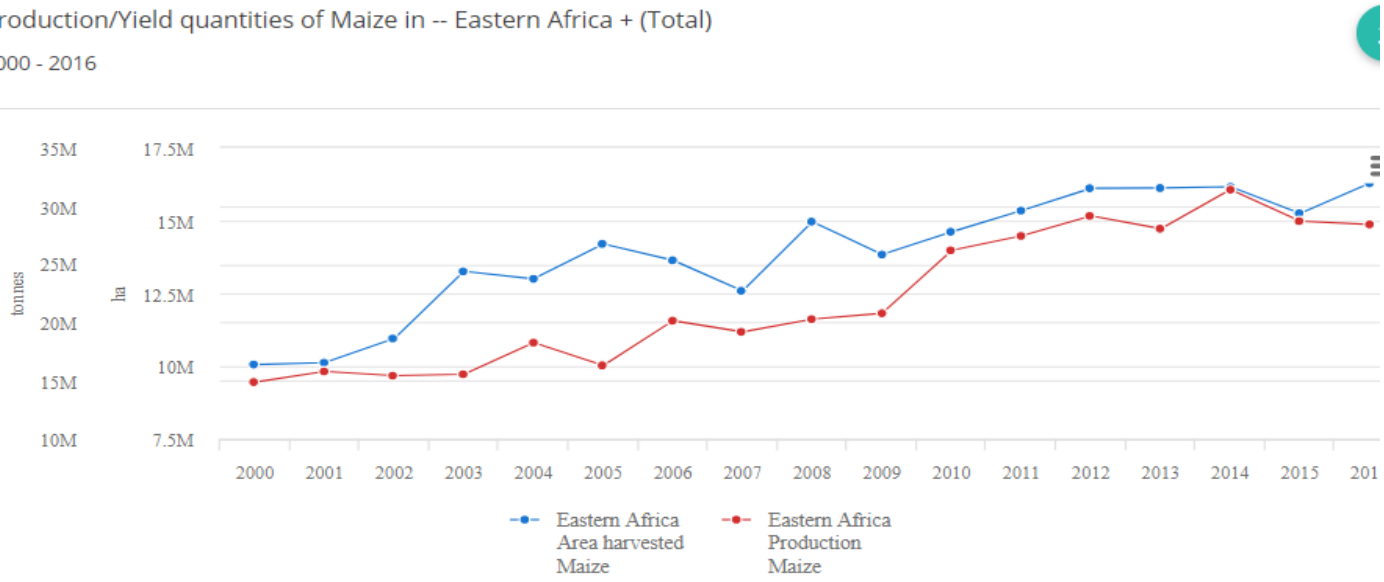
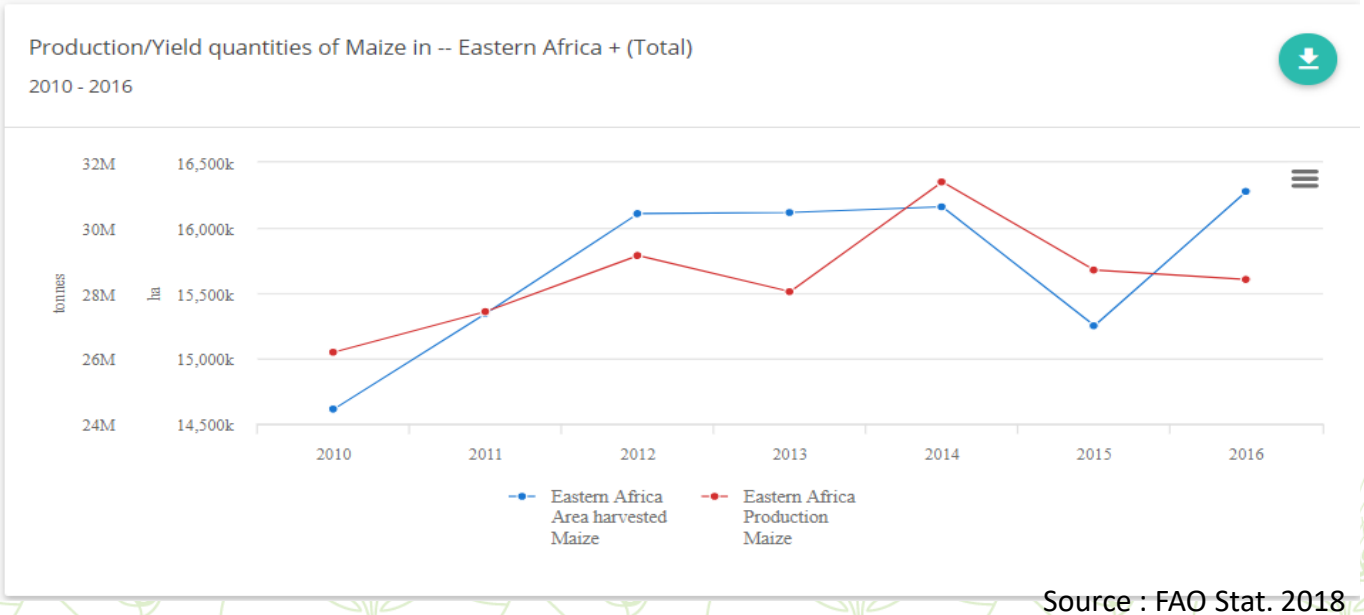
Maize – Area and production in eastern Africa





Source : FAO Stat. 2018

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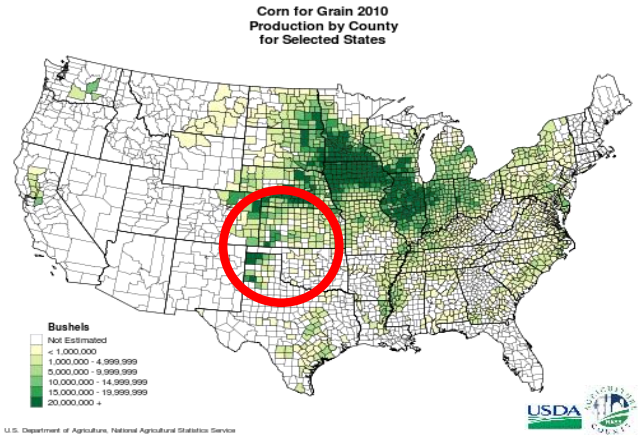
History of MLN

- First report of viral co-infection (MCMV+MDMV) leading to MLN was reported in Kansas and Nebraska (USA) in 1976 (Niblett). Disease was termed Corn Lethal Necrosis (CLN)



- In the 1990s, co-infection of MCMV+MMV was reported to cause extensive damage to temperate seed production in Hawai.

- **First report of MLN in Africa made in 2011 in Kenya (Wangai et al, 2012). Causal agents identified to be MCMV and SCMV by tissue blot immuno-assay and PCR.**

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MLN is a viral disease caused by combined infection of maize with Maize Chlorotic Mottle Virus (MCMV) and any of the Potyviruses infecting cereals, especially Sugarcane Mosaic Virus (SCMV)

The disease was first reported in Africa, particularly in Kenya in Sept 2011, and since then reported in Uganda, Tanzania, Rwanda, D.R. Congo, and Ethiopia.

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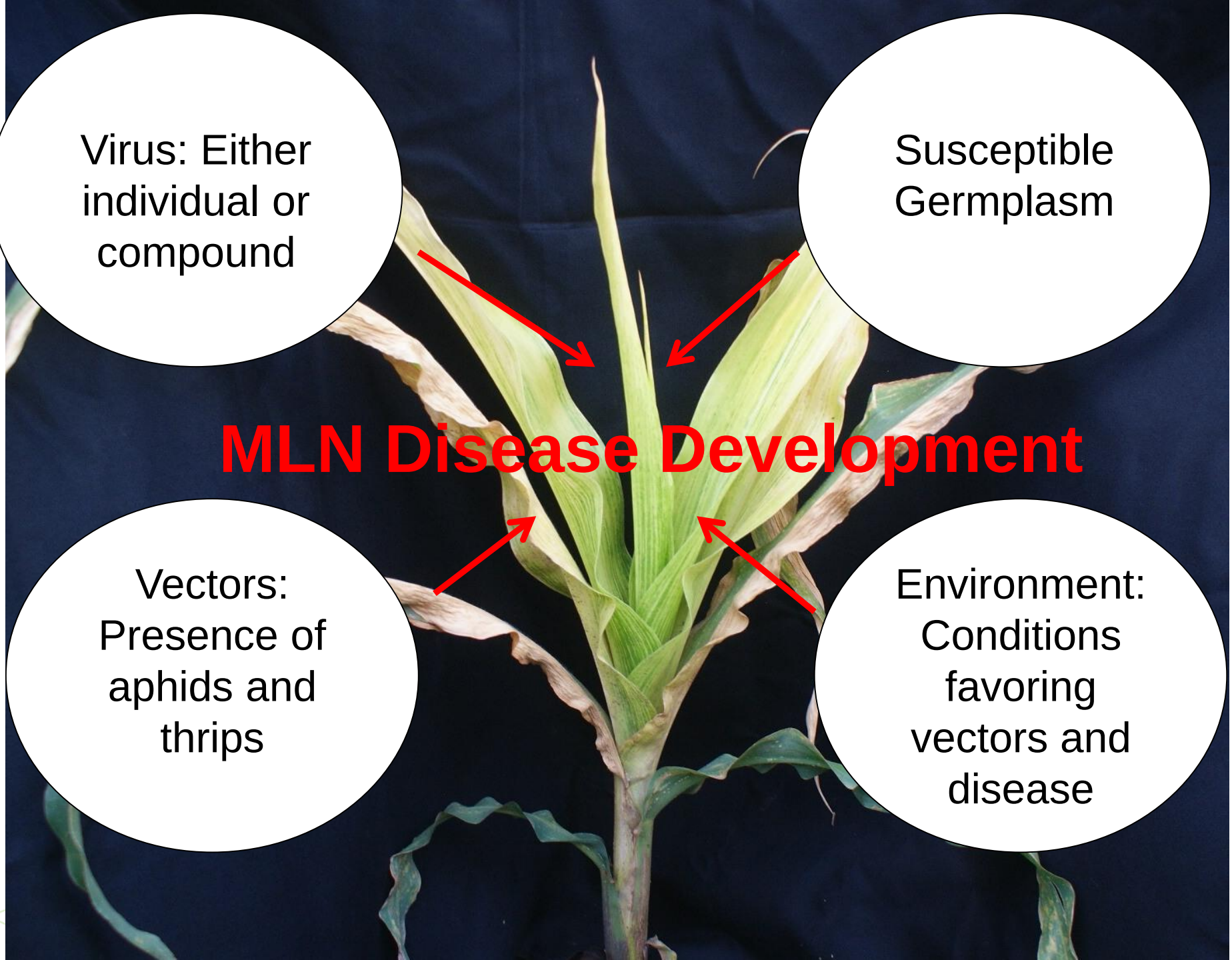

Virus: Either individual or compound
Susceptible Germplasm
MLN Disease Development
Vectors: Presence of aphids and thrips
Environment: Conditions favoring vectors and disease

What is Maize Lethal Necrosis

MCMV

Potyvirus :
MDMV,
SCMV,
WSMV

MLN

- Individual infection with each virus will also cause disease
- Typically infection with one virus results in milder symptoms than MLN, but reaction depends upon viral strain and germplasm.

What is MLN?

Viral Disease of Maize caused by double infection of:

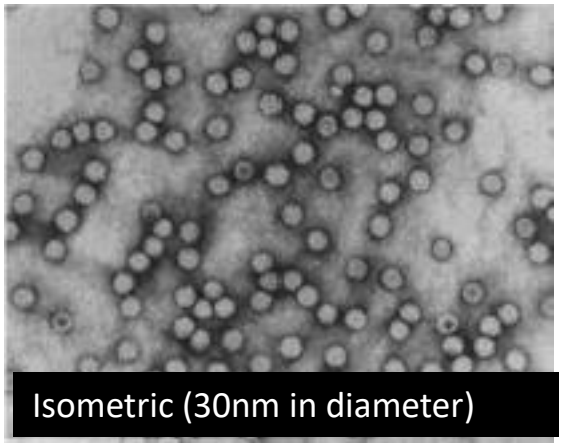
Maize chlorotic mottle virus (MCMV), Tombusviridae family

Cereal virus in Potyviridae family:

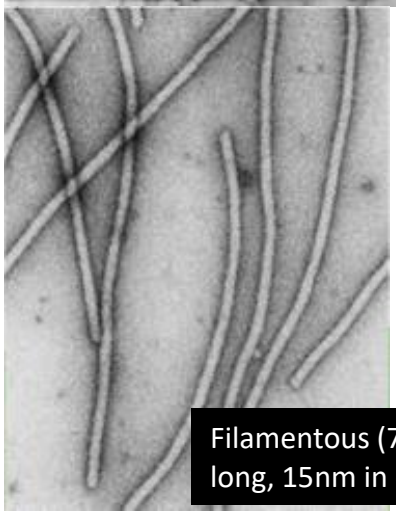
- Sugarcane Mosaic Virus (SCMV)
- Maize Dwarf Mosaic Virus (MDMV)
- Wheat Streak Mosaic Virus (WSMV)

Prior to 1989 (Shukla et al., 1989), MDMV was considered to be a strain of SCMV and the two names were used interchangeably.

Maize Mosaic Virus (MMV), Rhabdovirus family. Causes corn stunt and is spread by leafhoppers.



Isometric (30nm in diameter)



Filamentous (700nm long, 15nm in diameter

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Maize Lethal Necrosis (MLN)

- Symptoms: Severe mottling of leaves, dead heart, stunted growth (shortened internode distance), leaf necrosis and barren ears.

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Host Pathogen – Synergism

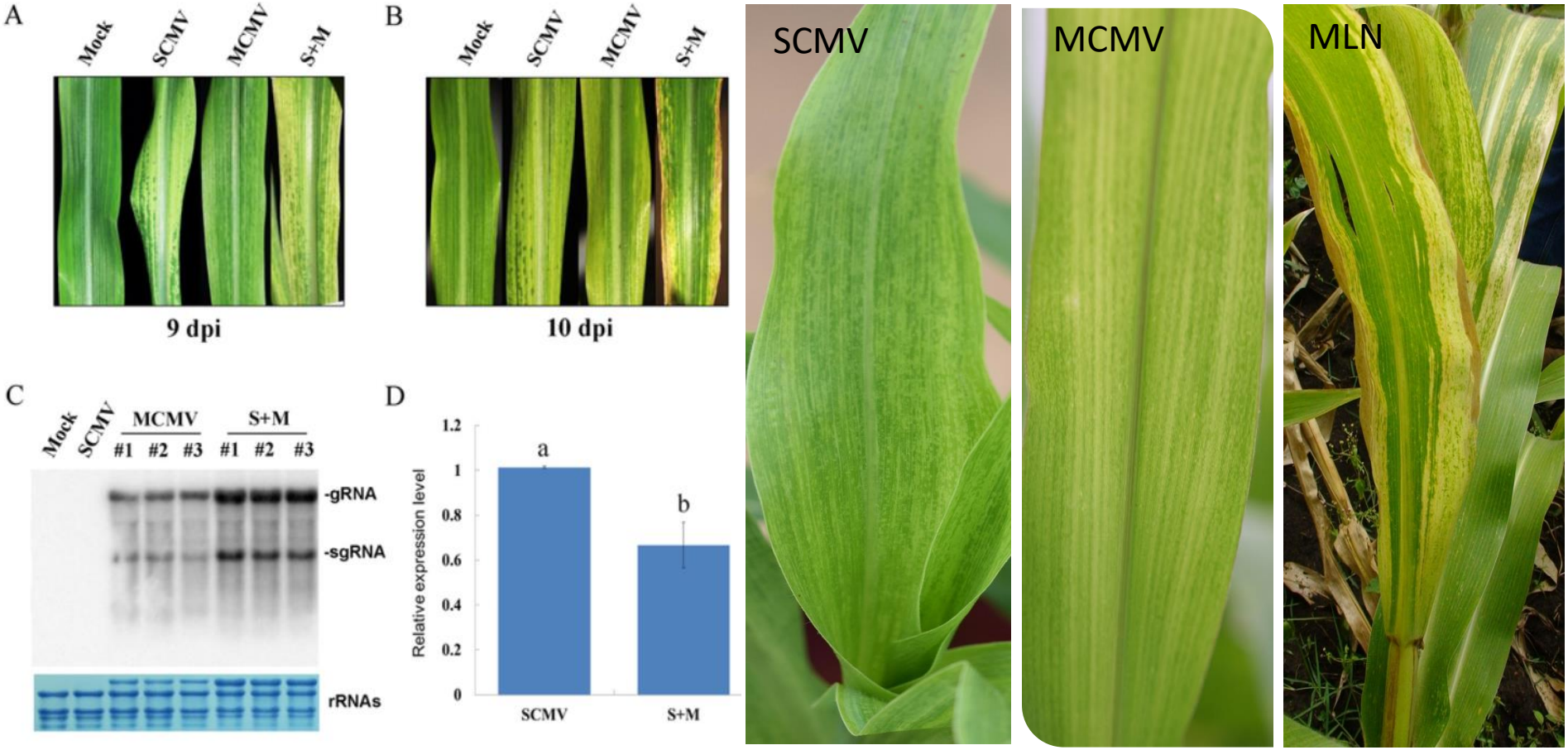

Figure 1. Co-infection of SCMV and MCMV increased the accumulation of MCMV. (A,B) The symptoms of the first systemically infected leaves at 9 and 10 dpi, respectively. (C) The accumulations of MCMV genome were determined by Northern blotting at 9 dpi in buffer (Mock), SCMV, MCMV and S + M inoculated maize plants. Three independent MCMV and S + M infected maize plants were used, and Mock and SCMV inoculated plants were used as controls. Methylene blue staining (bottom panel) of the same extracts was shown to demonstrate equal loading. (D) The relative expressions of SCMV RNAs were determined by qRT-PCR at 9 dpi in SCMV and S + M infected maize plants.

Source : Zihao Xia., Scientific Reports (2018) | 6:20520 | DOI: 10.1038/srep20520

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MLN/CLN is not a new disease in maize

But why was it so devastating in eastern Africa?

- Conducive environment – continuous maize cropping in certain areas in ESA (especially where there is bimodal rainfall pattern) leading to continuous build-up of virus inoculum
- **Seed contamination by** MLN-causing viruses, especially MCMV, besides local spread through insect vectors
- **Difficulty in finding elite maize germplasm with tolerance/resistance against MCMV / MLN.**

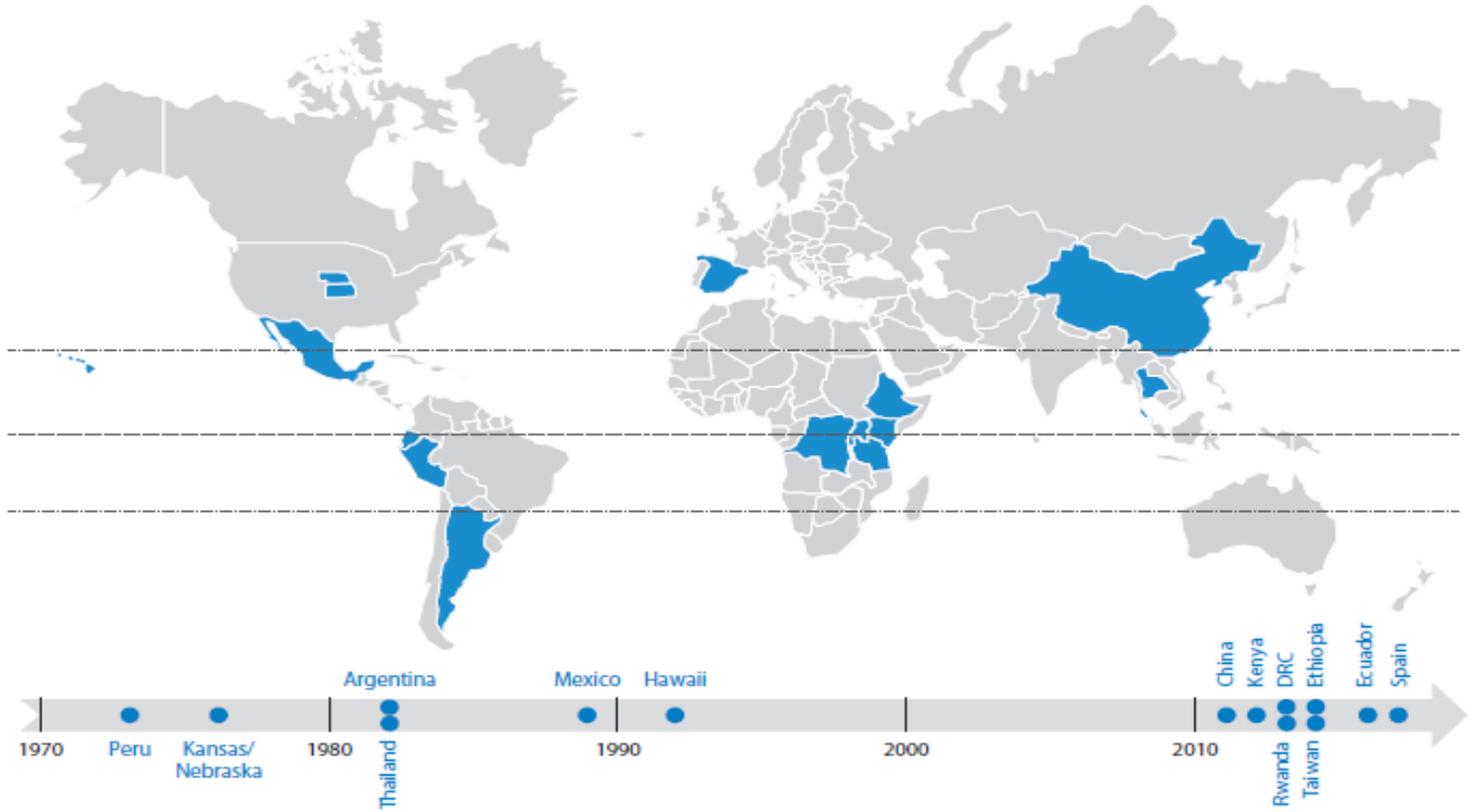

Figure 1

Emergence of maize chlorotic mottle virus (MCMV). MCMV has been reported in a number of countries (*blue*), and in the United States primarily in Kansas, Nebraska, and Hawaii. The reported year of MCMV emergence is indicated on the timeline. Abbreviation: DRC, Democratic Republic of the Congo.

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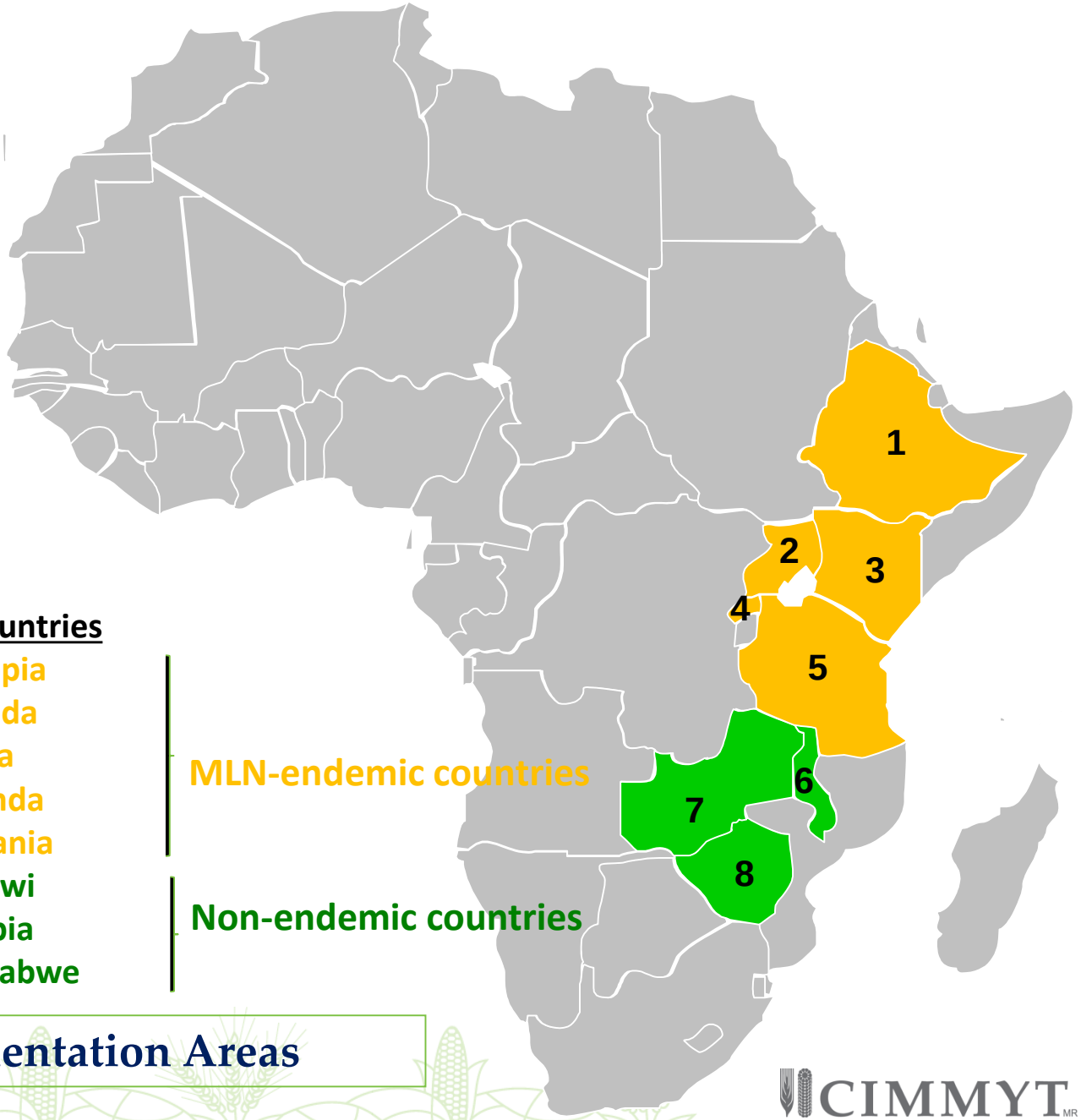
Need to control the spread and impact of MLN in sub-Saharan Africa



Target countries

1. Ethiopia
2. Uganda
3. Kenya
4. Rwanda
5. Tanzania
6. Malawi
7. Zambia
8. Zimbabwe

MLN-endemic countries

Non-endemic countries

Project Implementation Areas

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MCMV – diversity studies - SSA

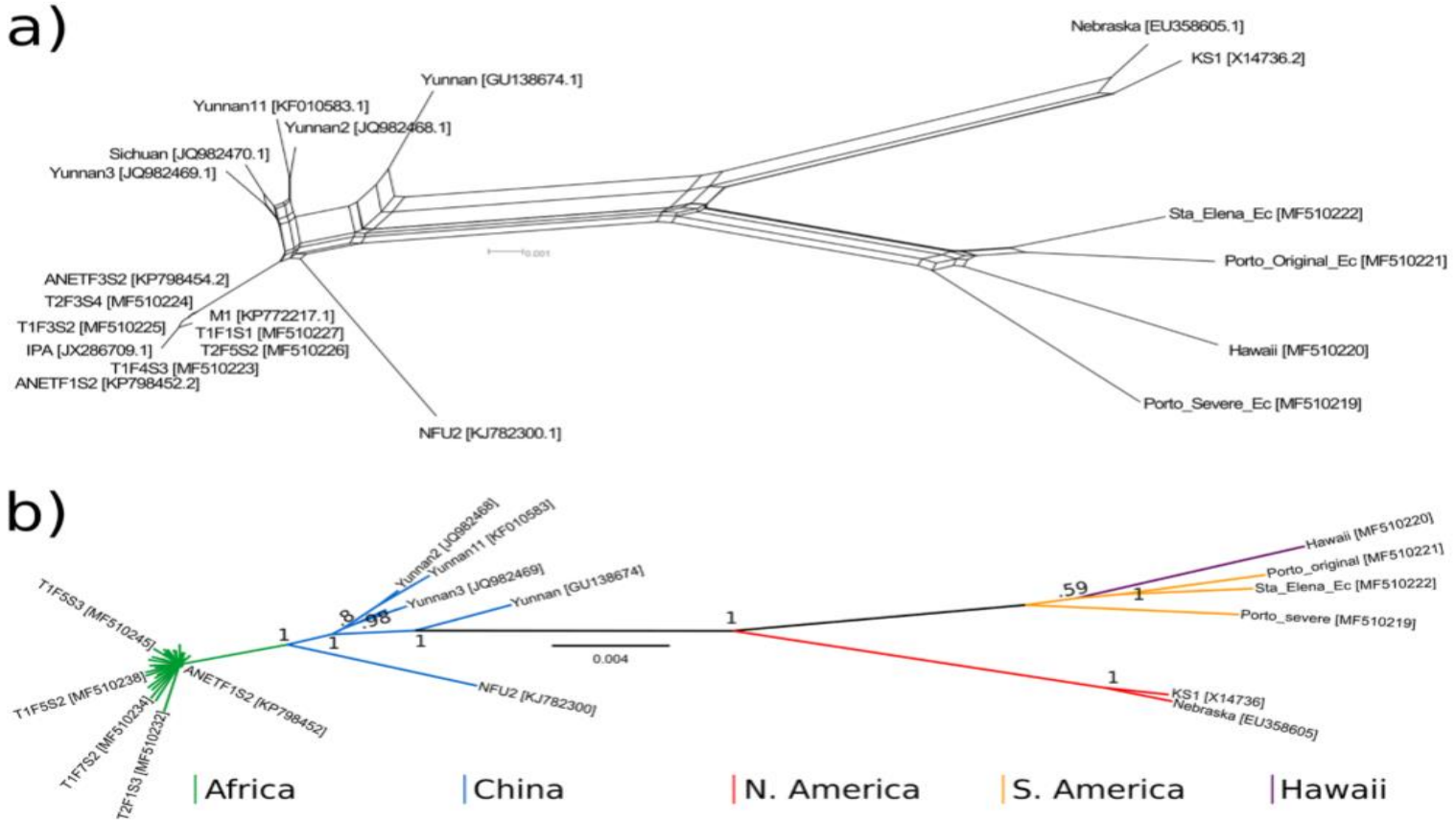

Figure 2. (**a**) Splits network of maize chlorotic mottle virus (MCMV) genomes, distances calculated with uncorrected P, and network generated by neighbour-net in Splitstree V4.6. (**b**) MCMV phylogeny generated using Bayesian inference in Mr Bayes 3.2. Scale bars are nucleotide substitutions per site.

Virus	Family	Isolate number	Sequence type	Number of continents	Average nucleotide diversity
MCMV	*Tombusviridae*	49	Genome	4	0.01
MCMV	*Tombusviridae*	49	CP	4	0.01

Source : Luke Braidwood et.al.,, Scientific reports | (2018) 8:1173 | DOI:10.1038/s41598-018-19607-4

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MCMV

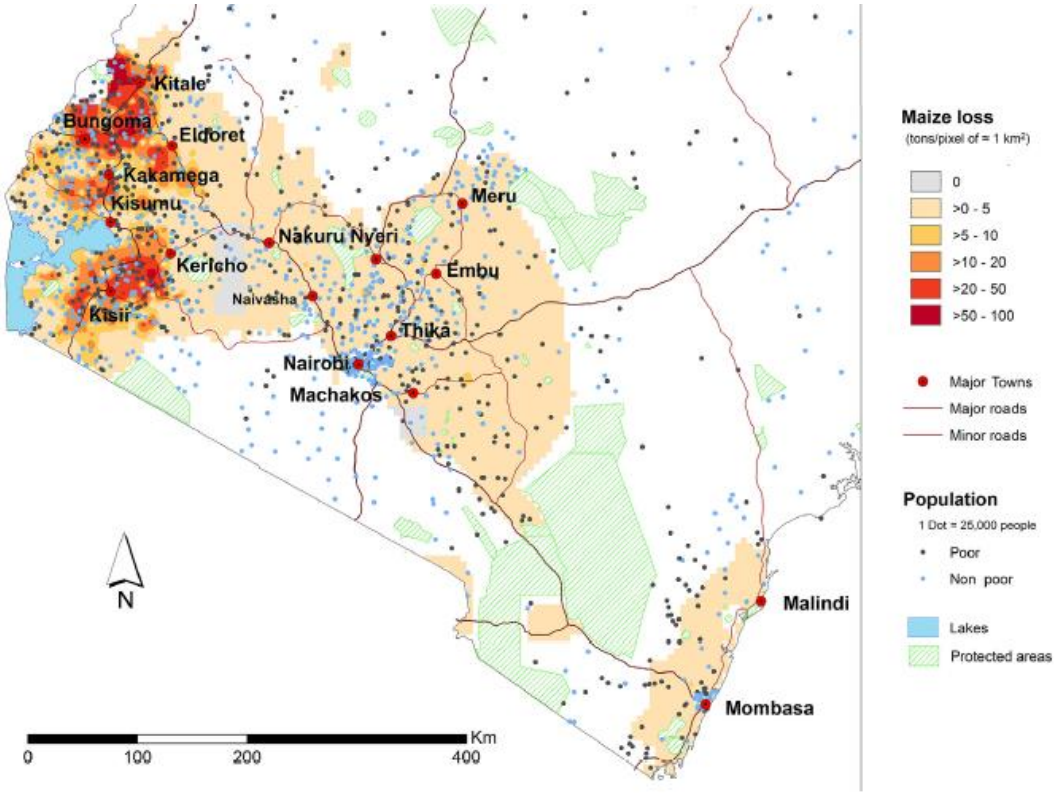
- MCMV first reported to infect maize in Peru (Herbert and Castillo, 1973) – yield loss of 10-15% in some cultivars (Nault et al, 1978)
- Subsequently reported in Argentina, Brazil, Peru, Mexico, USA, Thailand and China (Nyvall, 1999)
- Major problem for temperate seed production in Hawaii in the 1990s (Nelson et al, 2011)
- **From Kenya (Wangai et al., 2012)**

Two geographically and genetically different strains of MCMV have been reported (Nyvall,1999):

- MCMV (K) – Kansas
- MCMV (P) – Peru
- There may be others…

Losses due to MLN in Kenya (2014)

- Yield loss of 0.5m metric tons in Kenya in 2014 based on community based surveys. This is equivalent to US$187m.
- USDA Foreign Agric Service estimated yield losses of 10% in the 2014B cycle in Kenya equivalent to US$ 50m.



De Groote et al., 2016

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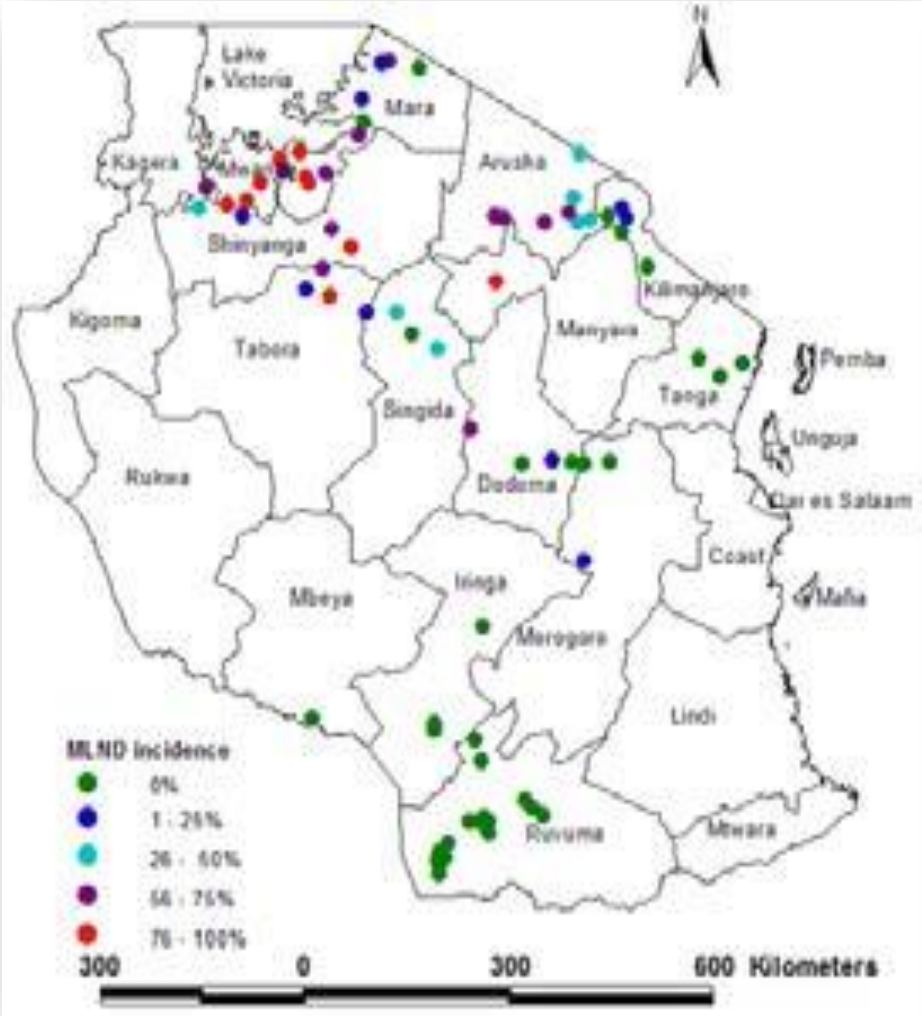
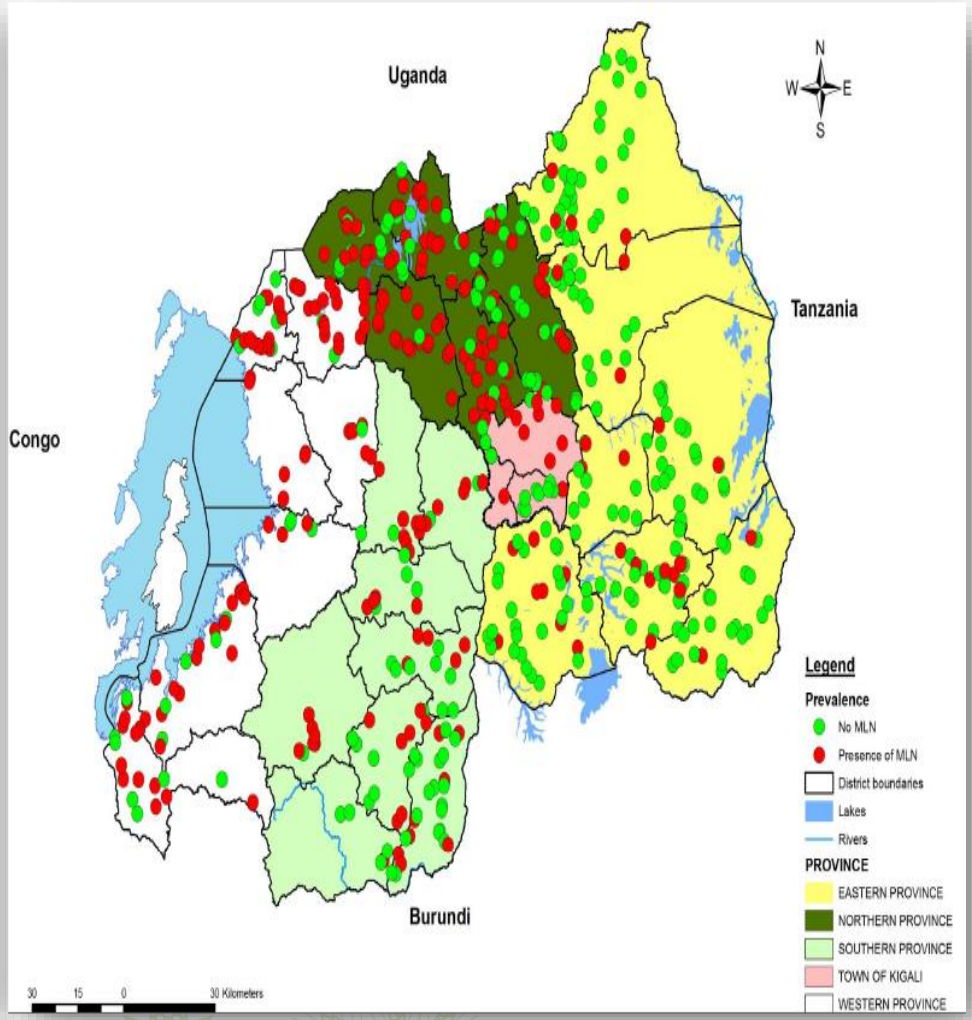
MLN in Eastern Africa

TANZANIA



RWANDA



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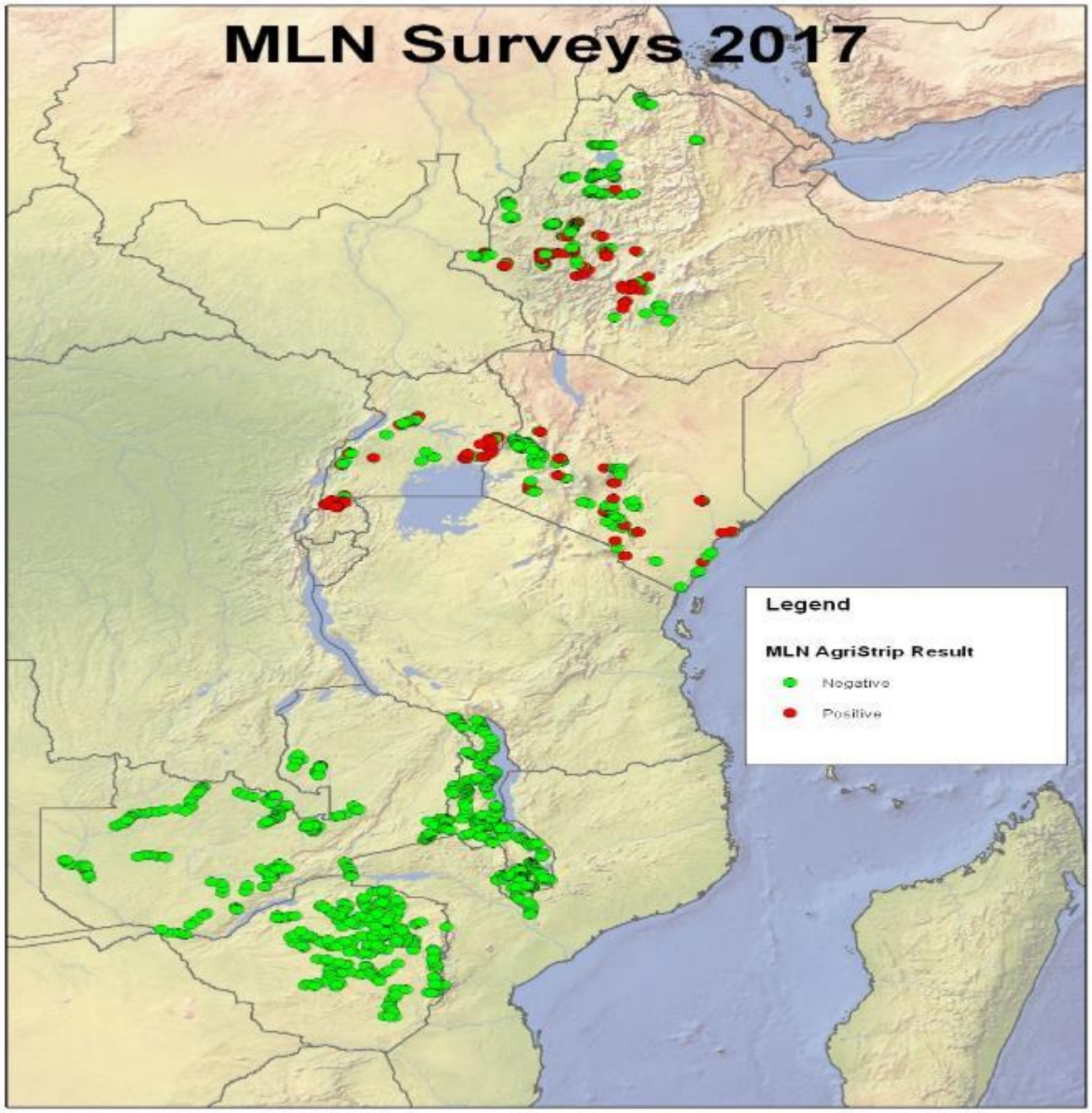

Surveyed Areas in the Region-2017

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Disease Symptoms

Symptoms of the disease

- Symptoms observed vary widely depending on;

  -Germplasm

  -Time of infection

  -Prevailing environmental conditions

  -Ratios of the viruses infecting the plant

- The symptoms can easily be confused with drought , micro- nutrient deficiency or stalk borer infestation

MLN Symptoms

Mild mosaic and mottling

Mild mosaic and mottling

Chlorosis and Mottling

Diffuse mottling and chlorosis

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Disease Symptoms

- Dying leaves, leading to premature plant death
- Failure to tassel and sterility in male plants
- Malformed or no ears
- Rotting cob

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Symptoms in artificially inoculated maize plants in screen house

MCMV

SCMV

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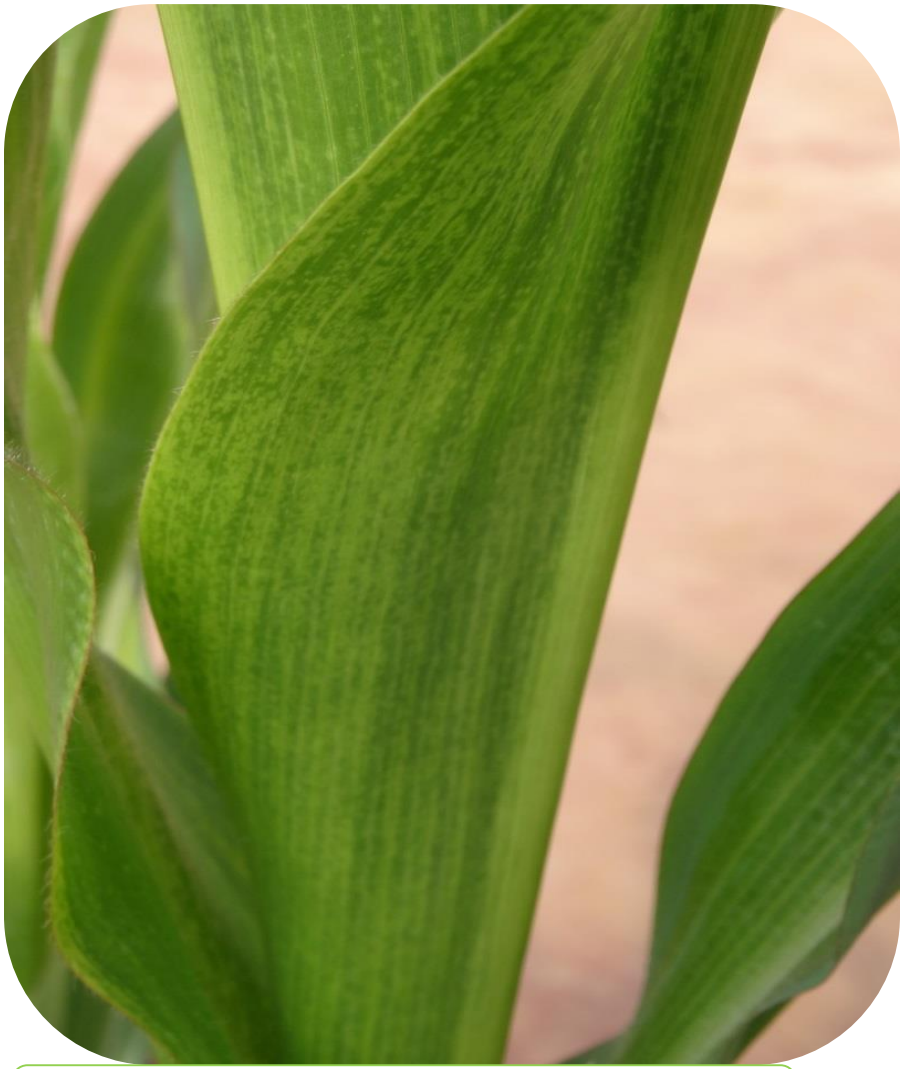
Early Symptoms

Mild mosaic and mottling

Mild mosaic and mottling

Early MLN Symptoms

Chlorosis and Mottling



Diffuse mottling and chlorosis

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Severe chlorosis and leaf necrosis

Shortened internodes and severe chlorotic mottle

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‘Dead Heart’ symptoms

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Premature drying of the husks

Poor or no grain filling

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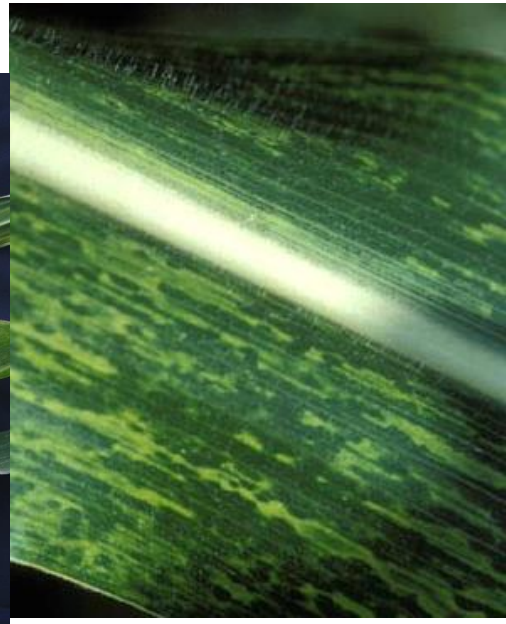
SCMV and MCMV symptoms

- Chlorotic spots/mosaics on lower leaves developing into streaks along leaf veins.
- As plants approach maturity, leaves become yellow
- Slight stunting (higher incidence of ear and stalk rot if early disease infection)
- Seed set is affected.



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Poor seed set and shrivelled ears

Shortened Internodes

Early leaf necrosis

MCMV symptoms

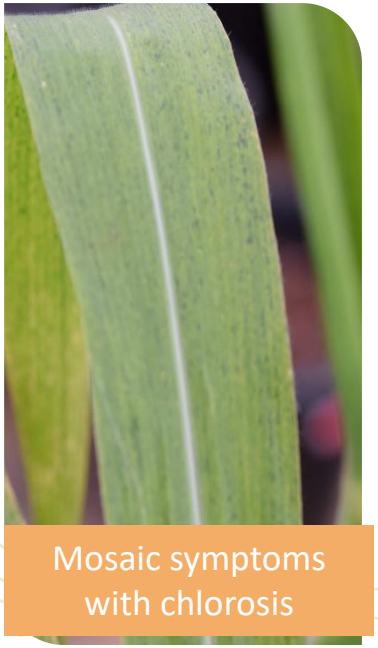
Mottling

Chlorotic stripes

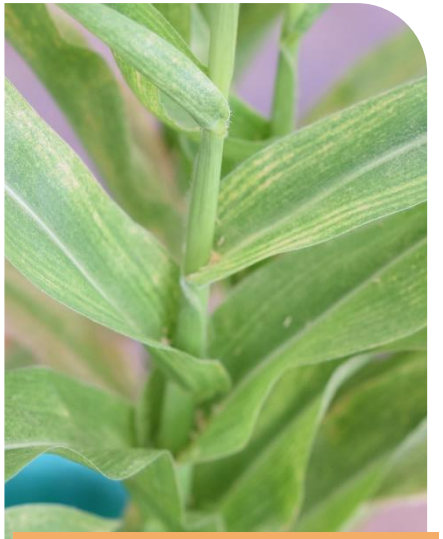
Inter-veinal necrosis / severe chlorosis

Coalition of chlorotic spots forms chlorotic stripes

Mild chlorotic stripes



Mosaic symptoms with chlorosis



Shorter intermodal distance

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MLN Symptoms

- Mottling symptoms on leaves, usually starting from base of young leaves in the whorl and extending outwards
- Stunting and shortened internodes
- Dead heart and necrosis
- Sterility, poor seed set, shrivelled seeds

Why is the MLN devastating in EA?

- MCMV is new to the region - Widespread cultivation of susceptible germplasm that has never been screened for MCMV
- Current strain of MCMV is highly virulent.
- Agronomic practices of year round maize cultivation, late planting, recycling seed
- Seed transmission rates are higher than earlier reported facilitating the rapid spread of the MCMV virus.
  - **Seed transmission could be higher than 15%**

MCMV: Transmission and Alternate Hosts

Transmission:

- Primary transmission through insect vectors
  - Thrips (Frankliniella williamsi)
  - Chrysomelid beetles
  - Rootworms
- Vectors can often survive on a wide variety of cultivated crops and weeds.
- Mechanically
- Seed : 0.04% (18 plants per 44,0000) – Jensen et al., 1991

Alternate hosts: grasses in Poacea – 73 species including wheat, sorghum, oats, johnson grass (Sheets, 2005)

Table 1. Plants tested for susceptibility to strains of MCMV (Scheets 2004).

Immune genera[1]	Susceptible genera	Genera with both immune and susceptible species
Axoponus	*Andropogon*	*Agropyron*
Chloris	*Avena*	*Bromus*
Elymus	*Bouteloua*	*Cenchrus*
Festuca	*Buchloe*	*Cynodon*
Lolium	*Calamovilfa*	*Dactylis*
Oryza	*Eleusine*	*Digitaria*
Paspalum	*Eragrostris*	*Echinochloa*
Poa	*Euchlaena*	*Panicum*
Saccharum	*Hordeum*	*Phalaris*
	Secale	*Setaria*
	Sorgastrum	*Zea*
	Sorghum	
	Spartina	
	Tripsacum	
	Triticum	

[1]Status of hosts listed in this table are a result of experimental inoculations, not natural field infection.

Insect vectors – MLN transmission

Tetranychus sp.

Frankliniella sp.

Rhopalsiphum sp.

Graminella sp.

Apion sp.

Callosobruchus sp.

Anthaxia sp.

Hippodamia sp.

Systates sp.

Anthrenus sp.

Selenocephalus sp.

Nariscus sp.v

Trialeuroide sp.

Myzus sp.

Species	Order	% insects
Apion sp.	Coleoptera	0.96
Callosobruchus sp.	Coleoptera	1.16
Anthaxia sp.	Coleoptera	1.19
Monolepta sp.	Coleoptera	0.89
Hippodamia sp.	Coleoptera	0.69
Systates sp.	Coleoptera	0.96
Anthrenus sp.	Coleoptera	1.16
Selenocephalus sp.	Hemiptera	5.49
Nariscus sp.	Hemiptera	6.75
Arocatus sp.	Hemiptera	4.64
Rhopalosiphum sp.	Hemiptera	4.85
Myzus sp.	Homoptera	5.11
Trialeuroides sp.	Homoptera	4.60
Graminella sp.	Homoptera	4.78
Tetranychus sp.	Trombidiformes	2.37
Frankliniella sp.	Thysanoptera	8.24

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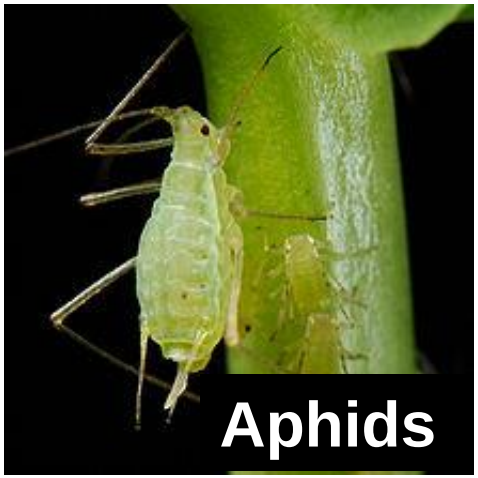
Methods : Observations on arthropods using yellow, bue sticky and water traps for 8 weeks after planting

Transmission of SCMV, MDMV

1. Primarily by insect vectors:

   Aphids (up to 15 species)

   WSMV is transmitted by mites



2. Seed Transmission – 0.007 to 0.4% reported (Hill et al., 1974; Mikel et al., 1984)

3. Mechanical

Virus can persist in infected overwintering crop residue

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Diagnostics

- Symptoms: Complicated as symptoms are similar and may differ according to environment, stage of infection and germplasm
- ELIZA (Enzyme linked immunosorbent assay)
- PCR based methods
- Tissue blot immunoassay
- Immuno strip

Accelerated Breeding for MLN Resistance

KALRO-CIMMYT MLN Screening Facility at Naivasha (Kenya)

- > **150,000 diverse germplasm entries (235,000 rows)** screened against MLN under artificial inoculation at the Naivasha facility since Sept 2013.
- Of these, **61% from CIMMYT, 18% from NARS, and 21% from the private sector**.

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Maize Streak Virus (MSV)

- Maize streak has been the most important and widespread virus disease of maize in sub-Saharan Africa and several Indian Ocean Islands for more than 100 years (Fuller, 1901; Lapierre & Signoret, 2004; Storey, 1925; Thottappilly, Bosque-Perez, & Rossel, 1993).

- The disease continues to cause significant losses and food insecurity across sub-Saharan Africa (Martin & Shepherd, 2009).

- The disease is caused primarily by *Maize streak virus*-strain A (MSV-A; family *Geminiviridae*; genus *Mastrevirus*), which is transmitted in a persistent manner by leafhoppers in the genus *Cicadulina*, especially *C.mbila* (Naudé).

- Typical symptoms include longitudinal chlorotic streaks along the leaf veins, and a reduction in plant growth and yield. Early planted maize crops serve as reservoirs of both virus and vectors in regions with staggered, overlapping growing seasons, and a number of wild grasses can also serve as virus reservoirs (Konate & Traore, 1992; Shepherd et al., 2010).

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Maize Steak Virus

Symptoms appear on the leaves 3-7 days after inoculation as pale spots or flecks, 0.5-2 mm in diameter. Symptomatology may vary depending on the host, cultivar or virus isolate. In severe cases, the initial pale spots become longer streaks which eventually coalesce. Maize plants infected before the 4-5 leaf stage can be severely stunted. In milder instances, the lesions do not develop to more than a few sparse flecks or dots. Isolates which infect grain crops cause an abnormal bunching of flowers and shoots. Some isolates from South Africa induce a reddish pigmentation on those leaves initially infected.

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Disease inoculation

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Aerial View of MLN Screening Facility at Naivasha

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MLN Screening Facility established by CIMMYT-KALRO at Naivasha, Kenya

MLN Phenotyping at a Glance - 2014 to till date

Season	CIMMYT		NARS		Seed companies	
	Entries	Rows	Entries	Rows	Entries	Rows
2014A	15712	32807	3409	6761	5594	9226
2014B	10003	20047	1724	3866	4827	7876
2015B	7022	10284	3372	5077	3263	4038
2016A	12335	19703	6785	6785	5410	7734
2016B	11454	13834	4128	6006	4807	5005
2017A	9046	16938	2671	3379	1569	4285
2017B*	7128	7128	1909	2488	2593	2593
Total	**72700**	**120741**	**23998**	**34362**	**28063**	**40757**

Season Starting Total	Location	Entries*	Total Rows
Nov-12	Olerai and Sunripe Farm	2,636	5,272
Jun-13	Olerai and Marula Farm	8,021	16,042
Mar-14	Naivasha Facility	19,539	39,078
Oct-14	Naivasha Facility	15,322	20,356
Nov-15	Naivasha Facility	17,000	30,000
May-16	Naivasha Facility	24,530	34,222
Oct-16	Naivasha Facility	20,389	24,845
May-17	Naivasha Facility	13286	24602
Nov-17	Naivasha Facility	11630	12209
	Total	**132,353**	**206,626**

Organization Type	Total number of entries	Total Rows	% of Rows
Public	23998	34362	17.54
Private	28063	40757	20.81
CIMMYT	72700	120741	61.65
Total *	**124,761**	**195,860**	

Private Partner
Monsanto
Seedco
Dupont - Pioneer
East Africa seed company
Western Seed
Syngenta
Kenya Seed
NASECO
Pannar Seeds
Advanta Seeds
ZAM Seeds
Tan seed
Aminata
Gicheha Farm
Victoria seed UG

Public Partner (Country / Organization)
KALRO - Kenya
Student - Uganda
EIAR NARO
NaCRRI - UGANDA
ZIMBABWE
Mozambique
RAB - RAWANDA
SARI - TANZANIA
University of Nairobi
KEPHIS - Kenya
South Sudan
MALAWI
Rongo University
Burundi

*** Note : updates from Naivasha ;2014 to till date**

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MLN Tolerant Hybrids released in EA

S.No.	CIMMYT-derived MLN-tolerant Hybrid	Year of Release	Country	Status
1	Bazooka UH5354	2014	Uganda	Being commercialized by NASECO
2	H12ML	2013	Kenya	Certified seed to be produced and commercialized by KSC in 2017
3	H13ML	2014	Kenya	Being commercialized by KSC.
4	Meru HB607	2014	Tanzania	Certified seed expected to be produced by Meru Agro in 2017
5	WE5135	2016	Kenya	Recommended for release through KALRO
6	WE5140	2016	Kenya	Recommended for release through KALRO
7	WE6109	2016	Kenya	Recommended for release through KALRO
8	WE6110	2016	Kenya	Recommended for release through KALRO
9	KATEH16-01	2017	Kenya	Licensed to Agri- seed by KALRO
10	KATEH16-02	2017	Kenya	Recommended for release through KALRO
11	KATEH16-03	2017	Kenya	Recommended for release through KALRO
12	WHMLN	2018	Kenya	Recommended for release through Western seed company
13	WE7117	2018	Kenya	Recommended for release through KALRO
14	WE7118	2018	Kenya	Recommended for release through KALRO
15	WE7119	2018	Kenya	Recommended for release through KALRO

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MLN - infected

MLN - free

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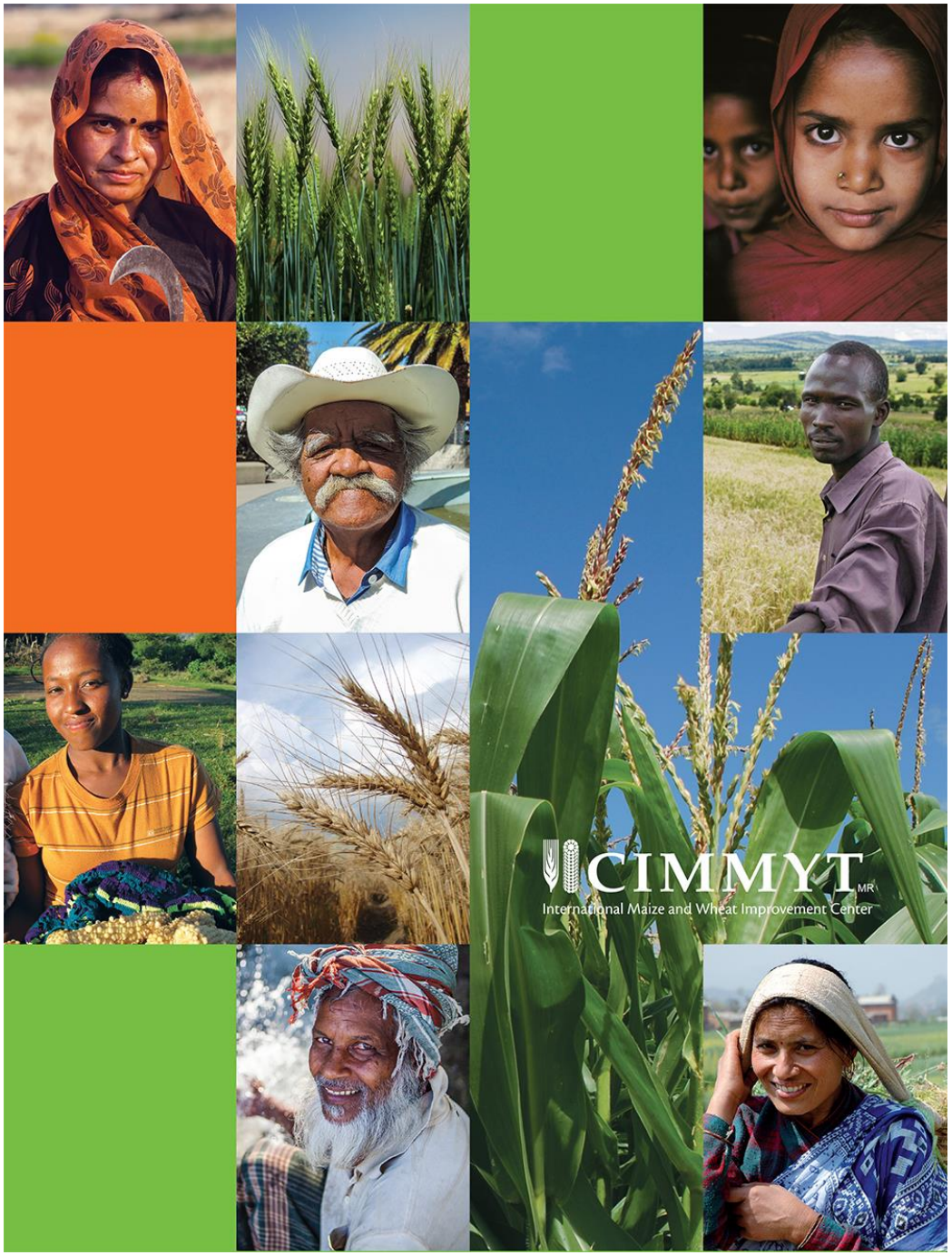

Thank you for your interest!