CGIAR Plant Health Initiative

MLN disease diagnostics, MLN disease-free seed production and MLN disease management

Tuesday 20th September 2022 at KALRO-AMRI, Katumani.

MLN disease diagnostics, MLN disease-free seed production and MLN disease management

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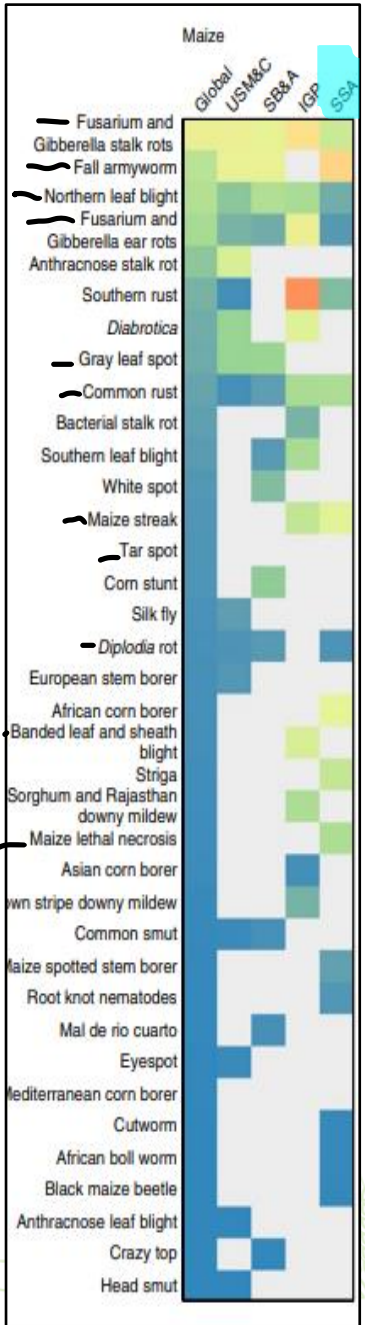
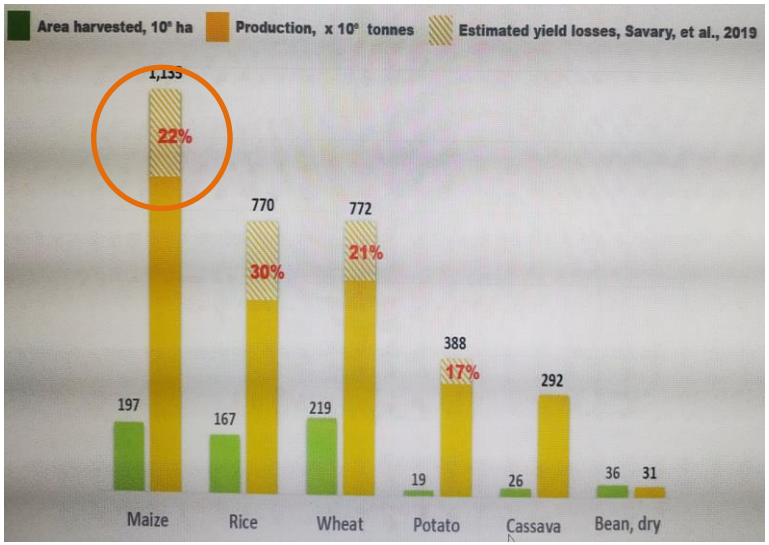
Yield losses due to plant disease, world (total) FAO, (2017)



Maize affected by several pest and diseases and affecting the crop, the crop loss globally is around 22%.

In Sub – Saharan Africa, the crop productivity is about 1.5 tons per ha as compared to 5.5 tons / ha globally, the decreased crop productivity is due to various stress and poor input management.



MLN | Wheat rust | Fall Army worm | Fusarium wilt TR-44

Invasive pest and Diseases in Africa

- Insect – pests and pathogen has no geographic boundaries
- Africa has seen occurrences of several devastating pests and diseases
  - (examples: MLN (maize), Ug-99 (wheat), FAW (Maize), Fusarium wilt TR-44 (Banana)

Figure 5.3 Relative yield losses from agronomic causes in maize crops in Sub-Saharan Africa. Soil fertility includes low soil organic matter, deficiencies in zinc, phosphorus and potassium, and soil acidity, but not low nitrogen status. Source: based on Gibbon et al. (2007)

ARTICLES
https://doi.org/10.1038/s41559-018-0793-y
nature ecology & evolution

The global burden of pathogens and pests on major food crops

Serge Savary[1], Laetitia Willocquet[1], Sarah Jane Pethybridge[2], Paul Esker[3], Neil McRoberts[4] and Andy Nelson[5*]

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Major maize diseases in Sub Saharan Africa

Turcicum Leaf Blight

Gray Leaf Spot

Fusarium Ear rot

Common Rust

Maize Lethal Necrosis

Maize streak Virus

Resistance to major diseases is important for varieties to be successful in the tropics

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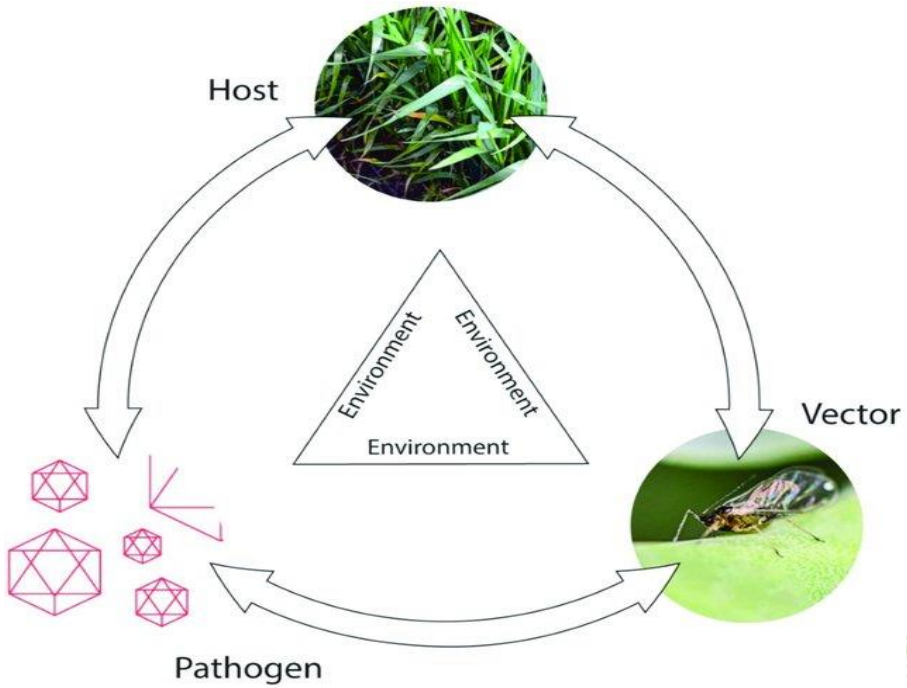
Few viruses affecting maize

Virus	Abbreviation	Genus	Family
Maize streak virus	MSV	Mastrevirus	Geminiviridae
Maize dwarf mosaic virus	MDMV	Potyvirus	Potyviridae
Johnson grass mosaic virus	JGMV	Potyvirus	Potyviridae
Maize dwarf mosaic virus	MDMV	Potyvirus	Potyviridae
Wheat streak mosaic virus	WSMV	Tritimovirus	Potyviridae
Sugarcane mosaic virus	SCMV	Potyvirus	Potyviridae
Maize rough dwarf virus	MRDV		Reoviridae
Maize sterile stunt virus	MSSV	Fijivirus	Reoviridae
Maize chlorotic dwarf virus	MCDV	Waikavirus	Sequiviridae
Maize stripe virus		Tenuivirus	Tenuiviridae
Maize chlorotic mottle virus	MCMV	Machlomovirus	Tombusviridae

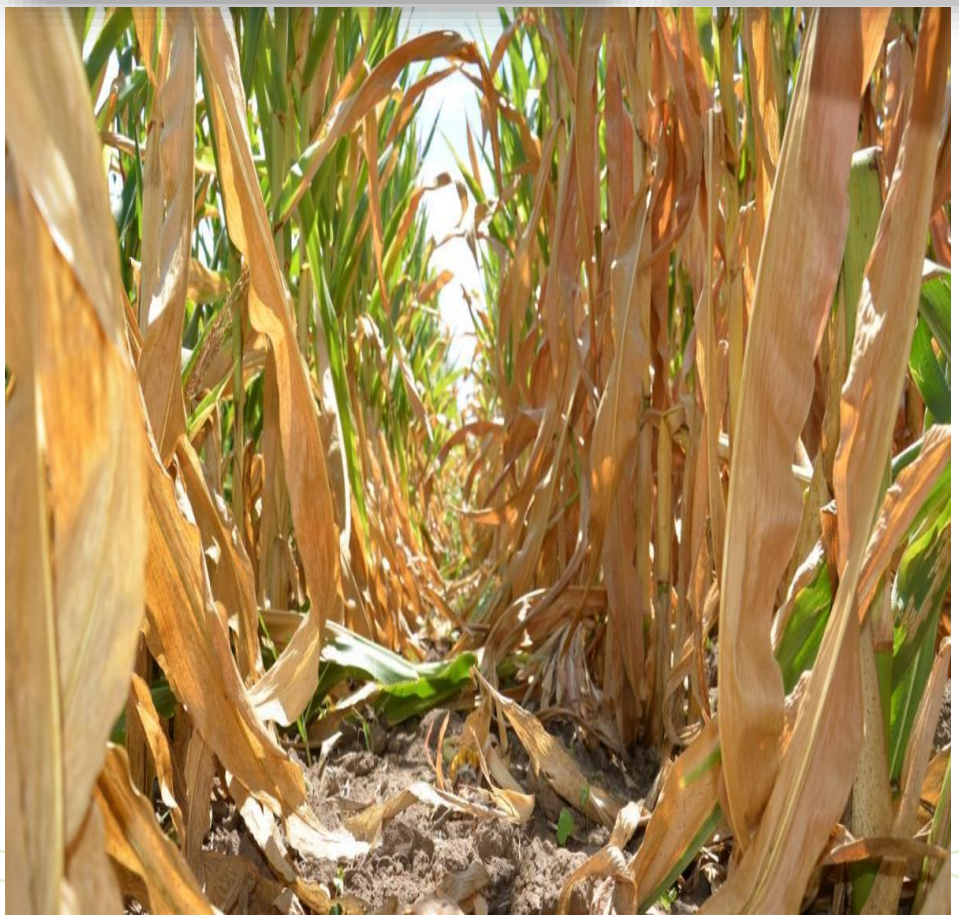


Transmission: Seed, Soil, Crop Debris, Mechanical, Insect vectors etc.,

Insect Vectors

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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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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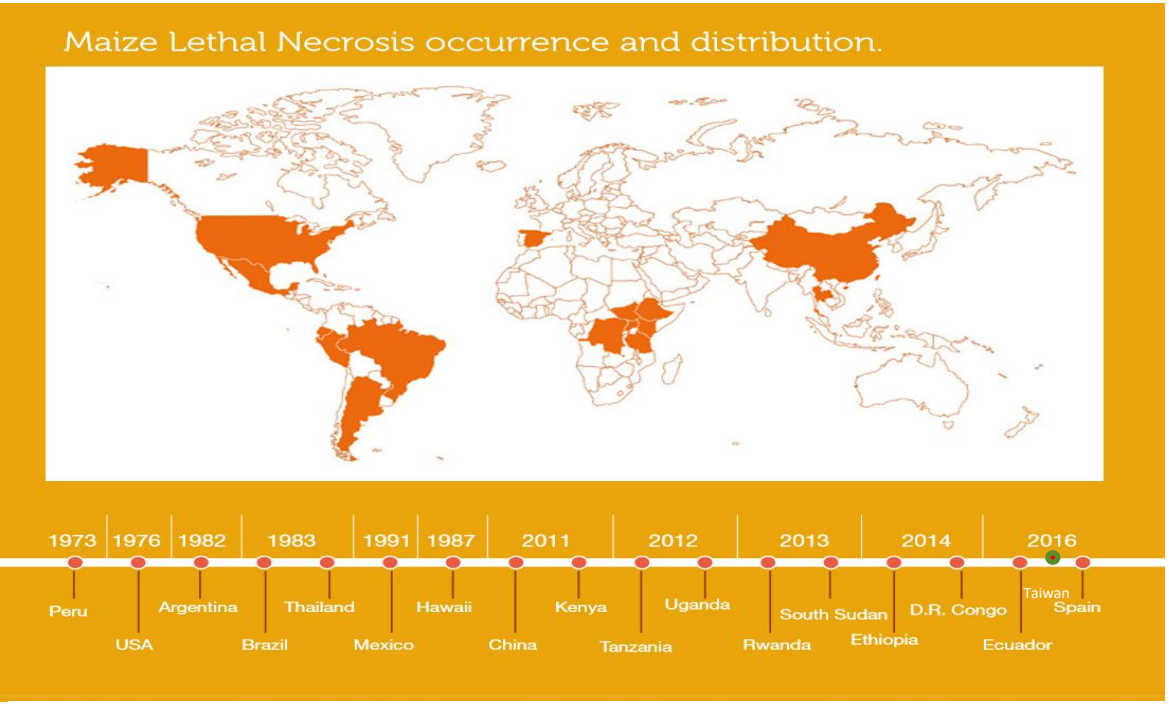
Global Occurrence and distribution of MLN/MCMV and SCMV



Source : https://doi.org/10.1038/s41598-019-56227-y

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Threats and options of emerging transboundary disease in Sub-Saharan Africa – Maize Lethal Necrosis

Maize lethal necrosis (MLN) emerged as a serious threat to maize production and livelihoods, income of smallholders in eastern Africa since 2011.

Favorable weather, crop grown through out year

Quarantine system for seed and grain movement

Cropping period, host prevalence.

Commercial - susceptible varieties grown in endemic region

Most conducive weather for host, vectors, less phytosanitation measures

Less input management

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Multi-disciplinary and multi-institutional strategy to curb the spread of MLN in sub-Saharan Africa

- Intensive germplasm screening and fast-tracked development and deployment of MLN-tolerant/resistant maize hybrids in Africa-adapted genetic backgrounds.

- Optimizing the diagnostic protocols for MLN causing viruses, especially MCMV, and capacity building of relevant public and private sector institutions on MLN diagnostics and management.

- MLN monitoring and surveillance across sub-Saharan Africa in collaboration with national plant protection organizations (NPPOs)

- Partnership with the private seed sector for production and exchange of MLN pathogen-free commercial maize seed.

- Awareness creation among relevant stakeholders about MLN management, including engagement with policy makers

"Is life"

Peter and Herrera, 1994)

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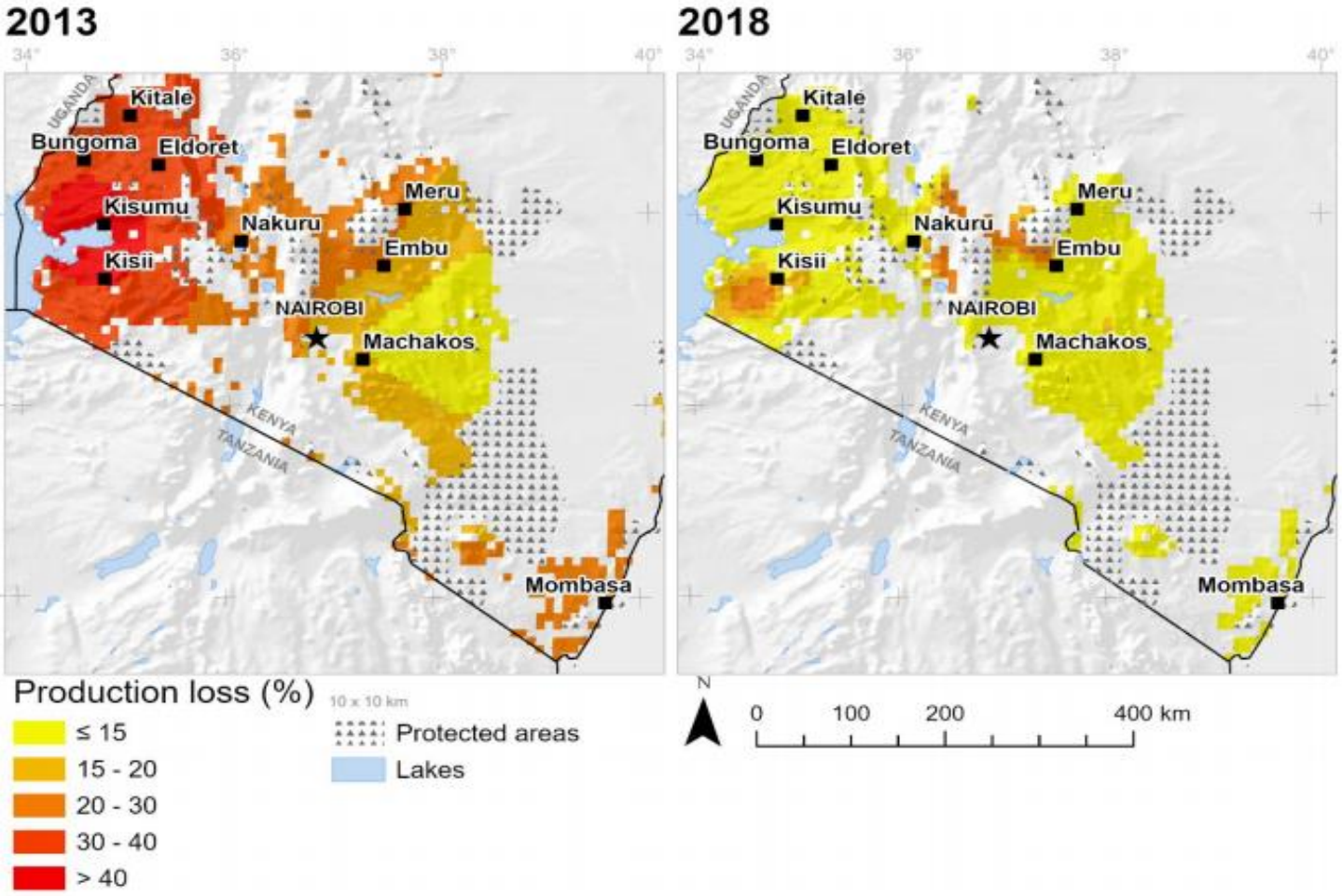
Economic Impact of MLN



In Kenya, the aggregate national loss of maize production due to MLN in 2013 was about 0.5 million tons at a value of US$ 180 million (De Groote et al., 2016).

2016: https://doi.org/10.1016/j.cropro.2015.12.003

2021: https://doi.org/10.1094/PDIS-08-20-1730-SR

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Maize Lethal Necrosis

MCMV + **Potyvirus**
SCMV
MDMV
WSMV
= **MLN**




- Individual infection with mixture of viruses can also cause disease
- Typically, infection with one virus results in milder symptoms than MLN but reaction depends on germplasm and viral strain.

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Disease Symptoms

- Chlorotic specs, streaks mosaic, mottling, Necrosis
- 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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MCMV symptoms

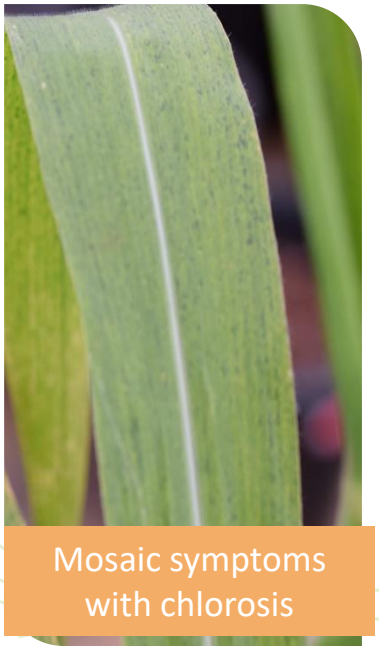
Mottling

Chlorotic stripes

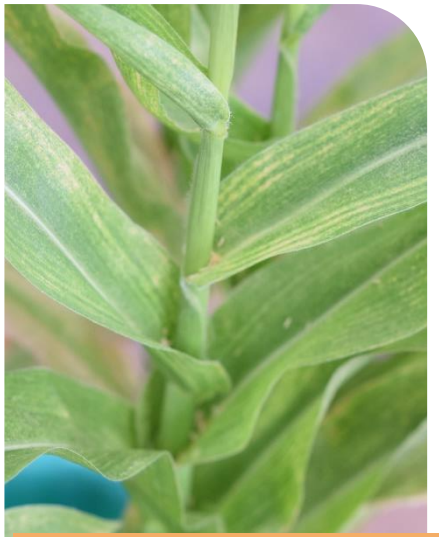
Inter-veinal necrosis / severe chlorosis

Coalition of chlorotic spots forms chlorotic stripes

Mild chlorotic stripes



Mosaic symptoms with chlorosis



Shorter intermodal distance

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MLN Symptoms

Mild mosaic and mottling

Mild mosaic and mottling

Chlorosis and Mottling

Diffuse mottling and chlorosis

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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

Poor seed set and shrivelled ears

Shortened Internodes

Early leaf necrosis

Why is the MLN devastating in EA?

- MCMV is **new** to the region
- Potentially new strains of SCMV/MDMV
- **Conducive environment** – continuous maize cropping in certain areas leading to continuous build-up of virus inoculum
- **Seed contamination by** MLN-causing viruses, especially MCMV, besides local spread through insect vectors
- Widespread cultivation of **susceptible germplasm** that has never been screened for MCMV
- **A very large proportion of commercial maize varieties in eastern Africa as well as other regions in sub-Saharan Africa are highly vulnerable to MLN.**

Developing and deploying MLN resistant hybrids

2011 2019

MLN Phenotyping Service [2014-till date]

Year	CIMMYT		NARS		Private Seed companies		Total	
	Entries	Rows	Entries	Rows	Entries	Rows	Entries	Rows
2014	25715	52854	5133	10627	10421	17102	41,269	80,583
2015	7022	10284	3372	5077	3263	4038	13,657	19,399
2016	23789	33537	10913	12791	10217	12739	44,919	59,067
2017	16174	24066	4580	5867	4162	6878	24,916	36,811
2018	29816	31954	8548	8165	8332	8735	46,696	48,854
2019	21563	35891	1047	2094	3169	5774	25,779	43,759
2020	4123	9680	939	3604	2397	6316	7,459	19,600
2021	5841	13718	2125	4250	2661	3973	10,627	21,941
Total	**134,043**	**211,984**	**36,657**	**52,475**	**44,622**	**65,555**	**215,322**	**330,014**

- **MLN – PSMS (Phenotyping management service system)** is in place for smooth operation, which is well appreciated by partners and EiB.
- **215,322 germplasm entries (>330,014 rows)** screened against MLN & MCMV under artificial inoculation at the Naivasha facility since 2014. **Heritability : 0.85 to 0.99 [CIMMYT : 65%; NARES : 15%; SME's : 20%]**
- Impact: so far, **22 MLN resistant/tolerant hybrids released** in eastern Africa.
- 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**

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1. Vehicle disinfection trough
2. Change room facility
3. Tools, implements, tractor, vehicle cleaning and disinfection zone
4. Laboratory complex
5. Solar power facility
6. Virus pure culture facility – Green house
7. Incineration area
8. Media sterilization and storerooms
9. Environmental controlled green house
10. Net house for single virus research
11. Automated fertigation drip irrigation facility

MLN Phenotyping facility – Center of Excellence

Inoculation Protocol

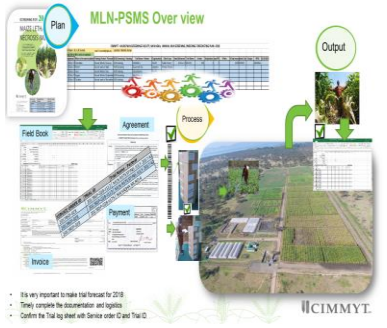




Facilities




Process

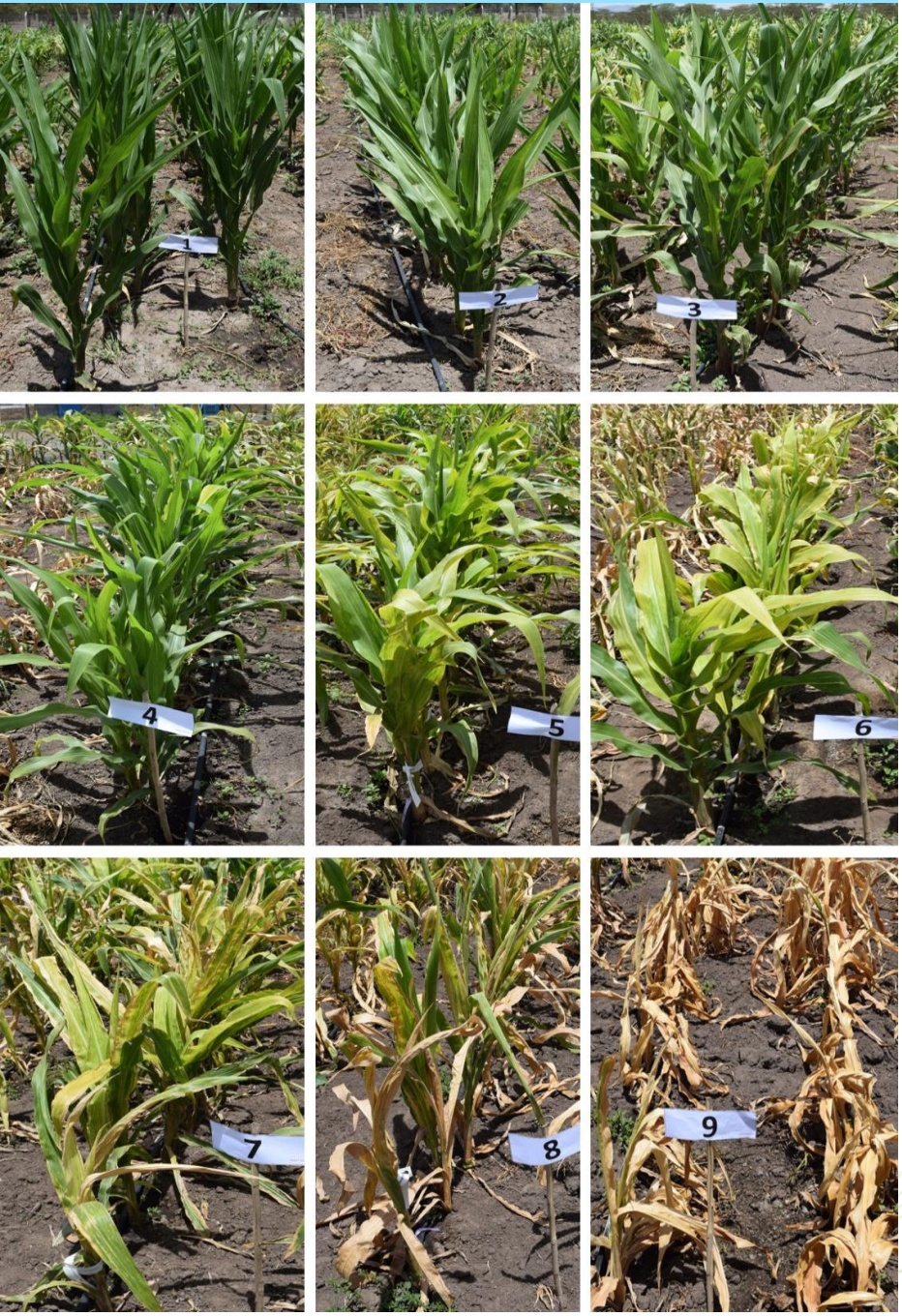




Phytosanitation




Lines



Hybrids

Demonstration plots of tolerant hybrids and susceptible check under natural MLN infestation in Tanzania and Artificial inoculation in Kenya

Natural Hot Spot – Site Babati, Tanzania

•Artificial Inoculated – Site Naivasha, Kenya

Performance of MLN tolerant hybrids under natural MLN

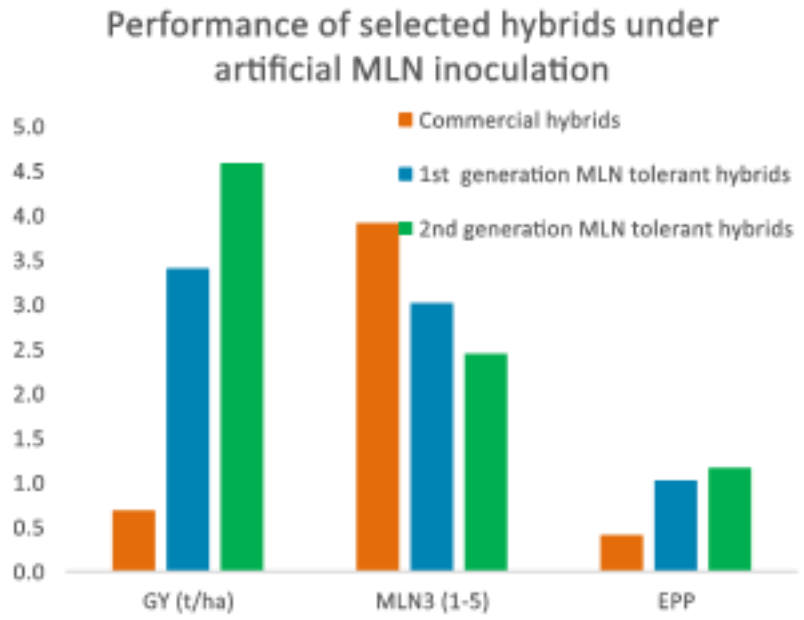
Babati (Tanzanian)

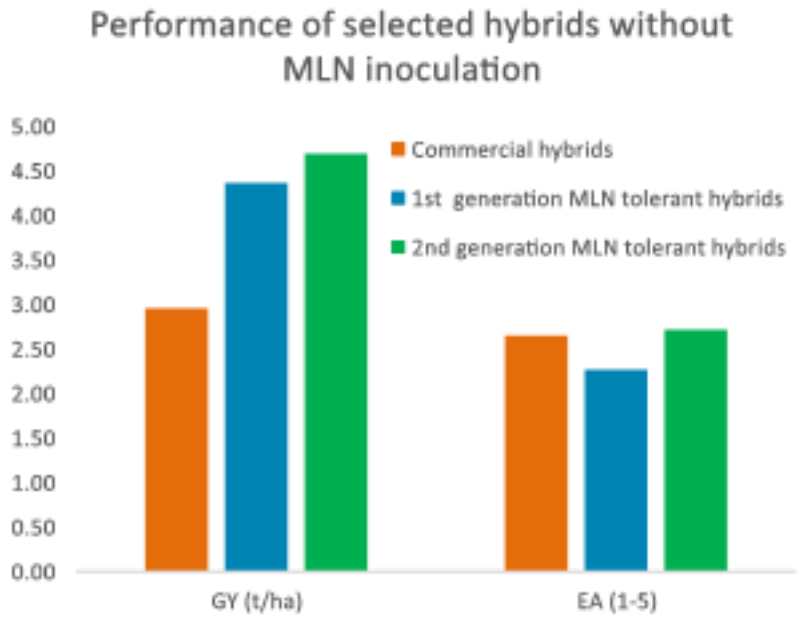
•Wondogent (Ethiopia)

Yield at Babati (Tanzanian)

Kiboko (Kenya)-Optimum

Results of Yield loss study due to MLN





	GY (t/ha)	EPP	MLN3(1-5)
Heritability	0.8	0.5	0.93
n Replicates	3.0	3.0	3.00
n Locations	2.0	2.0	2.00

	GY (t/ha)	EA (1-5)
Heritability	0.69	0.59
n Replicates	3	3

	Yield (t/ha)without MLN	Yield (t/ha) under MLN	Yield loss duet to MLN (%)
Commercial hybrids	3.0	0.7	77.7
1st generation MLN tolerant hybrids	4.4	3.4	22.9
2nd generation MLN tolerant hybrids	4.7	4.6	3.1

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MLN tolerant / resistant Hybrids released in Eastern Africa

CKMLN150084
(2nd generation MLN-tolerant hybrid)

CKMLN146069
(1st generation MLN-tolerant hybrid)

MLN-susceptible commercial check

S.No.	CIMMYT-derived MLN-tolerant Hybrid	Year of Release	Country	Status
1	H12ML	2013	Kenya	Certified seed to be produced and commercialized by KSC in 2017
2	H13ML	2014	Kenya	Being commercialized by KSC.
3	Meru HB607	2014	Tanzania	Certified seed expected to be produced by Meru Agro in 2017
4	Bazooka UH5354	2014	Uganda	Being commercialized by NASECO
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	Recommended for release through KALRO
10	KATEH16-02	2017	Kenya	Recommended for release through KALRO
11	KATEH16-03	2017	Kenya	Recommended for release through KALRO
12	WE7117	2018	Kenya	Recommended for release through KALRO
13	WE7119	2018	Kenya	Recommended for release through KALRO
14	WE7118	2018	Kenya	Recommended for release through KALRO
15	CKMLN150074	2019	Kenya	Recommended for release through Seed Co. Ltd
16	WE5135	2019	Tanzania	Recommended for release through COSTEC
17	WE7118	2019	Tanzania	Recommended for release through COSTEC
18	WE5141	2019	Tanzania	Recommended for release through COSTEC
19	WE7133	2019	Tanzania	Recommended for release through COSTEC

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Release of MLN-tolerant/Resistant Hybrids

Comparison of MLN-tolerant/resistant hybrids versus MLN-susceptible commercial checks

19 CIMMYT-derived, MLN-tolerant/resistant hybrids released so far in Eastern Africa

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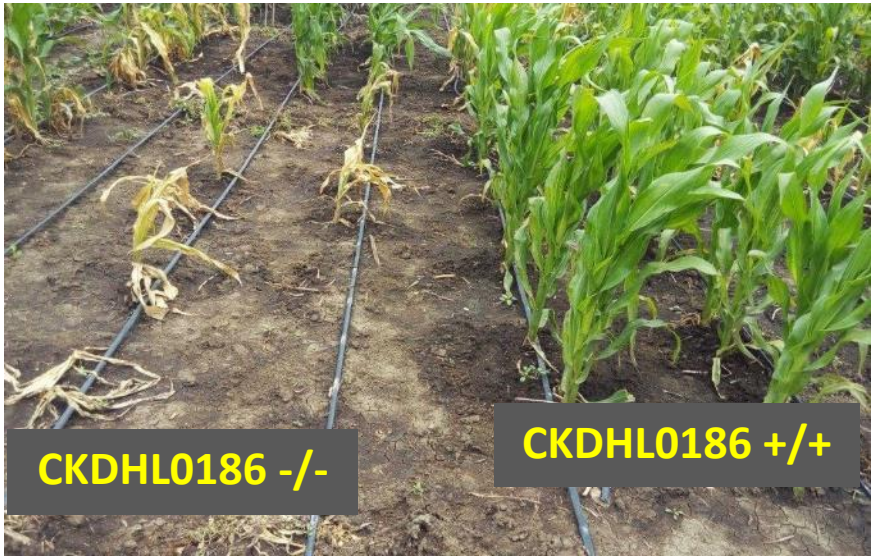
Introgressing MLN Resistance through Molecular Breeding



Fast-tracked conversion of **52** elite DT but MLN susceptible CIMMYT lines into MLN-resistant versions

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MLN Quarantine Facility at Mazowe, Harare

- Established with support from USAID, DR&SS-Zimbabwe, and CRP MAIZE.
- Enables CIMMYT maize germplasm flow from eastern Africa to southern Africa after evaluation under quarantine conditions

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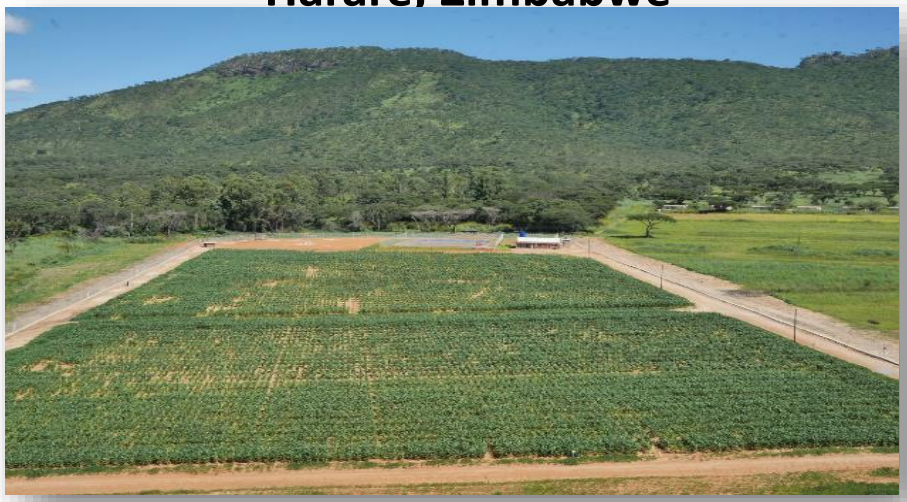
MLN Quarantine and Screening facilities in SSA

MLN Quarantine facility

Harare, Zimbabwe



- Surveillance and diagnosis for MLN
- Seed production, selfing and Crossing.
- Destroy the maize crop in the farm and 50 KM vicinity, if found MCMV positive.
- Distribute Maize germplasm, after confirming negative to MCMV

•MLN Screening and Quarantine facility

•Naivasha, Kenya

- Germplasm Screening for MLN, MCMV & SCMV
- No seed production, selfing and Crossing.
- No return of seeds after screening, only provide the data.

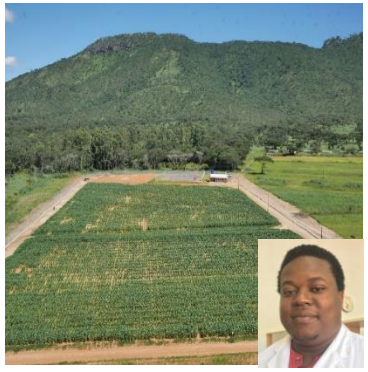
Safe seed movement with effective quarantine measures

Field scouting CIMMYT & KEPHIS at Kiboko

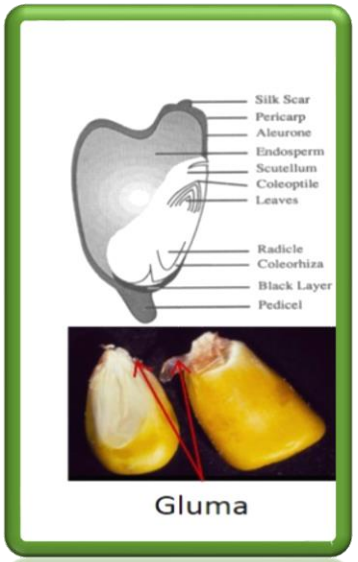
Seed samples test – CIMMYT lab, Nairobi

Kenya Plant Health Inspectorate Service

Seed test in KEPHIS



Seed test in CIMMYT quarantine site and Harare lab

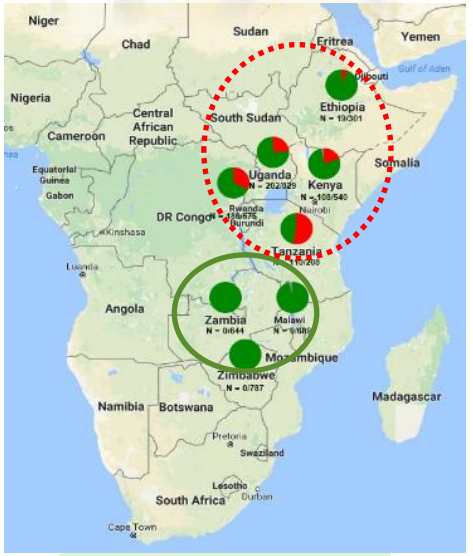


Progress made in Kenya

Progress made in Zimbawe

Year	Field inspection	Seed testing	Samples tested for causing MLN tested	Lines planted and released from facility	Incidence of MLN in pre-released seed
2017	750	1335	Established Quarantine facility in Mazowe, Harare, 2016		
2018	854	1444			
2019	503	3451	>35000	>6800	0
2020	649	2345			
2021	450	1991			

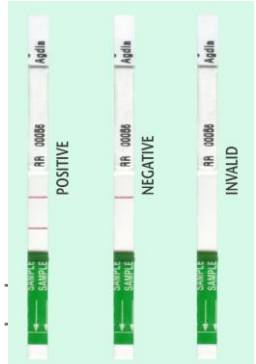
>10,000 composite seed samples (>40,000 entries) were tested and sent the seeds to Harare, endemic countries, no single samples found positive so far after the seeds received since 2016.



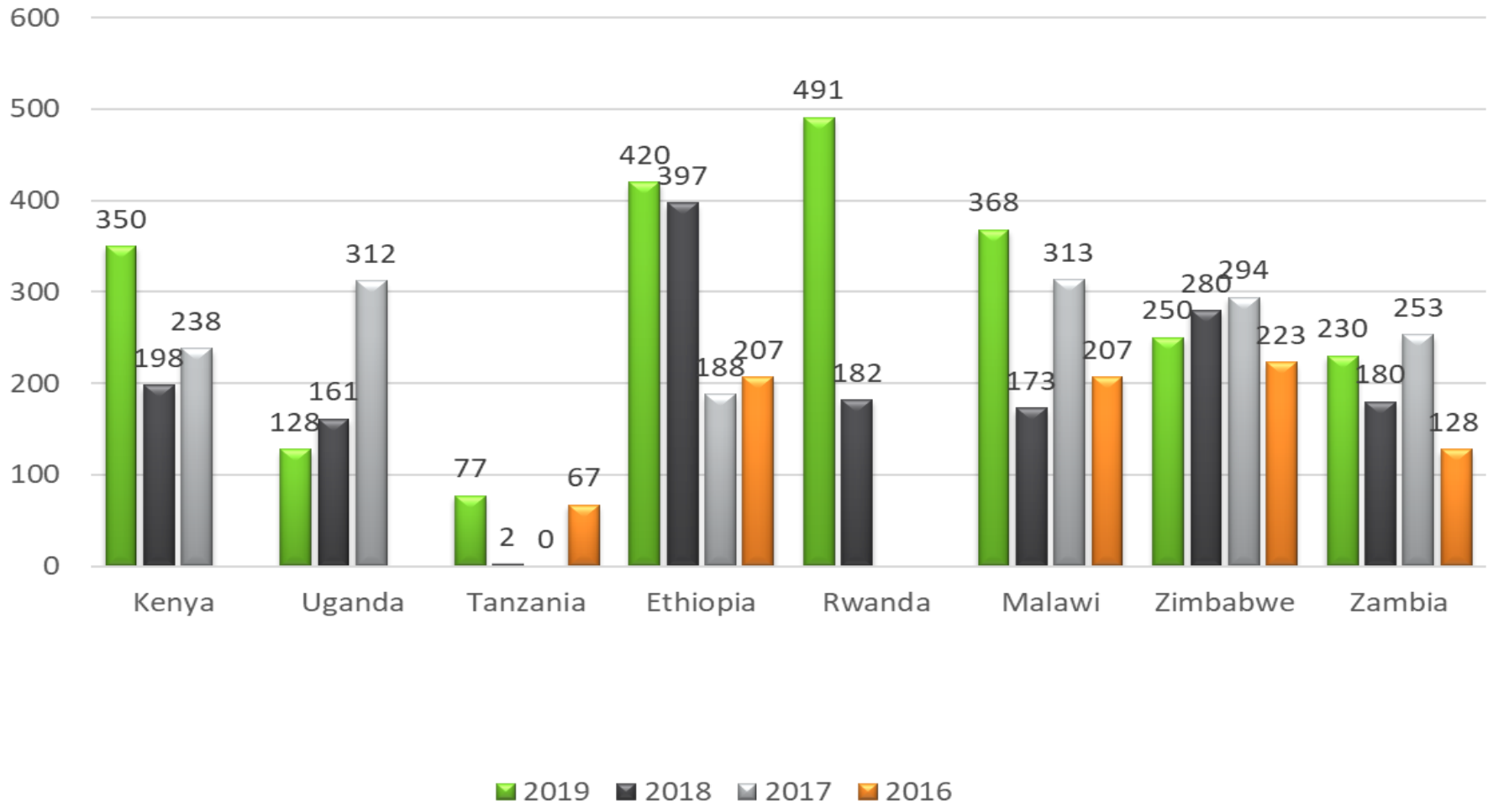
Current MLN Status As on Nov, 2021

MLN farmers' fields Surveillance

Using ODK and Immunostrip



MLN Surveillance points 2016 - 2019



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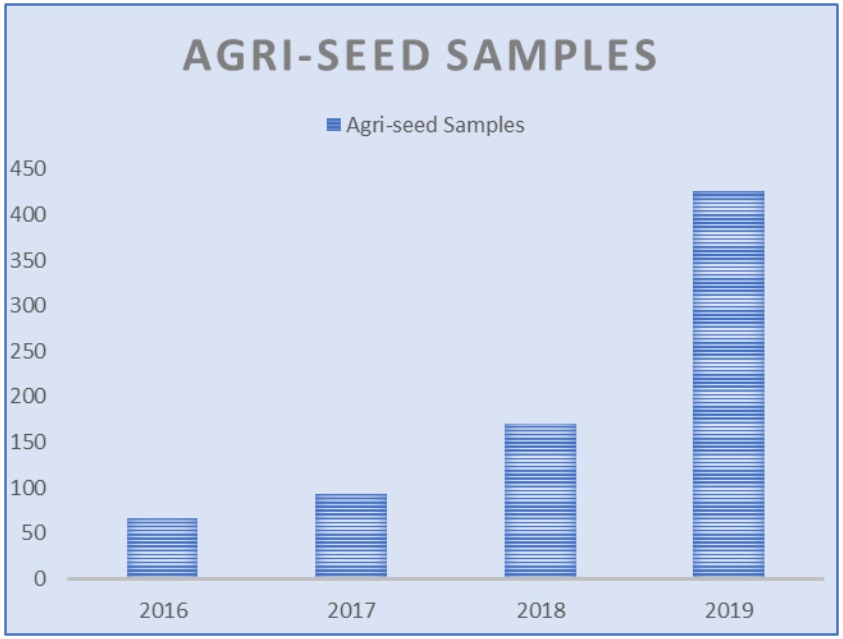
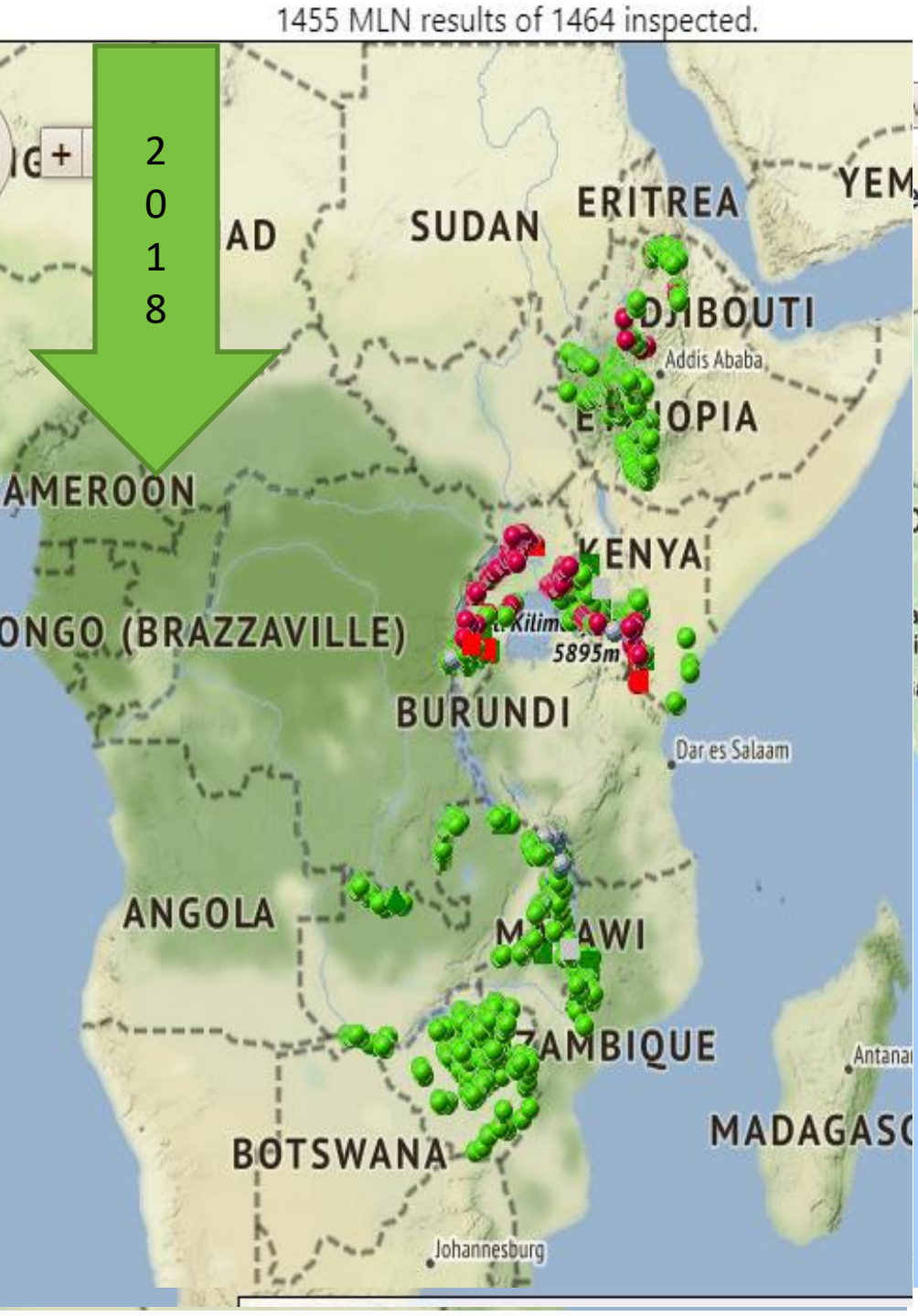
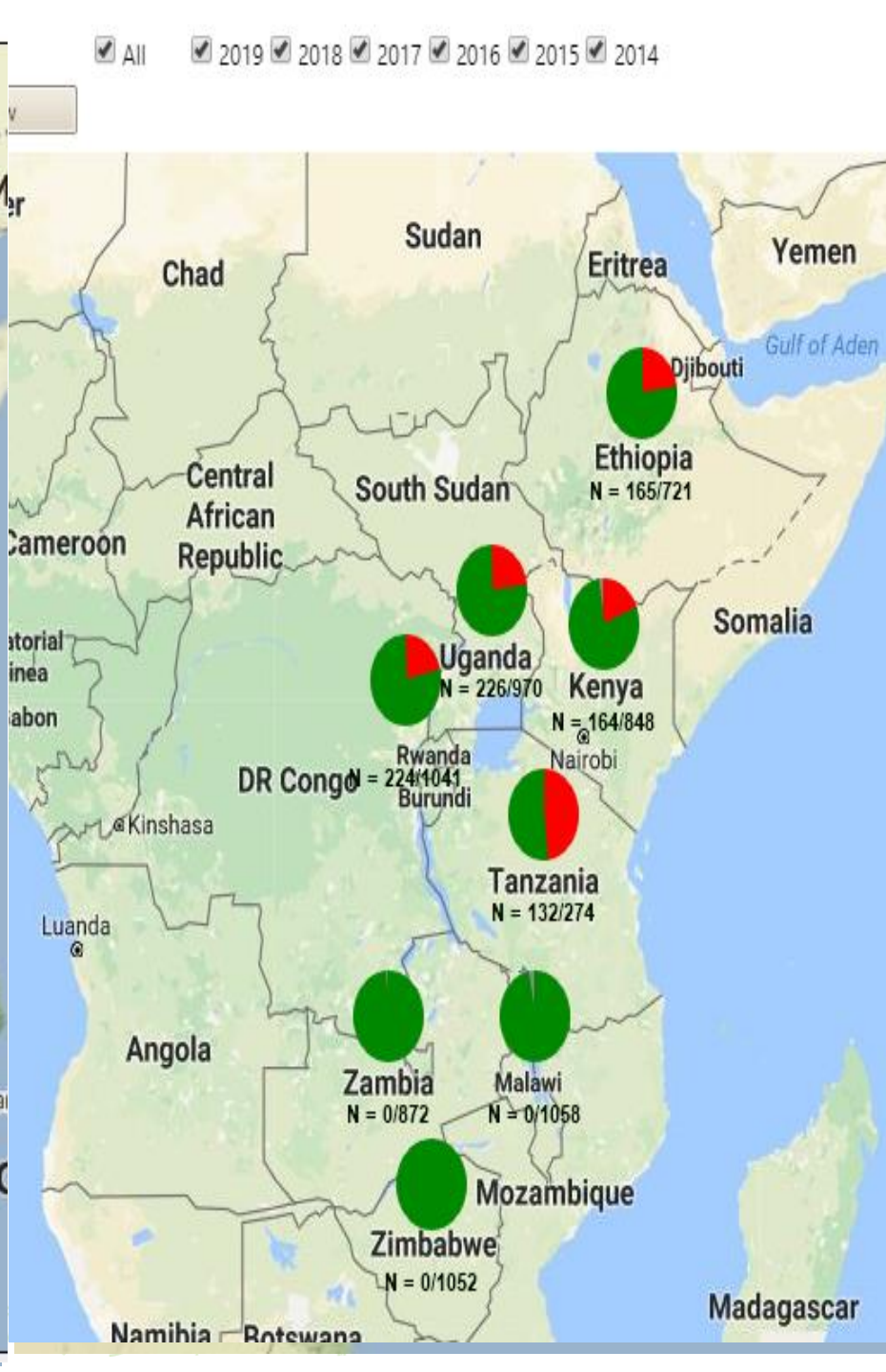
Agri -seed dealers' surveillance 2019

2016 - 2019

Country	No. of maize Agri-seed dealers	No. seed samples tested	
		+ve	-ve
RWANDA	0	N/A	N/A
MALAWI	0	0	144
ZAMBIA	53	N/A	53
ZIMBABWE	118	0	118
KENYA	10	0	10
UGANDA	0	0	0
ETHIOPIA	220	0	220
TANZANIA	25	0	25
TOTAL	**426**	**0**	**426**




1455 MLN results of 1464 inspected.
2018
SUDAN
ERITREA
DJIBOUTI
Addis Ababa
KENYA
5895m
BURUNDI
Dar es Salaam
ANGOLA
BOTSWANA
Johannesburg


All
2019
2018
2017
2016
2015
2014
Sudan
Chad
Eritrea
Yemen
Gulf of Aden
Djibouti
Ethiopia
N = 165/721
Central African Republic
South Sudan
Cameroon
Somalia
Uganda
N = 226/970
Kenya
N = 164/848
Nairobi
Rwanda
N = 224/1041
Burundi
DR Congo
Kinshasa
Tanzania
N = 132/274
Luanda
Angola
Zambia
N = 0/872
Malawi
N = 0/1058
Mozambique
Zimbabwe
N = 0/1052
Madagascar
Namibia
Botswana

MLN free seed production activities achievements

Year	No. of Seed company on farm visits	No. of Seed Farmer on farm visits	No. of field days facilitated and attended	Number NARS Programs and Seed Companies involved in MLN Tolerant Variety Promotion	Number of Stakeholders Trained on Rapid Diagnostics	Number of IEC Materials Developed and Distributed
2016	-	-	-	-	-	11,000
2017	38	225	1	13	55	8,200
2018	35	336	3	21	60	6'000
2019	52 (45 AGRA)	125	1	12	80	4,100
TOTAL	170	686	4	46	195	29,300

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Ensuring MLN pathogen-free commercial seed production and exchange

- Intensive efforts are being made to prevent spread of MLN, especially MCMV, through contaminated seed from the MLN-endemic to non-endemic areas in Africa
- Several seed companies now implementing **voluntary MCMV control programs** and SOPs to minimize the risk of seed transmission
- **Key staff of both public and private sector institutions trained** on MLN diagnostics and management

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Capacity Building to Partners

- Regular training to all NPPO's, private company staff and NARS staff on carrying out MLN survey.
- Regular technical back stopping for all the partners for successful survey.

ODK App practical use - Naivasha

Sampling demonstration

Seed testing training at Harare

Country	NPPOs				Seed companies				Research institutions				Seed Growers			
	2016	2017	2018	2019	2016	2017	2018	2019	2016	2017	2018	2019	2016	2017	2018	2019
Kenya	12	28	35	25	22	68	25	6	12	25	12	8	68	260	230	121
Uganda	8	15	22	8	12	35	25	11	5	12	5	8	45	150	158	89
Tanzania	8	18	28	21	15	32	68	6	4	8	6	13	33	60	320	78
Ethiopia	8	12	18	47	18	61	18	12	5	18	12	16	28	93	335	125
Rwanda	2	14	55	67	12	21	13	8	7	13	28	21	23	125	138	225
Malawi	12	8	2	13	3	16	4	2	5	4	4	12	14	21	35	-
Zambia	18	18	3	12	4	25	5	3	4	6	12	4	18	28	45	-
Zimbabwe	13	12	8	4	4	32	6	2	4	8	8	3	22	32	32	-
TOTAL	**81**	**125**	**171**	**197**	**90**	**290**	**164**	**50**	**46**	**94**	**87**	**85**	**251**	**769**	**1,293**	**638**
				574				**444**				**227**				**2,258**

Training on MLN Surveillance and testing

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Community of Practice

COP Outcomes

- Increased MLN knowledge sharing amongst stakeholders in SSA

- Harmonization of surveillance and Diagnostics protocols in SSA

- Adoption of rapid diagnostics using immunostrips by seed companies and NARs

Objectives of CoP:

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;

f) to **provide information** for the review of maize seed certification and import/export procedures in relation to MLN for formulation of appropriate SOPs.

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Regional meetings organization and participation

- East African Community(EAC) / CIMMYT organized a regional MLN meeting on 22nd to 24th May 2018 in Nairobi where 28 participants attended.
- Training workshop on MLN free seed SOPs and rapid MLN field diagnostics – KEPHIS on 30th – 31st July 2018.


East African Community (EAC) Meeting


KEPHIS Meeting

MLN Information Portal

mln.cimmyt.org

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search...

MLN in the news

- Deadly virus threatening maize
- Maize Lethal Necrosis: Possible threat to local maize production
- New facility to help Zimbabwe deliver healthy seeds
- Zimbabwe: Government unveils crop disease quarantine

MLN disease symptoms
Sampling protocol for MLN and
Introduction to Immunostrips for MLN pathogen detection

Dr. SURESH, L.M.

Global Maize Program (GMP)
CIMMYT, Nairobi, Kenya

Training on MLN rapid diagnosis Kits and MLN management,
Dates 19th July
Katumani, Kenya

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Overview

- Field sampling
- Sample processing
- Sample testing
- Documentation
- Sample shipping for ELISA testing.

How to collect and ship Fresh samples for the VIRUS diagnosis

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FIELD INSPECTION and LEAF SAMPLING:

•The sampling procedure must ensure that all parts of the field are adequately and proportionately represented in the plants inspected with in the various usual microclimates of the field.

•The pattern of field inspection can vary as far as the above condition is met

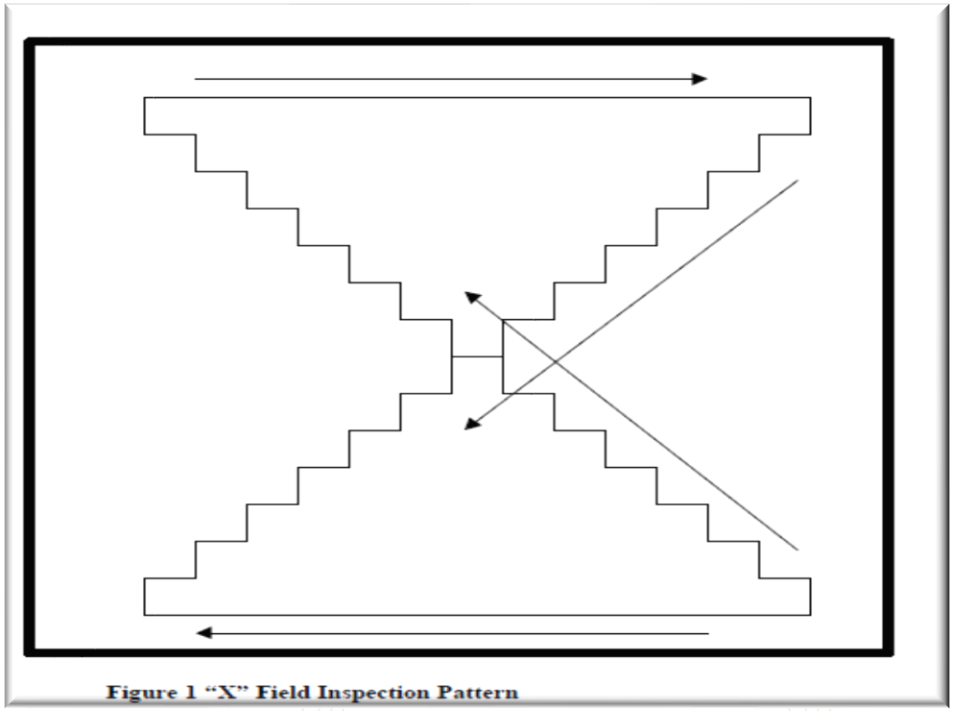

Figure 1 "X" Field Inspection Pattern

(CDFA Phytosanitary Certification Manual, 1985)

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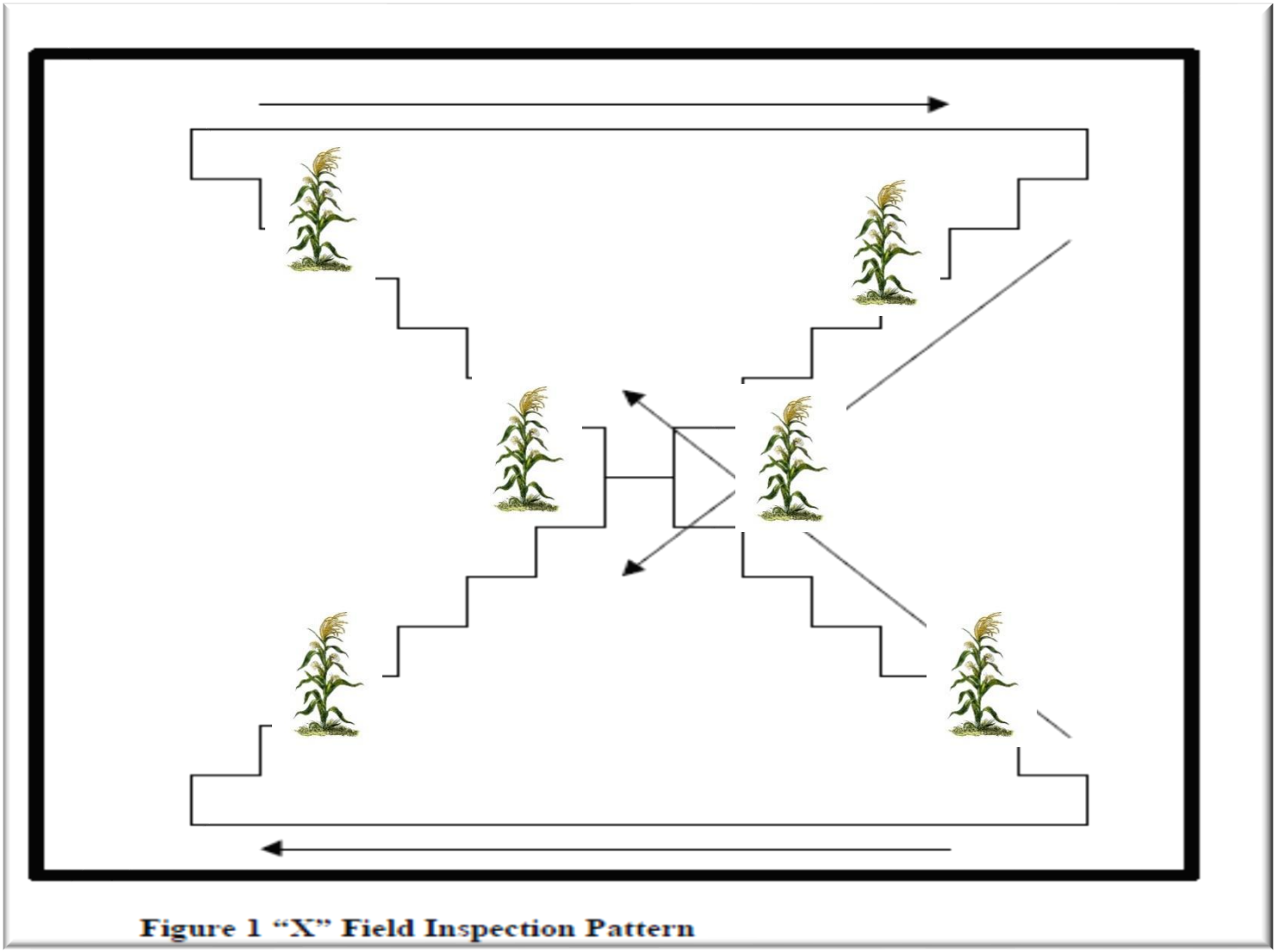


Figure 1 "X" Field Inspection Pattern

(CDFA Phytosanitary Certification Manual, 1985)

Procedure for leaf sampling

Precautions :

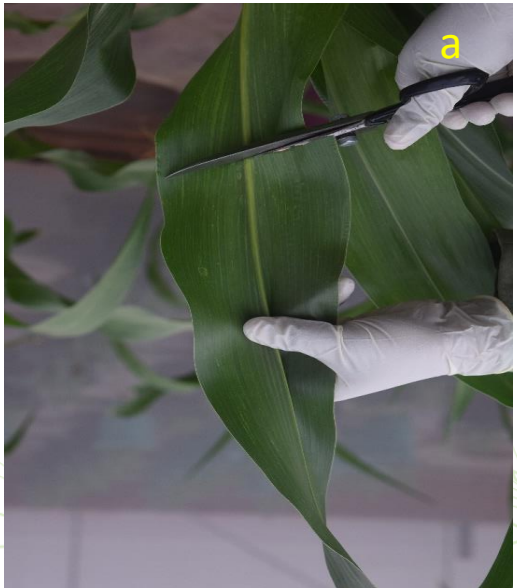
- Choose the **right plant and leaf**.
- **Avoid** the plants with **abiotic symptoms** such as (mechanical) wilt, nutrition deficiency, pesticide injury, due to drought.
- Try to **avoid samples with multiple symptoms** such as leaf blight, yellowing along with mosaic.
- If you are in doubt, please look for fresh plant sample.
- It is important that the samples should be collected before pesticide application.

Selection of plant and collection of leaf tissue

- The sample should be taken from the youngest leaf of the plant.
  To carry out the sampling procedure, the diagnostician should wear laboratory gloves.

- Leaf samples should be collected as follows:
  - Select approximately a leaf of **newly developed symptomatic tissue.**

    Using a clean sheet of fresh tissue paper, enclose and hold the leaf to be sampled. Use bleach-cleaned scissors to cut off a 5-6 cm leaf segment (Fig 2). Carefully avoid cross-contamination of samples with exudate from leaves onto hands or implements. Scissors or any other implements must be cleaned thoroughly with bleach solution between each sample (between farm).



2. Samples with no symptoms (a) and symptoms (b)

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Cover the leaf tissue and place it in perforated paper bag:

Fold the tissue paper so that it covers the leaf sample. Place the single collected leaf sample into a paper bag perforated with air holes (Fig 3) [NB: Only 1 leaf sample should be placed into each leaf sample bag]. Caution must be used not to touch the interior of the sample bag with fingers, implements or any other leaves.

3. Placing the samples Between the layers of Tissue paper (a) and Inserting the samples In the perforated paper bag (b).

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Labelling and packing

- Stick completed sample labels and a unique QR code on each sample bag and record the unique sample code. Place each labelled sample bag inside a zip lock plastic bag, this will protect the label from damage).

Fig 4. A. Individual leaf sample bag lableled with QR code and sample label. B. Labeled leaf sample bag inside ziplock bag

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Sample bulking

- Place 6 individually labelled leaf sample bags from the same plot inside one large plastic bag (Bulk sample bag). This will keep the 6 leaf samples from the plot to be used for one bulk immunostrip assay together (Fig. 5). It is essential to stick another unique QR label onto this bulk sample bag and record the unique bulk sample code. Always use separate bags for each set of 6 leaf samples i.e., if 12 leaf samples in total are collected from a plot there should be 2 uniquely labeled bulk sample bags for the plot.

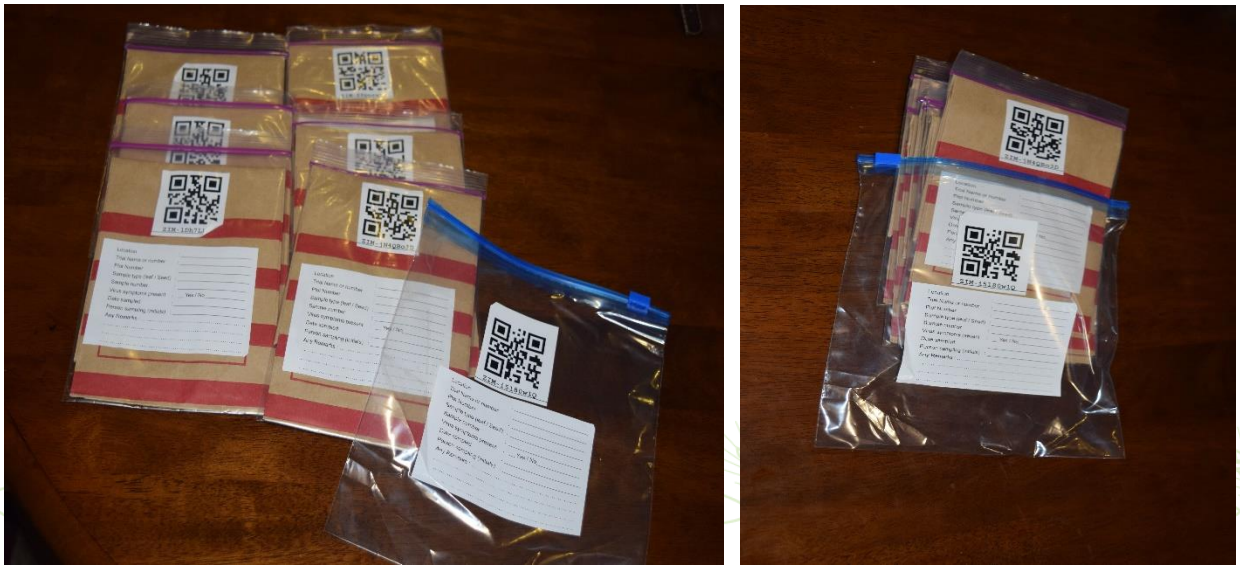

Fig. 5. Labelled bulk sample bag containing 6 individual labelled leaf sample bags

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Storing the samples in the container

Place the labeled bulk sample bags inside a cool box, and ensure that ice packets are placed around the samples to keep them cool during sample processing and subsequent transportation.

❖ **Keep samples in a cool place until shipping. (Do not place in the refrigerator).**

❖ **When ready to ship, place the double bagged box containing in a styrofoam container with a coolpack. Place the styrofoam container in a cardboard box and ship as one unit.**

❖ Label the outside of the box (Styrofoam) with sample ID, date sample was collected, grower and field where sample was collected. (place a sample log details inside the box)

❖Fill shipping container with packing material to avoid movement of the sample in the container

Fig. 6. Keeping the samples inside the ice box using ice pack

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Immunostrip test steps

- Selection of test place
- Keep at most hygiene as possible
- Carefully handle the leaf sample using glouce
- Keep all the items sanitized
- Keep all the material check list ready
- Ensure the test documentation is done before you leave the place
- If needed or in doubt please contact the nearest contact or send mail to : l.m.suresh@cigar.org or whats up +254392664

- Avoid cross contamination between the bulk samples
- Avoid taking food, using phone, smoking etc..
- After the test is done, leave the sample in the same farm

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Preparation of the sample:

1. For the correct execution of the test the ratio between leaf tissue and extraction buffer must be as close as possible to 1:40 w/v (or the one indicated in the instructions of the kit)
2. To maintain this proportion the total amount of tissue to be processed in the test must not exceed the size of a coin
6. Wearing gloves, open the first collection bag and detach a small portion of the tissue, introduce it in the plastic extraction bag
7. Close the collection bag where the samples was preserved and store the bag again in the cool box
8. Change gloves between samples
9. Wear a new pair of gloves and proceed as in point 6 until you obtain the 6 leaf fragments to test in the extraction bag
10. Add the buffer as indicated on the instructions included with in the kit
11. Homogenize the sample with …..on a flat surface; for this purpose a plastic box or a plastic tray can be used as working bench. This action should not be longer than 2-5 seconds
12. Transfer a total of 4 drops of the extract into the disposable cuvette
13. Insert 1 strip as indicated on the instructions included with the kit
14. Leave the strip for at least 10-15 minuets before reading the results
15. Interpretation of the results:

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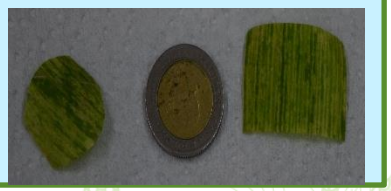
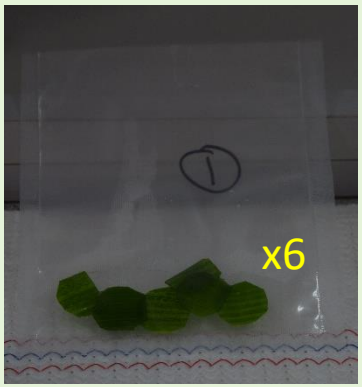
Immunostrip test steps

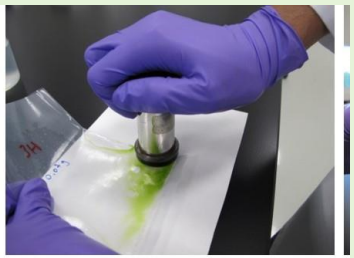
Sample collection



5 min

Sample Preparation





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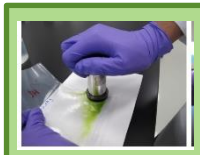
Immunostrp test

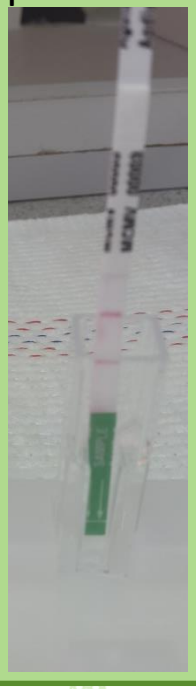


1 drop

3 drop

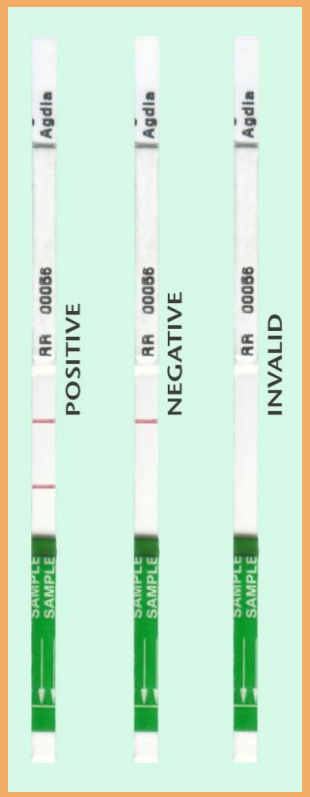
sap

buffer



15 min

Documentation



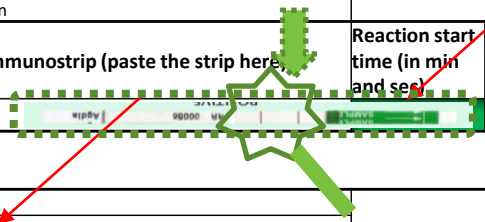
5 min

MYT

MCMV immunostrip documentation

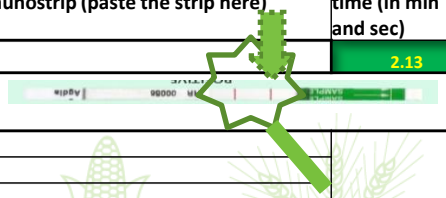
MCMV Diagnostic Testing of Maize Plant Samples

Location :	Harare
Farmer's Name :	Jan De Vries
Trial Name or Number (if applicable):	
Plot Number (if applicable):	
Virus symptoms present: Yes / No	Yes
Date of sample :	3rd March 2014
Sample location :	Harare
Name of the person who sampled:	Kevin Conn

Bulk Sample ID	Immunostrip (paste the strip here)	Reaction start time (in min and sec)	Reaction confirmation time (in min and sec)	Remarks
ZWEweodfrtdds12			4.13	Positive

Signature :	
Name :	Kevin Conn
Institution :	ZARI

Location :	Harare
Farmer's Name :	Jan De Vries
Trial Name or Number (if applicable):	
Plot Number (if applicable):	
Virus symptoms present: Yes / No	Yes
Date of sample :	3rd March 2014
Sample location :	Harare
Name of the person who sampled:	Kevin Conn

Bulk Sample ID	Immunostrip (paste the strip here)	Reaction start time (in min and sec)	Reaction confirmation time (in min and sec)	Remarks
ZWEweodfrtdds12		2.13	4.13	Positive

Signature :	
Name :	Kevin Conn
Institution :	ZARI

Disclaimer:
CIMMYT does not specifically endorse any specific commercial product / brand / company mentioned in this experiment.

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❖ Sending Samples

1. *If MLN symptomatic plants (or suspected MLN symptomatic plants) are observed on the survey (i.e., any bulk sample that test positive with the immunostrip test), the samples should be sent to a recommended laboratory for ELISA testing for confirmation as soon as possible (within 48 hours).*
2. *If possible, mail the positive / suspected samples to a recommended laboratory in the country* ***[NB: NO samples should be mailed to another country]****. Place the bulk sample bag(s) inside a small cardboard box. Please mention on the box* ***"Plant sample" "RUSH IMMEDIATELY"*** *and mail using either a courier service or express mail to the laboratory.*
3. *If the samples cannot be mailed immediately, keep them in a refrigerated condition or in a cool dark place. Samples must be refrigerated at 4°C and kept for no longer than 48 hours. After that time, leaf samples can deteriorate and the results will not be reliable.*

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Packing and shipping samples :

- After placing the bulk sample bags into the cool box take the samples to a suitable site / laboratory in the plot to undertake the ELISA
  - After completing the immunostrip tests and recording all sample information, place the Styrofoam / cool box with all the collected plot samples inside a cardboard box, to provide a good and sturdy transport arrangement (Fig. 9).
- **Note:** Inside each box, please keep a list of samples that has all the information collected during the sampling .

Fig. 9. Sample box ready for transportation

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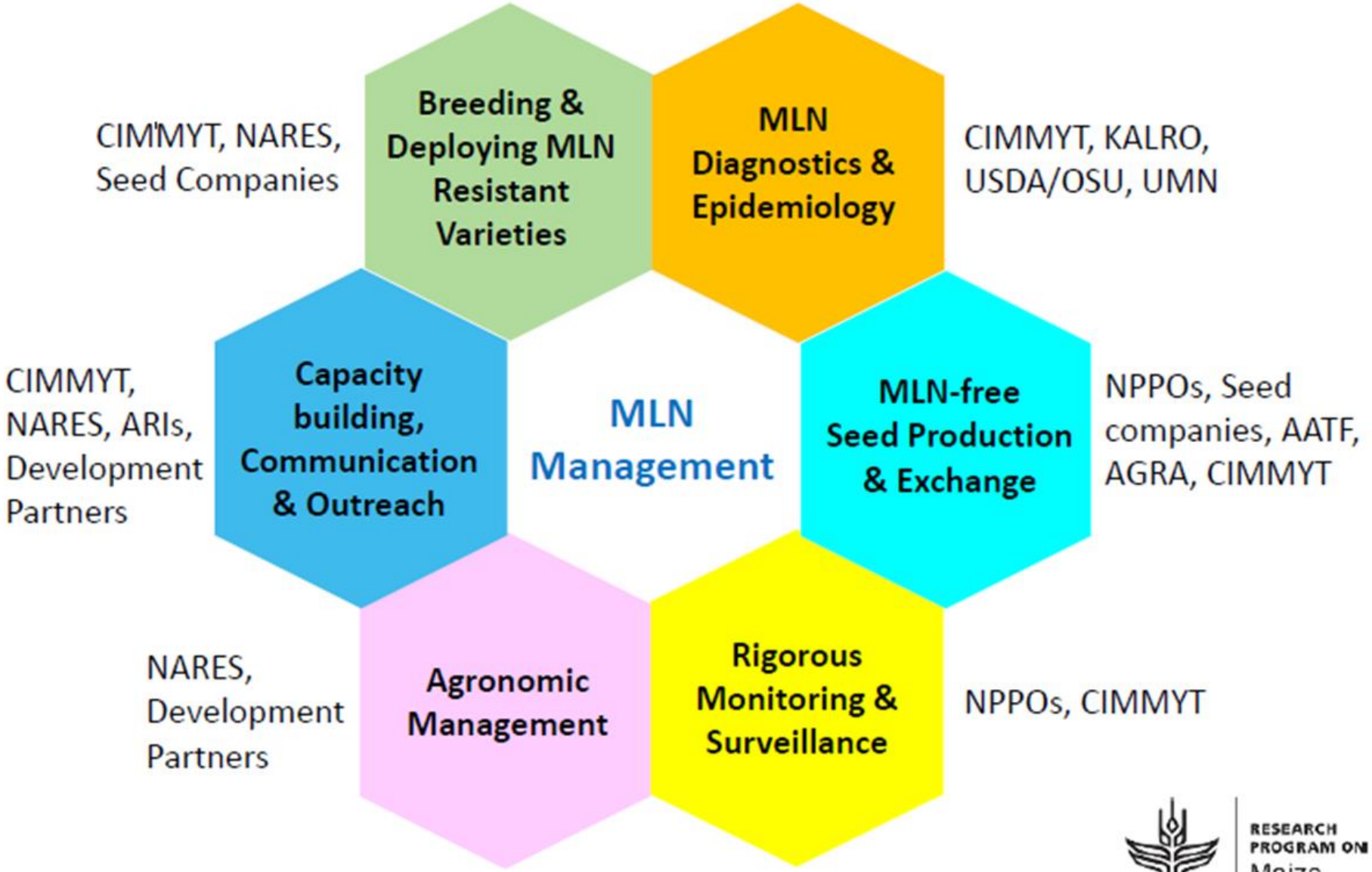
Tackling a Complex Challenge

Success through Integration of Various Components



CGIAR RESEARCH PROGRAM ON Maize

MLN-Free Seed production Objectives

To accomplish these objectives our Partners AATF and AGRA had the following activities;

- On-farm farmer and seed company visits to ascertain the status of MLN disease and monitor implementation of harmonized MLN management checklists.
- Training of stakeholder on the use of the checklists
- Promotion of MLN tolerant maize varieties during field days and other forums for uptake .
- Various (IEC) materials on MLN disease management and MLN –Free seed production were distributed to stakeholders in MLN endemic countries.

Note : A total of 574 participants from NPPOs and NARS institutions,
544 participants from commercial seed companies, and
2313 small-scale contract seed growers in eastern Africa were trained during 2016–2019 on the SOPs for MLN pathogen free seed production.
The course content included on-farm MLN diagnostics, disease scouting, leaf and seed sampling, and testing using immunostrips and ELISA.

Producing MLN free seeds using Harmonized Checklists

HARMONIZED MLN MANAGEMENT CHECKLIST & SOPs (KENYA)					
No.	TASK	START DATE	DUE DATE	% COMPLETE	NOTES
1.	Monitor crop disease history of seed production fields to enable adequate control plans				
2.	Maintain adequate levels of soil fertility based on soil tests to ensure healthy crops				
3.	Timely planting to facilitate disease escape and eliminate disease incidence due to late planted crop				
4.	Use of disease free seed stocks in subsequent seed production				
5.	Clean farming equipment to remove contaminated soil debris and minimize spread of disease from one field to another				
6.	Eliminate grasses and other weeds from fields and plot borders to remove vector hosting plants				
7.	Monitor and control insect vector population through tested spraying regimes				
8.	Scout for viral symptoms to ensure early detection and control				
9.	Rogue symptomatic plants and burn/bury to minimize spread of disease				
10.	Sample suspect plants for diagnostic testing within the internal quality control to confirm disease presence				
11.	Post-harvest cob selection and seed testing for MLN causing viruses				
12.	Seed treatment using systemic insecticides to ensure early control of the disease				
13.	Crop rotations with non-cereal crops for 1- 3 seasons depending on disease history				
14.	Closed maize season where appropriate				

BEST PRACTISES TO MANAGE MAIZE LETHAL NECROSIS (MLN):

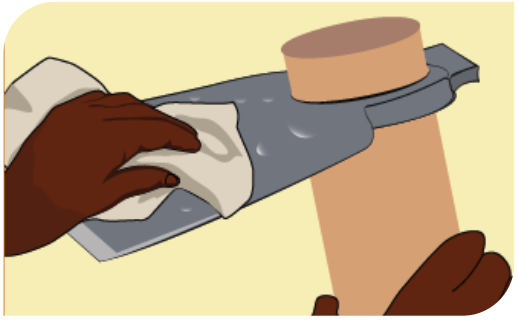
Avoidance

FIELD ACCESS RESTRICTION:
Avoid visiting your maize field once in contact with any MLN-affected maize field.

DON'T FEED INFECTED MLN PLANTS TO LIVESTOCK (CATTLE, SHEEP, GOAT, etc)
Animals feeding on MLN infected plants shall graze on healthy plant shall spread MLN

DISCUSS WITHIN COMMUNITY AND GET CONTROL MEASURES IN CONSULTATION WITH THE EXPERTS AND MINISTRY OF AGRICULTURE:

Exclusion



FARM TOOLS AND IMPLIMENTS DISINFEACTION
Clean farming equipment before and after use to remove MLN contaminated debris.

USE CERTIFIED MAIZE SEEDS:
Use certified maize seeds from a reputed seed agency or company each growing season.

AVOID SEEDS FROM PREVIOUS MAIZE CROP
Do not use seeds from infected maize plants or fields for planting.

Eradication

AVOID ALTERNATIVE HOST DURING OR PRIOR TO MAIZE CULTIVATION:

INSECT MANAGEMENT:

Effectively monitor the potential air borne insect vectors in the maize field by placing blue and yellow insect sticky traps in a 40 meter grid pattern throughout the maize field.

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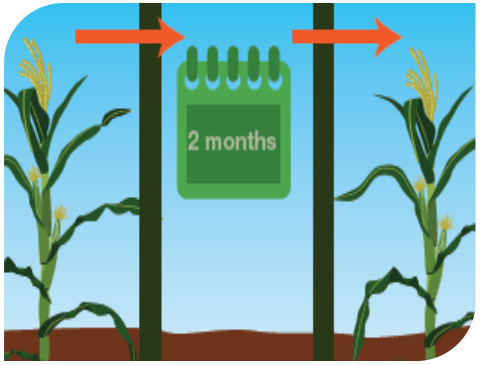
BEST PRACTISES TO MANAGE MAIZE LETHAL NECROSIS (MLN):

Protection



HOST- FREE PERIOD:
Crop practice a closed maize season of at least 2 months where applicable.

CROP ROTATION
Grow non-maize crop like legumes after the maize crop to avoid regular MLN hots

ROGUE MLN SUSPECTED PLANTS AND BURN THEM

GROW BARRIER CROP WITH MAIZE: Grow non MLN host crop as barrier in the maize plot, so that vector shall not get primary source of inoculum

USE Resistant Hybrids
Grow resistant hybrids that are developed by CIMMYT

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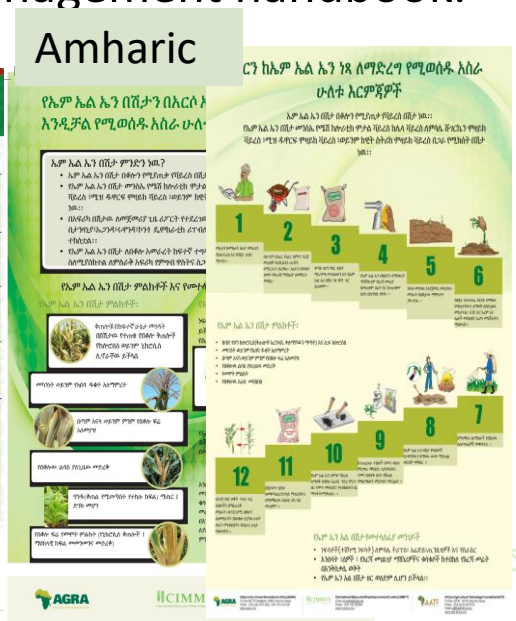
Develop and dissemination of appropriate communication materials on specific topics

- The MLN Web Portal post card updated with summary of achievements in MLN management efforts and distributed
- The project brief (Fact sheet) updated developed and distributed to various interest groups
- MLN Management materials development and distributed AATF/AGRA. Translated in Kiswahili and Amharic
- Development of the MLN catalogue in progress
- Development of MLN management handbook.

English

Kswahili

Amharic



Spanish

Highlights

- Maize lethal necrosis (MLN) emerged as a serious threat to maize production and livelihoods of smallholders in eastern Africa since 2011.

- An intensive multi-disciplinary and multi-institutional strategy is being implemented to curb the spread of MLN in sub-Saharan Africa, and mitigate the impact of the disease.

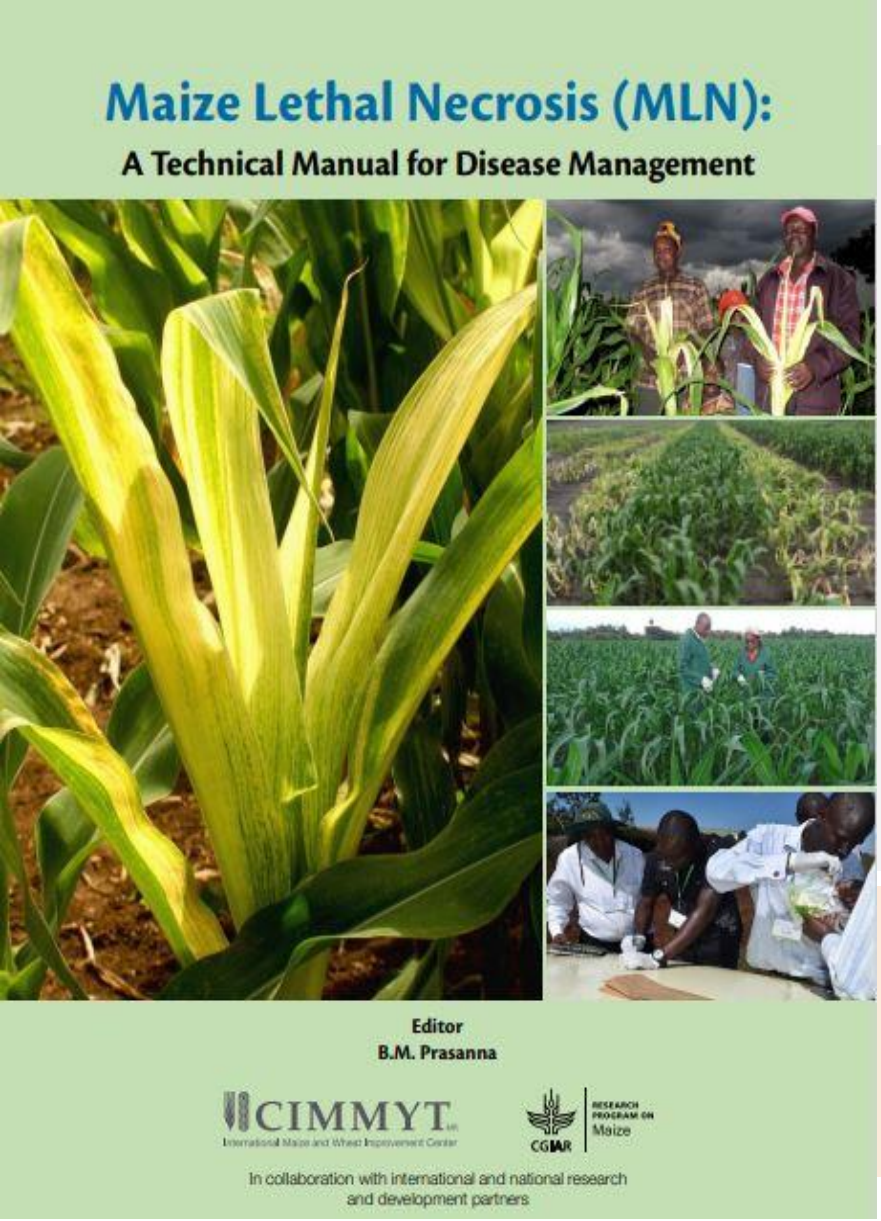
- Intensive germplasm screening led to identification of MLN-resistant sources, and fast-tracked development and commercial release of 19 MLN-tolerant/resistant hybrids in eastern Africa.

- Marker-assisted breeding led to successful conversion of 52 elite but MLN-susceptible inbred lines into MLN-resistant versions.

- MLN/MCMV diagnostic protocols have been optimized, and personnel from relevant public and private sector institutions trained on MLN diagnostics, monitoring and surveillance.

Summary

- MLN disease in eastern Africa is in control, with constant survilense, better quarantine measures, host free period, producing MLN disease free seeds
- Southern Africa is maintained free of MLN with safe exchange of MLN free seeds and emergency preparedness is ready
- Focus on MLN resistance breeding using various novel tools, technologies and deployment of MLN resistance hybrids in eastern Africa.
- Strong private and public partnership on producing MLN free seeds and MLN disease management



- The manual is organized in 10 chapters, as below:

- **Chapter 1:** Maize Lethal Necrosis (MLN) in Africa: Incidence, Impact, Rapid Response, and Management
- **Chapter 2:** MLN-causing Viruses in Africa, and their Symptoms
- **Chapter 3:** Modes of Transmission of MLN-causing Viruses
- **Chapter 4:** Maize Germplasm Phenotyping for MLN, MCMV and SCMV under Artificial Inoculation at the MLN Screening Facility, Naivasha, Kenya
- **Chapter 5:** MLN Surveillance, Leaf and Seed Sampling Protocols
- **Chapter 6:** MCMV, SCMV, and MLN Diagnostic Protocols
- **Chapter 7:** Managing MLN Quarantine Facilities: Phytosanitary Guidelines
- **Chapter 8:** MLN Pathogen-free Commercial Seed Production: Standard Operating Procedures
- **Chapter 9:** MLN Early Warning and Emergency Preparedness Plans
- **Chapter 10:** MLN Management: Conclusions and Future Perspective

This publication on Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management is intended as a comprehensive guide on best practices and protocols for sustainable management of the MLN disease in countries where the disease is already prevalent as well as for technically supporting “high-risk” countries globally for proactive implementation of practices that can possibly prevent the incursion and spread of the disease

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Virus Research 282 (2020) 197943

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Review

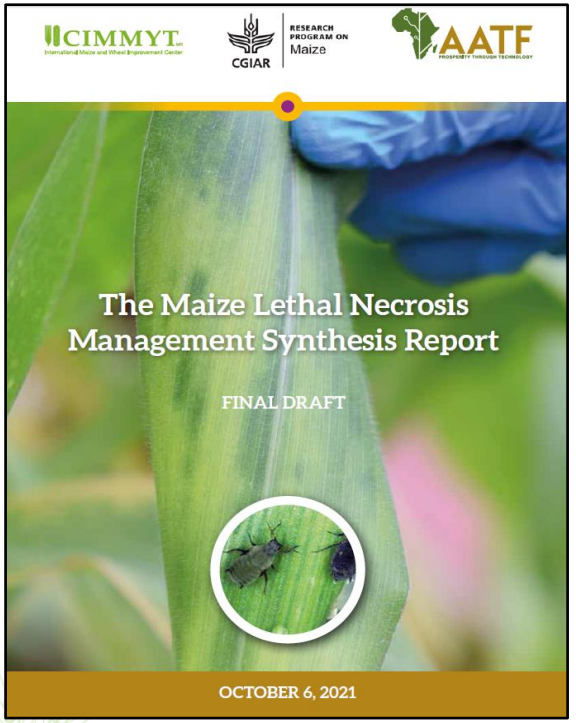
Maize lethal necrosis (MLN): Efforts toward containing the spread and impact of a devastating transboundary disease in sub-Saharan Africa

Prasanna Boddupalli[a,*], L.M. Suresh[a], Francis Mwatuni[a], Yoseph Beyene[a], Dan Makumbi[a], Manje Gowda[a], Mike Olsen[a], David Hodson[b], Mosisa Worku[a], Monica Mezzalama[b,1], Terence Molnar[b], Kanwarpal S. Dhugga[b], Anne Wangai[c], Lilian Gichuru[d], Samuel Angwenyi[e], Yoseph Alemayehu[f], Jens Grønbech Hansen[g], Poul Lassen[g]

https://doi.org/10.1016/j.virusres.2020.197943

THE MAIZE LETHAL NECROSIS MANAGEMENT SYNTHESIS REPORT

https://archive.maize.org/download/the-maize-lethal-necrosis-management-synthesis-report/



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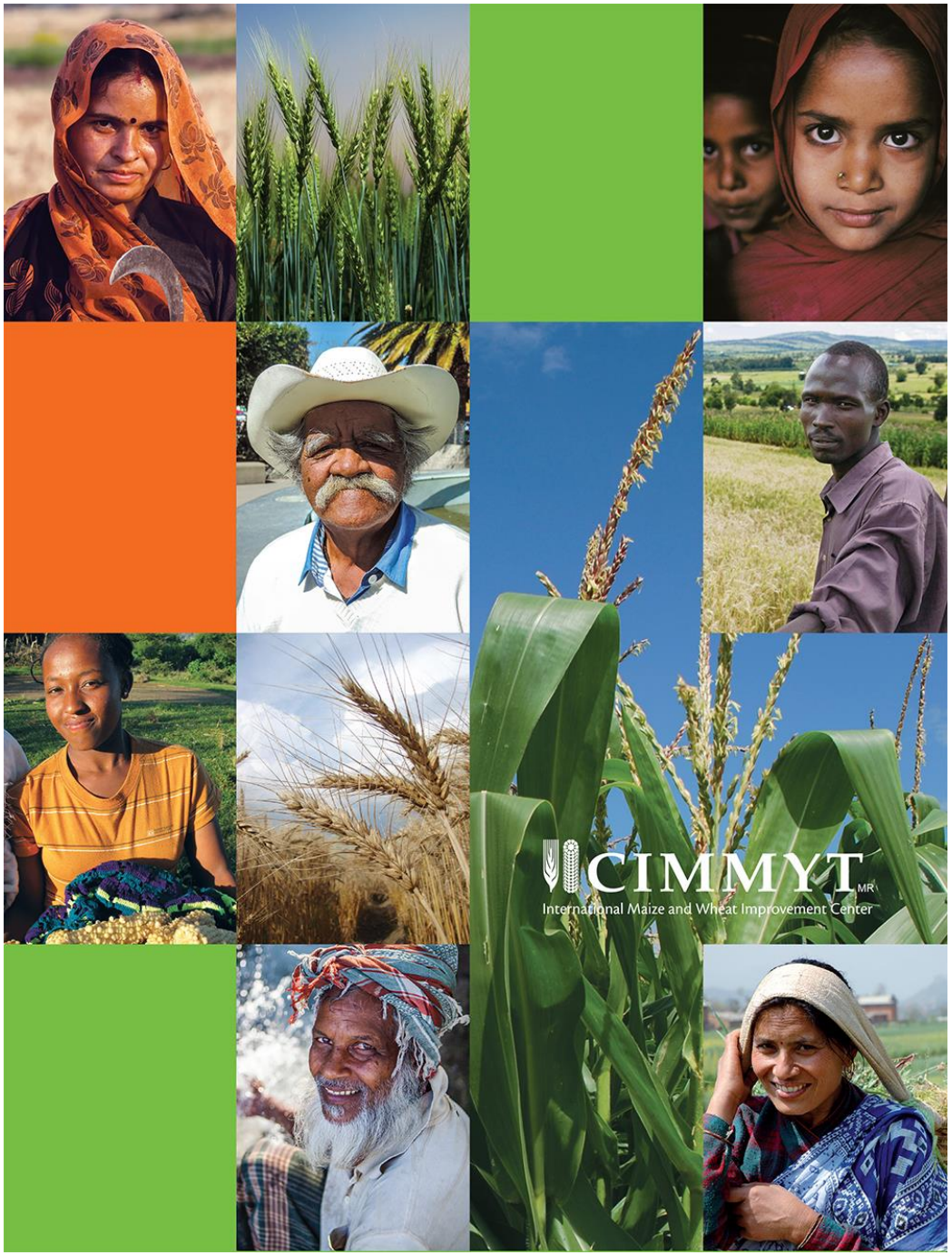
Thanks to…

- USAID, Bill & Melinda Gates Foundation, SFSA and CRP MAIZE, for supporting the work on MLN
- KALRO, NARES and Private sector partners



Thank you for your interest!