AGG-MAIZE TECHNICIAN TRAINING COURSE FOR EASTERN AFRICA

Diseases of Maize and Phenotyping

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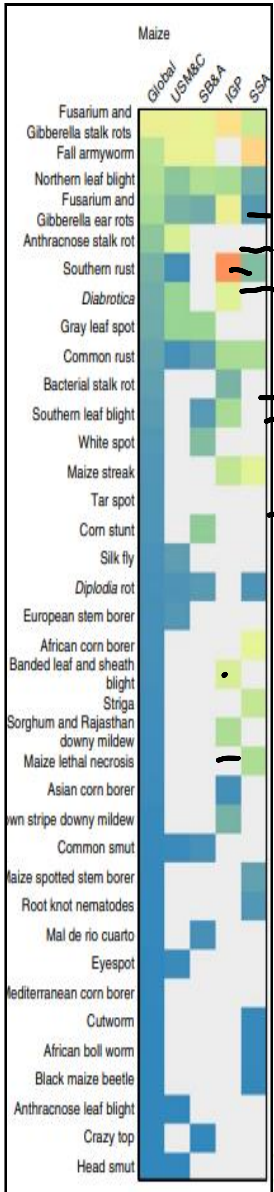
CIMMYT

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%.

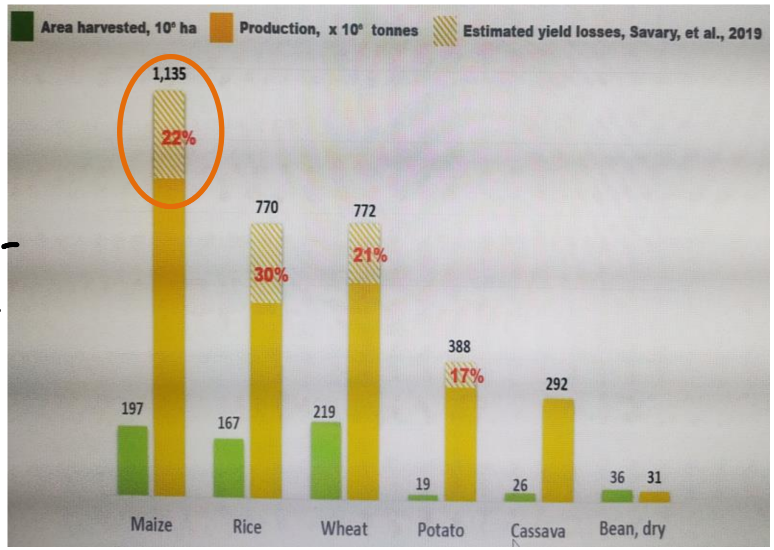
In Sub – Saharan Africa, the crop productivity is about 1.5 tons per ha as compared to 5.5 tons / ha globally, the decreased crop productivity is due to various stress and poor input management.

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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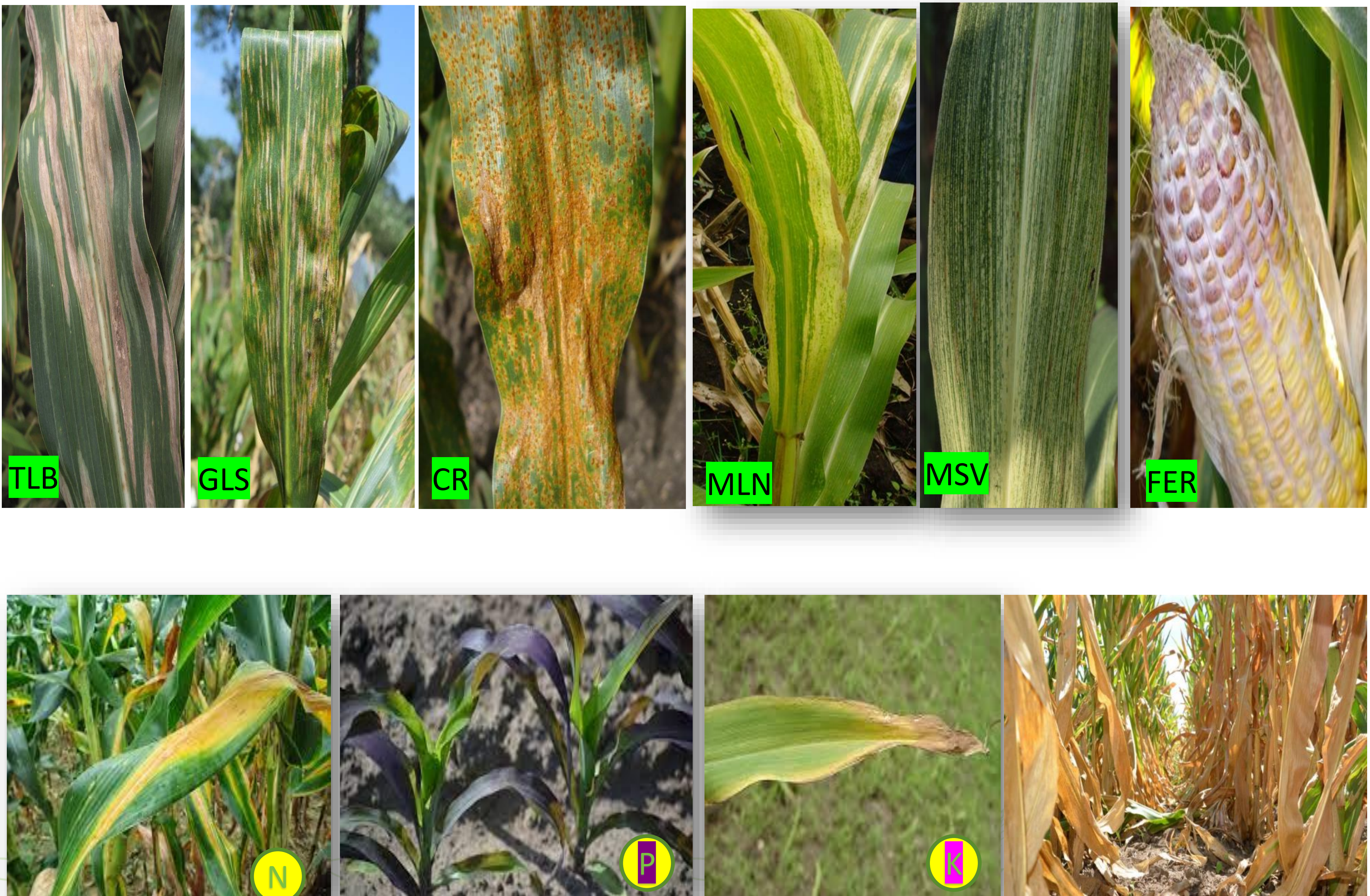
Maize Diseases

- Maize production in sub-Saharan Africa is affected by a wide array of diseases
- Environmental conditions prevalent in the different agro-ecological zones are conducive to the growth and spread of pathogens
- Different disease complexes affect maize production in the lowlands and mid-high altitudes
- Diseases often reduce production and cause up to 100% yield loss under severe epidemics depending on environmental conditions

Diseases of Maize and Phenotyping

- Foliar diseases
- Viral diseases
- Ear rot diseases
- Scoring for foliar and viral diseases
- Rating ear rot diseases

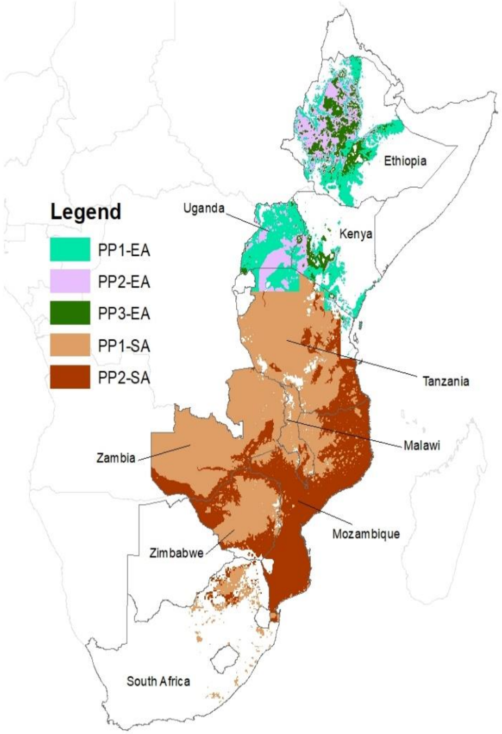
Maize Diseases and other stress



Topic covered

- Product profile – Trait matrix
- Why disease resistance
- What is disease [Host-Pathogen-Environment]
- Source of disease resistance
- Screening methodology
- Methods of disease resistance
- Efficacy of disease resistance

CIMMYT Maize Product Profiles in ESA



Region	Product Profile	Target Agro- ecology	TLB	GLS	CR	FER	MLN	MSV
Eastern Africa	EA-PP1-Int	Mid-altitude; wet	✓	✓		✓		✓
Eastern Africa	EA-PP1-Early	Mid-altitude; dry	✓	✓			✓	✓
Eastern Africa	EA-PP2	Upper Mid-altitude; wet	✓	✓	✓	✓		✓
Eastern Africa	EA-PP3	Highland	✓	✓	✓			
Southern Africa	SA-PP1	Mid-altitude (wet, dry)	✓	✓	✓	✓		✓
Southern Africa	SA-PP2	Mid-altitude & Lowland (wet, dry)	✓	✓	✓	✓		✓
Southern Africa	NuMPVA		✓	✓	✓	✓		✓

✓ Essential Traits

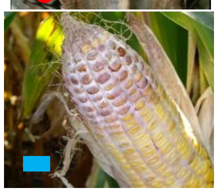
Disease	Causal Organism	Abbreviation	Trait Threshold
Maize Lethal Necrosis	Maize Chlorotic mottle Virus + Sugarcane Mosaic Virus	MLN	<4 MLN score (on 1-9 scale)
Maize Streak Virus	Maize Streak Virus	MSV	<4 MSV score (on 1-9 scale)
Turcicum Leaf Blight	*Exserohilum turcicum*	TLB	<4.0 TLB score (on 1-9 scale)
Grey Leaf Spot	*Cercospora zeina*	GLS	<4.0 GLS score (on 1-9 scale)
Fusarium Ear Rot	*Fusarium verticillioides*	FER	Less than 10% incidence
Common rust	*Puccinia sorghi*	CR	≤4.0 Common Rust score (on 1-9 scale)
Sorghum Downy Mildew	*Peronosclerospora sorghi*	SDM	<4.0 SDM score (on 1-9 scale)

Maize Disease Phenotyping Sites

Natural hot spots	Screening Sites under Artificial Inoculation
9	3

Natural hot spots	Screening Sites under Artificial Inoculation
9	3

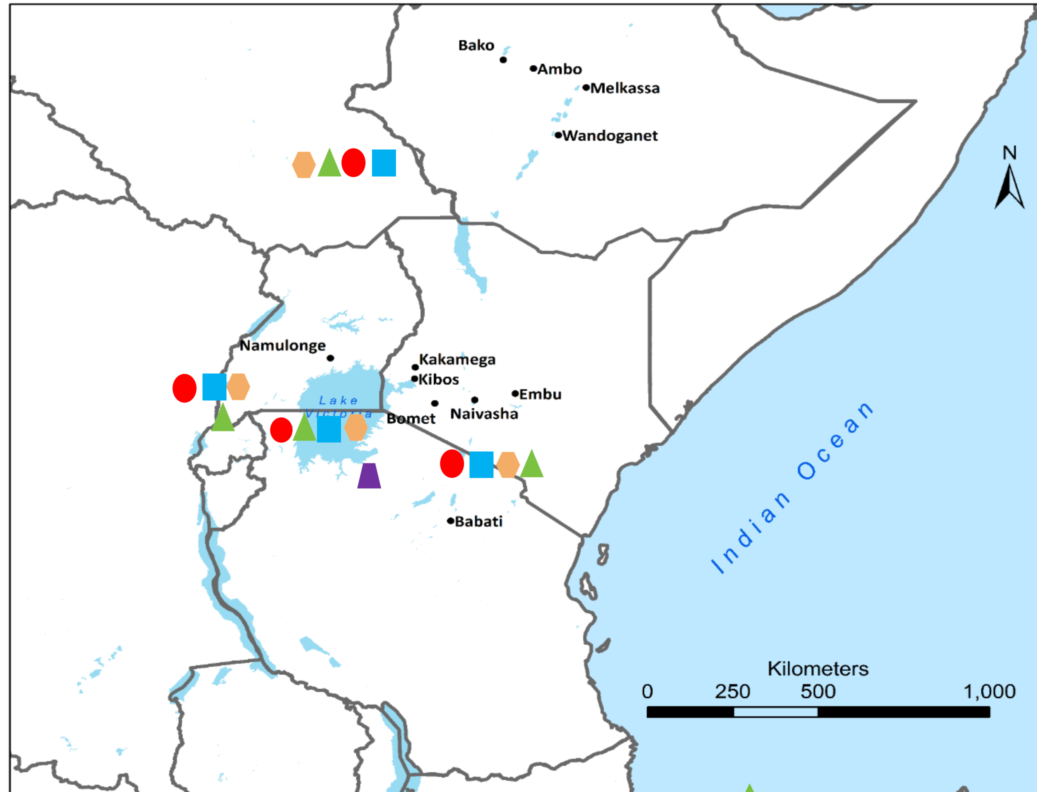
Natural hot spots	Screening Sites under Artificial Inoculation
9	-

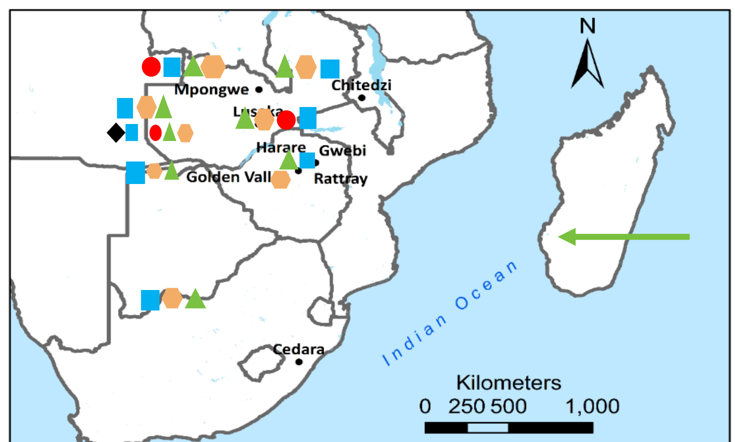


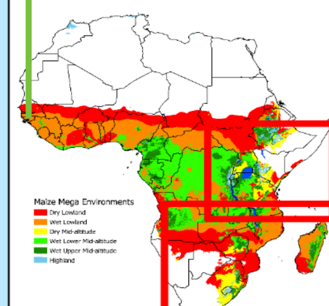
Natural hot spots	Screening Sites under Artificial Inoculation
9	1

Natural hot spots	Screening Sites under Artificial Inoculation
-	1

Natural hot spots	Screening Sites under Artificial Inoculation
-	1







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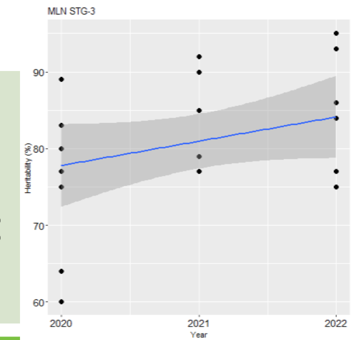
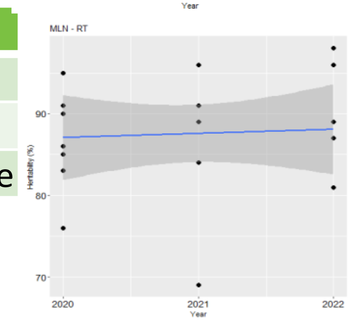
MLN Phenotyping Service (2014-till date)

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
2022	6,604	16,159	1,571	2,939	1,570	3,120	9,745	22,218
2023	**2,943**	**6,777**	**892**	**1,798**	**1,225**	**3,932**	**5,060**	**12,507**
Total	**143,590**	**234,920**	**39,120**	**57,212**	**47,417**	**72,607**	**230,127**	**364,739**

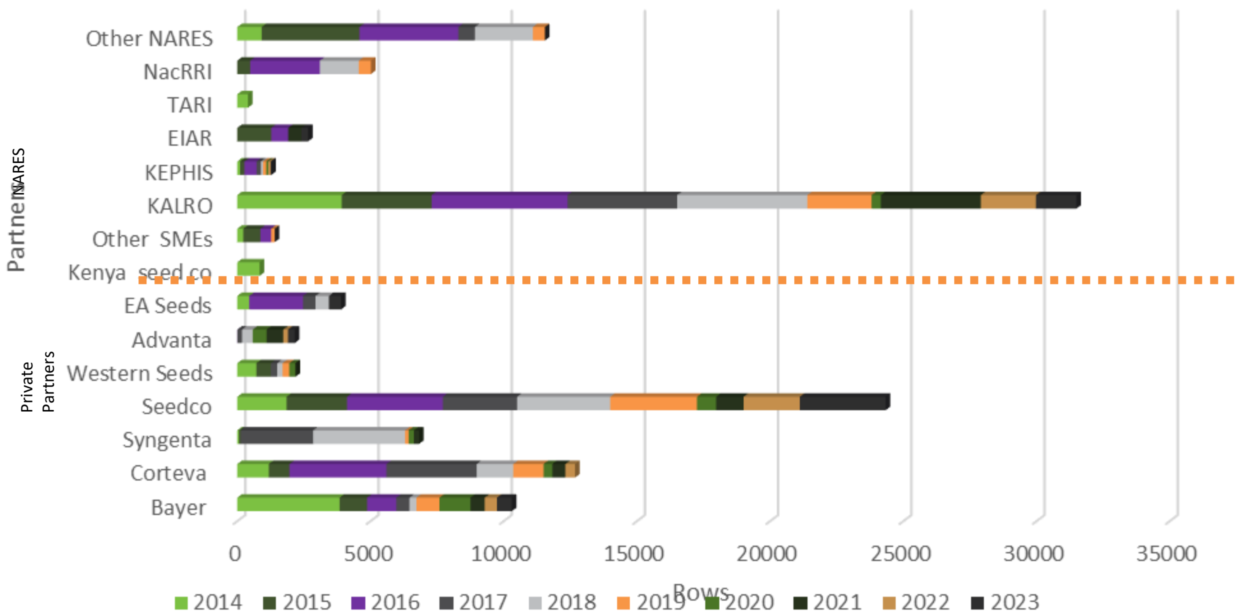
Responses of 262 advanced lines evaluated against MLN at Naivasha

Rating	# Lines	Response
1-4	**147**	Resistant
5-6	**84**	Tolerant
7-9	**31**	Susceptible



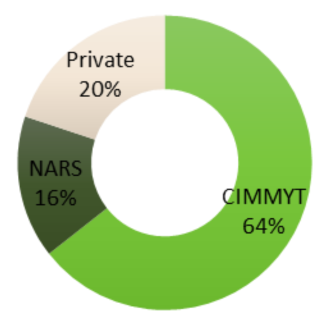


MLN Phenotyping Support for Public and Private Sector Partners



Other NARES: TARI, RAB, ZARI, DRSS, DARS, IIAM, SSARO (South Sudan)
Other SMEs: Zamseed, Aminata, Gicheha Farms, Victoria Seeds, Rasi Seeds.

MLN Phenotyping (2014 till date)



Private Partners: 17
NARES: 10
NPPO's: 1
CGIAR: CIMMYT, IITA

Disease Phenotyping Support to Breeding Pipelines

Example: 2022

CIMEAPP1-Early: Mean grain yield, agronomic traits and disease reaction of early maturing CIMMYT ːern Africa, 2022

						Agronomic traits across locations															reaction under natual and artificial inno			
EntNo	Entry code	Name	Pedigree	Vote 22	FAW artificial	AD	MOI	ASI	PH	EH	EPO	RL	SL	EPP	BHC	ER	TEX	NP	EA	PA	Rust Natural	MLN_AI	GLS_AI	TLB_AI
				n	t/ha	d	%	d	cm	cm	ratio	%	%	n	%	%	1-5 scale	n	1-5 scale	1-5 scale	1-9 scale	1-9 scale	1-9 scale	1-9 scale
21	CCK22EAPP1-02-01	SAWA(DSLH103)	SAWA(DSLH103)		4.64	66.83	18.97	1.29	217.30	98.48	0.49	4.93	2.56	0.98	18.11	8.71	2.74	34.84	2.63	2.87	3.03	5.99	4.02	4.15
22	CCK22EAPP1-02-02	DUMA43	DUMA43		2.33	65.35	17.11	3.08	223.84	99.03	0.48	5.79	3.12	0.84	16.54	17.56	3.03	34.07	3.69	3.45	3.50	8.00	4.19	3.86
23	TCK22EAPP1-02-01	CKHMLN181140	CKHMLN181140		3.78	69.86	20.09	1.02	248.77	128.59	0.51	8.10	3.57	0.99	5.48	2.54	3.68	35.56	2.49	2.84	2.80	4.01	3.93	4.40
24	XCK22EAPP1-02-01	DK777	DK777		4.62	69.17	19.42	0.09	234.82	107.67	0.49	4.29	2.63	1.01	5.89	3.08	2.34	36.16	2.25	2.45	2.69	4.99	4.43	4.66
25	RCK22EAPP1-02-01	CKHMLN181066	CKHMLN181066		5.70	69.13	19.78	0.09	240.87	135.95	0.54	6.41	4.09	1.04	3.35	2.78	2.24	35.56	2.40	3.02	2.56	4.00	3.34	5.03
29	WE7118	WE7118	WE7118																				7.00	6.76
9	CIM22EAPP1-02-09	CKHMLN200543	(CKDL0323LNT22/CKL05017LNT21)//CKDHL1800890	15	3.54	71.32	19.17	0.20	226.61	120.50	0.63	8.11	2.77	1.01	13.28	3.94	2.21	34.58	2.40	2.75	2.60	3.49	3.43	3.42
18	CIM22EAPP1-02-18	CHKE21W016	CML591LNT21/CML444LNT21//CML540LNT22	13	4.13	66.56	18.49	0.94	228.70	101.47	0.48	3.77	2.92	0.99	6.58	4.02	3.61	35.73	2.57	2.64	2.89	3.02	2.86	3.60
2	CIM22EAPP1-02-02	CHKE21W017	CML591LNT21/CML444LNT21//CML548LNT23	16	4.33	70.19	18.81	0.20	225.46	113.93	0.51	5.72	3.23	1.00	12.54	3.31	2.81	35.35	2.61	2.78	2.79	3.00	3.97	3.51
8	CIM22EAPP1-02-08	CHKE21W012	CML511LNT21/CML569LNT23//CML539LNT22	13	3.13	67.30	18.71	1.56	229.93	111.08	0.49	4.39	2.94	1.01	7.25	4.12	2.76	35.65	2.54	2.85	2.93	4.50	3.89	4.90
12	CIM22EAPP1-02-12	CKHMLN200520	(CML444LNT21/CML442LNT21)//CKDHL1800637	7	4.47	69.53	19.76	1.29	216.56	110.70	0.51	4.49	2.65	1.03	5.06	3.76	4.19	35.26	2.76	2.71	2.74	3.01	4.46	4.97
	Heritability	Heritability	Heritability		0.65	0.97	0.72	0.87	0.92	0.95	0.44	0.67	0.27	0.68	0.73	0.84	0.87	0.68	0.84	0.59	0.48	0.98	0.86	0.87
	Genotype Variance	Genotype Variance	Genotype Variance		1.53	5.82	0.74	0.66	107.59	131.28	0.00	8.33	0.84	0.00	27.96	11.20	0.51	0.76	0.09	0.06	0.12	1.94	0.73	0.72
	GenxLoc Variance	GenxLoc Variance	GenxLoc Variance		NA	0.38	0.61	0.24	40.22	20.97	0.00	12.54	4.94	0.00	26.39	10.10	0.31	1.01	0.08	0.13	0.35	NA	NA	NA
	Residual Variance	Residual Variance	Residual Variance		1.66	2.24	2.20	1.06	85.99	87.06	0.05	31.41	7.93	0.01	49.05	23.51	0.16	3.57	0.12	0.15	0.65	0.11	0.21	0.21
	Grand Mean	**Grand Mean**	**Grand Mean**		**4.18**	**69.12**	**18.75**	**0.69**	**229.80**	**114.37**	**0.51**	**6.72**	**3.00**	**1.00**	**8.08**	**5.17**	**2.92**	**35.16**	**2.68**	**2.84**	**2.86**	**4.26**	**4.09**	**4.36**
	LSD	LSD	LSD		2.14	1.29	1.31	0.82	9.60	8.62	0.10	5.07	2.21	0.08	7.74	3.86	0.74	1.37	0.36	0.45	0.73	0.75	1.07	1.03
	CV	CV	CV		30.80	2.16	7.91	149.69	4.04	8.16	42.53	83.42	93.84	9.40	86.71	93.82	13.56	5.37	12.66	13.84	28.13	7.71	11.07	10.50
	n Replicates	n Replicates	n Replicates		2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	n Environments	n Environments	n Environments		1	10	6	8	9	10	9	7	4	7	5	10	5	8	8	5	5	1	1	1
	Genotype significanc	Genotype significan	Genotype significance		0.01	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.00	0.00	0.00
	GenxEnv significance	GenxEnv significanc	GenxEnv significance		NA	0.07	0.02	0.01	0.00	0.02	0.73	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA	NA

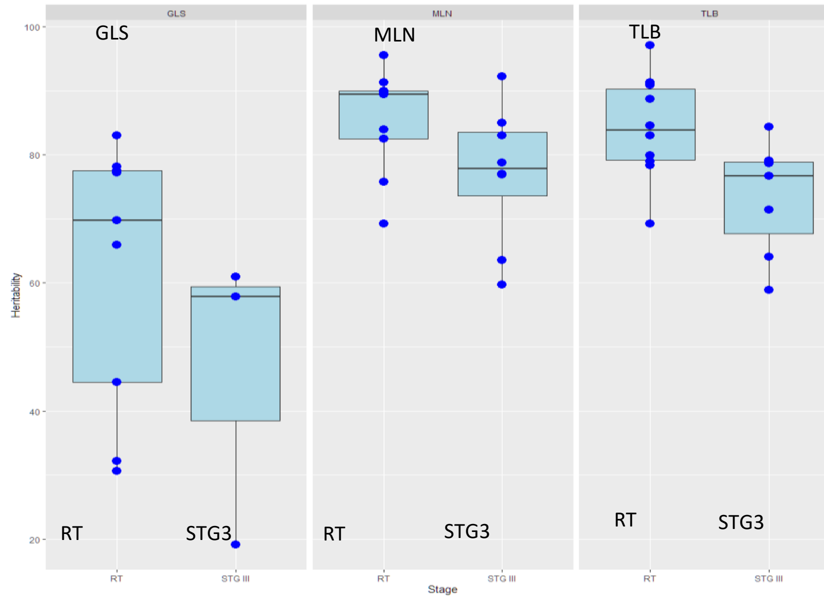
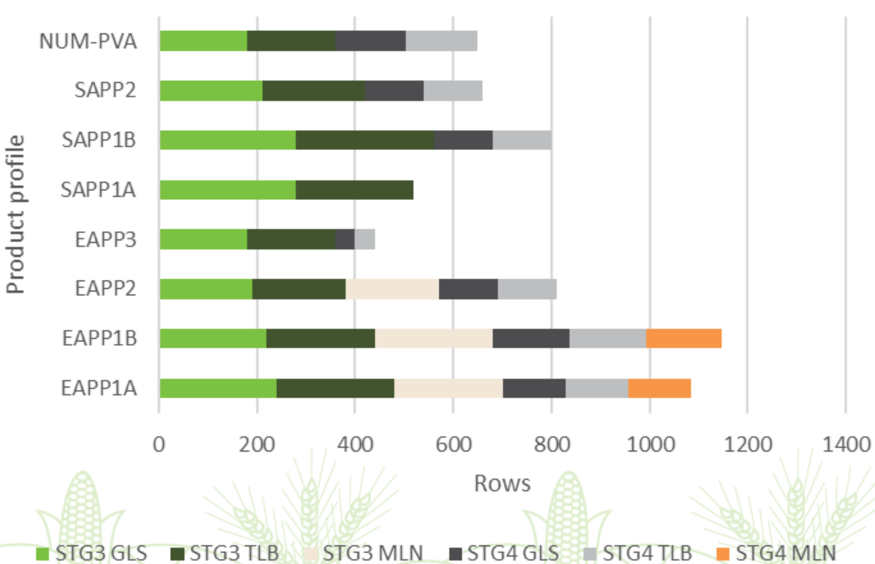


Figure: Disease phenotyping accuracy for STG III and RT trials for MLN and foliar diseases during 2020 and 2021

Disease Phenotyping Support for Breeding Pipelines - 2023



MLN Phenotyping - Naivasha

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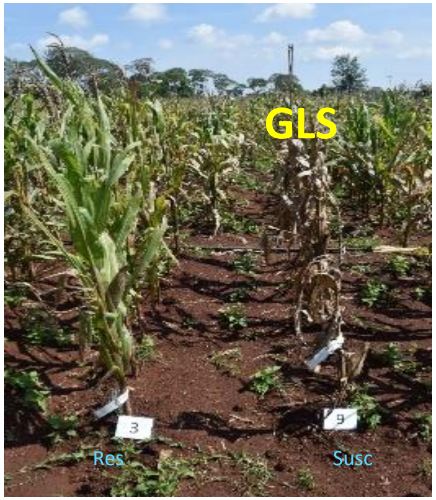
432 elite lines were evaluated in Kakamega for TLB and GLS for the last 4 years, and resistant lines identified.

Best performing lines for GLS and TLB (under artificial inoculation) and other agronomic traits were evaluated across four environments.

Genotype	GLS-R1	GLS-R3	GLS-AUDPC	TLB-R1[a]	TLB-R3[a]	TLB-AUDPC
CKDHL142989	2.47	2.55	34.85	4.17	5.91	70.71
DL14500	2.1	2.89	35.46	2.95	4.83	53.93
CML559	2.13	3.01	38.38	3.13	5.08	57.27
CML566	2.99	3.14	48.8	3.82	5.56	65.63
DL14501	3.22	3.16	45.47	2.92	4.43	50.6
((BRAZIL1546) DH4/CML395)-B-1-2-1-B	2.81	3.35	49.92	4.16	5.9	70.7
DL155	2.55	3.5	44.59	4	5.95	69.81
CKDHL120423	2.97	3.58	43.24	3.03	4.63	53.15
CKLMARS1C3S50196	3.41	3.67	49.35	4.48	6.48	77.22
CML540	3.42	3.68	49.71	3.28	4.99	57.55
CML536	3.24	3.75	59.91	2.75	4.01	46.57
CML574	2.34	3.87	45.46	3.33	5.21	59.63
DL14529	4.74	3.88	45.3	3.43	4.89	57.78
CML172	4.84	4.01	45.08	4.53	5.89	73.62
Susceptible						
CKL131275	7.56	8.53	114.70			
CKL13716	7.64	8.53	114.99			
CKL13802	7.02	8.70	111.18			
Mean	4.49	5.67	71.95			
σ2G	1.77**	0.40**	72.03**			
σ2GxE	0.40**	0.60**	101.64**			
σ2e	1.13	1.25	185.92			
h2	0.85	0.57	0.60			
CV %	19.83	17.95	16.55			
LSD at 0.05	1.98	1.38	17.97			

Genotype	TLB-R1	TLB-R3	AUDPC-TLB	GLS-R1a	GLS-R3a	AUDPC-GLS
CLWN211	2.24	2.36	32.02	3.65	5.17	63.07
CKL131049	1.81	2.73	31.68	3.53	4.58	59.18
ML-1A (IMAS-387)	2.83	2.99	41.32	4.61	5.4	72.26
CKDHL120918	1.39	3.06	31.23	3.11	5.23	59.18
DL15181	1.81	3.24	35.26	3.95	5.28	61.65
CML536	2.19	3.29	38.35	3.24	3.75	59.91
DL12102	2.72	3.31	42.3	4.36	4.06	58.05
ML-16 (IMAS-403)	1.2	3.41	32.13	3.55	4.93	60.56
CKL131305	2.79	3.41	43.17	4.6	5.52	71.23
CKL13870	1.88	3.5	37.57	4.12	4.6	63.06
DL12110	2.2	3.51	39.94	2.91	4.65	54.29
(CML488*2/S1920Z)-B-11-1-6-1-B	2.65	3.53	43.49	3.68	5.12	66.24
CKL131056	2.43	3.54	41.78	2.86	4.66	53.63
CKL131280	2.61	3.59	43.42	4.11	5.22	66.74
CKDHL0106	3.89	3.6	52.54	4.04	6.39	78.65
DL14501	2.19	3.62	40.6	3.22	3.16	45.47
CKDHL120331	2.74	3.63	44.62	5.58	6.1	73.09
CKL13814	3.55	3.66	50.62	3.21	4.69	56.53
((GEMS-0019xCML-312)-1-1-1-1/CML395)-B-5-1-1-B	2.34	3.68	42.26	4.42	5.61	71.98
CML543	2.87	3.7	45.92	4.71	5.29	67.79
DTPYC9-F38-4-3-1-1-B	3.8	3.7	52.45	4.75	5.45	72.98
VL05552	3.67	3.71	51.96	5.16	5.63	76.54
CML567	2.45	3.74	43.2	4.28	4.96	64.32
CKLT10045	2.22	3.8	42.14	4.39	6	95.98
CKDHL142833	1.69	3.9	38.9	4	4.87	64.26
DL141248	2.3	3.9	43.53	4.5	5.95	74.9
CKDHL0378	2.06	3.91	41.49	3.81	5.51	66.17
KIL-1 (IMAS-405)	2.75	3.92	46.57	4.16	5.28	67.44
((GEMS-0011xCML-373)-1-2-2-1/CML312)-B-31-2-1-B	2.69	3.93	46.34	4.3	5.55	70.82
DL14533	3.27	3.97	50.82	3.97	4.99	65.57
CKLTI0368	2.82	4.00	47.7	4.07	6.08	84.26
Susceptible						
CKL13731	6.44	8.52	104.77			
CKL05017	2.54	8.57	78.13			
CKDHL0364	5.45	8.68	99.05			
Mean	3.85	5.53	65.68			
σ2G	0.58**	0.69**	119.85**			
σ2GxE	1.01**	0.73**	94.36**			
σ2e	1.169	1.49	204.57			
h2	0.75	0.75	0.78			
CV %	18.41	13.96	14.96			
LSD 0.05	1.39	1.52	19.31			



Evaluation of elite lines for GLS and TLB

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Objective

- **Identify superior disease resistant germplasm for incorporation into breeding programs**
- **Collect good disease phenotypic data**
- Use association mapping approaches to understand the organization of disease resistance genes in the maize genome
- Develop markers for marker assisted selection breeding

Breeding for resistance to diseases

- Use of disease resistant cultivars is the most valuable and practical means to control diseases

- It is also inexpensive, effective, and simple to apply over a wide area in a target production zone

Requirements for development of disease resistant maize cultivars

1. Diverse germplasm

2. Screening tools

3. Test locations with consistently high disease pressure

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Types of resistance

- Resistance is available for most of the economically important diseases in maize
- Resistance is controlled mainly by
  - One or a few genes (monogenic or oligogenic)
  - Many genes (polygenic)
    - With additive and dominance effects

1.Vertical resistance

- Complete resistance of a host to a specific race of a pathogen
- The host plant exhibit hypersensitive reaction that prevents the establishment and multiplication of the pathogen
- Controlled by one (monogenic) or a few (oligoginic) genes
- Plants show distinct resistant and susceptible categories
  - Selection is thus easy in segregation populations
- Transfer from source to other germplasm is also easy
- It is less durable
- Has been used to control very few disease in maize

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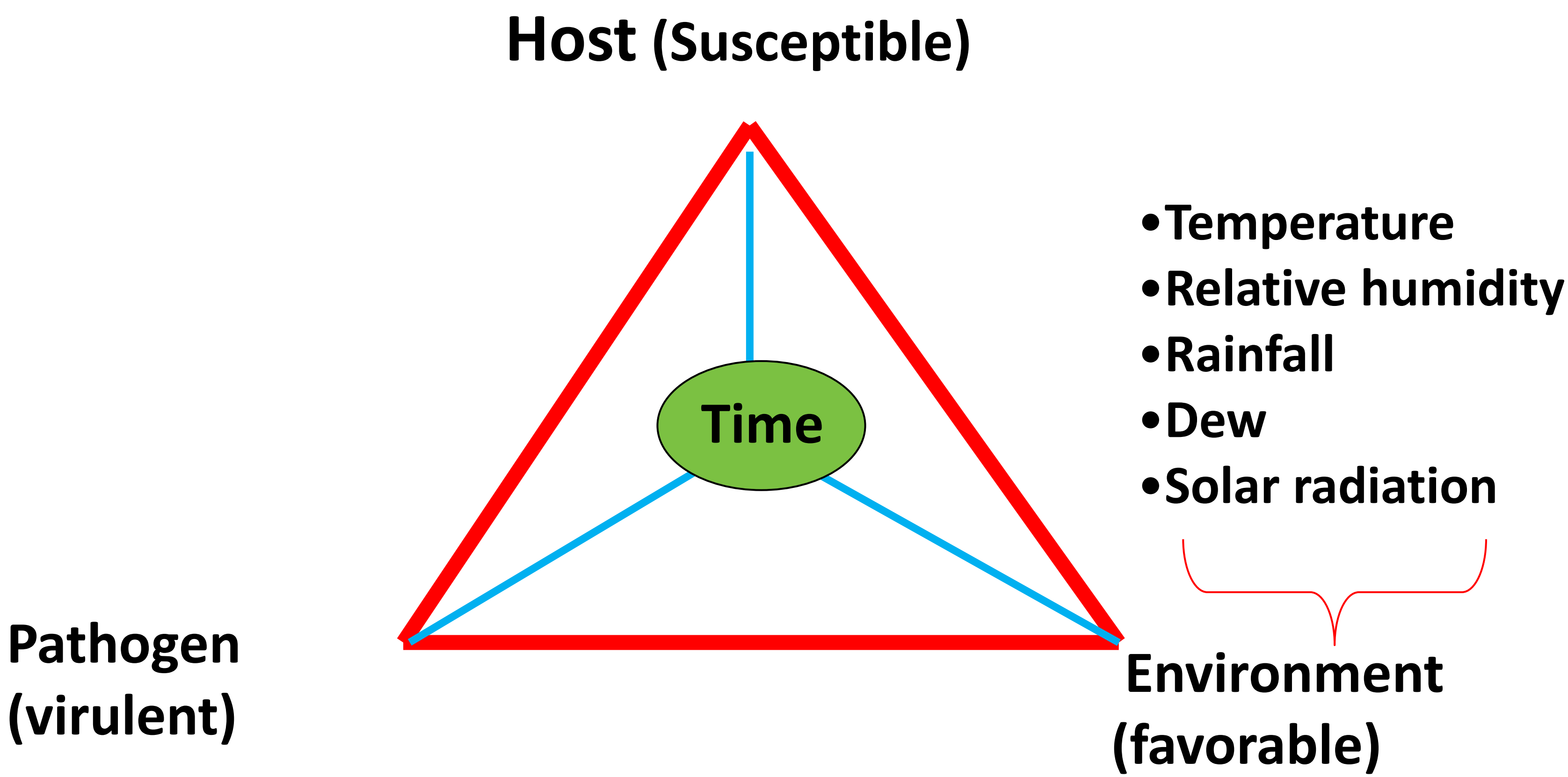
Types of resistance

2. Horizontal resistance

- Effect of resistance on the survival and reproduction of the pathogen is less complete
  - Provides less selection pressure on the pathogen
- It retards the infection process and slows down the spread of the disease
- Controlled by many genes (polygenic) each with small effect
- Resistance shows continuous variation
- It is more durable and stable due to the buffering effect of polygenes
- Has been used for controlling most diseases in maize

The two types of resistance can co-exist

Factors affecting disease occurrence



* Climate change impacts the host, agent (pathogen) and environment

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Disease Triangle Components

- Pathogen
  - Strains or pathotypes
  - Period of interaction/introduction
- Host
  - Breeding for host resistance
- Environment
  - Conducive to disease dev – usually high humidity and low standing water
- Time
  - Infection prior to grain filling is most vulnerable stage

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Environment

- Temperature
- Rain
- Moisture
- Wind
- Insect vectors
- Humans
- Agronomic practices

Host Resistance

- Vertical vs Horizontal
- Major gene vs Minor gene
- Qualitative vs Quantitative
- Genes vs QTLs
- Resistance vs Tolerance
- Resistance vs Escape

Breeding for Disease Resistance

Basic Requirements

1. Understanding of Pathogen Variability
2. Germplasm Diversity and Genetic basis for R
3. Reliable Phenotyping Protocols and Networks

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Mechanisms for disease resistance

1. Resistance to pathogen establishment

- *Immunity*
  - Prevent pathogen from establishing itself due to innate structural or functional properties of the host
- *Hypersensitivity*
  - Prevents pathogen survival and reproduction due to rapid death of the host plant cells

2. Resistance to an established pathogen

- Restricts the ability of the pathogen to spread and reproduce after becoming established in a host

3. Tolerance

- The plant exhibits severe disease symptoms without a serious loss in yield

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Resistance screening methods

- Field, greenhouse (screenhouse) and laboratory-based screening techniques are available for the major diseases of maize
- Use established screening techniques
  - Effective
  - Cheap
  - Easy to handle depending on available facilities and personnel
  - High throughput for screening a large number of breeding materials
- Field screening of breeding nurseries at hot-spot locations / artificially inoculated condition with consistently high disease pressure is also effective
- Evaluate selected resistant genetic materials in one location (greenhouse, laboratory) at multiple-locations to expose them to different populations (races) of the pathogen

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Objective of disease evaluations

- Test entries are exposed to adequate and uniform disease pressure.
- Guarantee greatest differentiation of genotypes.

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Disease screening methods

- Rate of progress to develop stable and durable disease resistance or marker development depends on:
  - the use of reliable screening techniques
  - use of as wide a spectrum of the pathogen as possible and at an appropriate disease pressure
- Take note that:
  - Low disease pressure
    - ✓ Unreliable results that slow down rate of genetic gain
  - High or severe disease pressure
    - ✓ Eliminate low level resistance inherent in adapted germplasm and may drastically narrow the germplasm base

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Disease screening techniques

Two major groups:

1. Naturally occurring epidemics

2. Artificially created epidemics

Naturally occurring epidemics

- Hot spots
- Use of a location known for its high level of infection for a particular disease
- Used for a pathogen with a local concentration of alternate hosts

Advantages

- ✓ Cheap and easy to manage
- ✓ Test materials are exposed to all pathogen races

Disadvantages

- ✓ Success depends on year– to– year consistent expression of epiphytotic
- ✓ adequate and uniform natural infections can rarely be achieved in most locations
- ✓ Disease might not be evenly distributed within the field

Naturally occurring epidemics

- Enhanced natural infections to ensure adequate disease levels
  - Manipulation of planting dates
  - Create favorable environmental conditions (e.g. irrigation, enhanced drought etc)
  - Use of spreader rows & use susceptible checks every few rows (e.g. every tenth row)
  - Sufficient replications (minimum of three)
  - Multiple locations

Increasing phenotyping accuracy and efficiency

Accuracy :

- Protocol optimization
- Capturing the host defense mechanism to improve the genetic gain
- Epidemiological studies for emerging diseases
- Exploring proximal phenotyping in controlled environments
- Management practices
- Capacity building
- Leveraging the internal capacity

Efficiency :

- Increasing the frequency of phenotyping
- Using remote sensing / Image analysis

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Disease Scoring and data recording – key notes

- **Field severity :**
  - Disease pressure should be uniform and consistent to differentiate the resistant and susceptible disease reaction
- **Trait Check :**
  - Resistant, Tolerant and Susceptible trait designated check should be included in each trial to understand and compare the disease reaction of the trait check.
- **Disease incidence:**
  - Disease reaction is observed and recorded based on per plant basis especially wilt diseases
- **Disease Severity :**
  - Disease reaction is observed and recorded based on plant or plot basis, most of the foliar, and MLN, MSV disease
- **Plot Score :**
  - Disease reaction is observed and recorded based on plot per se
- **Individual Plant Score :**
  - Similar to disease incidence
- **Number of Scores :**
  - Normally as many as 2 to 3 scores are recorded, but in case of MLN, four scores are taken, but finally 4th scores is analysed, which coincides with maturity
- **Critical stage – Disease score**
  - Same as above, but 3rd or 4th scores is considered as it coincides with maturity
- **Multiple season Score**
  - In order to compare the consistent disease resistant / tolerant reaction of a selected trials, is good to analyse multiple season under similar disease pressure
- **Multiple Site score**
  - In order to under stand the disease reaction of a line or hybrids from various hot spots, is good to consider the trials under natural hot spot with similar disease pressure.
- **Indexing**
  - Disease reaction for a line trial or hybrid trial is recorded, just to confirm the disease reaction
- **Screening**
  - Disease reaction is recorded for a hybrid or line trial, but yield is also harvested under disease pressure.

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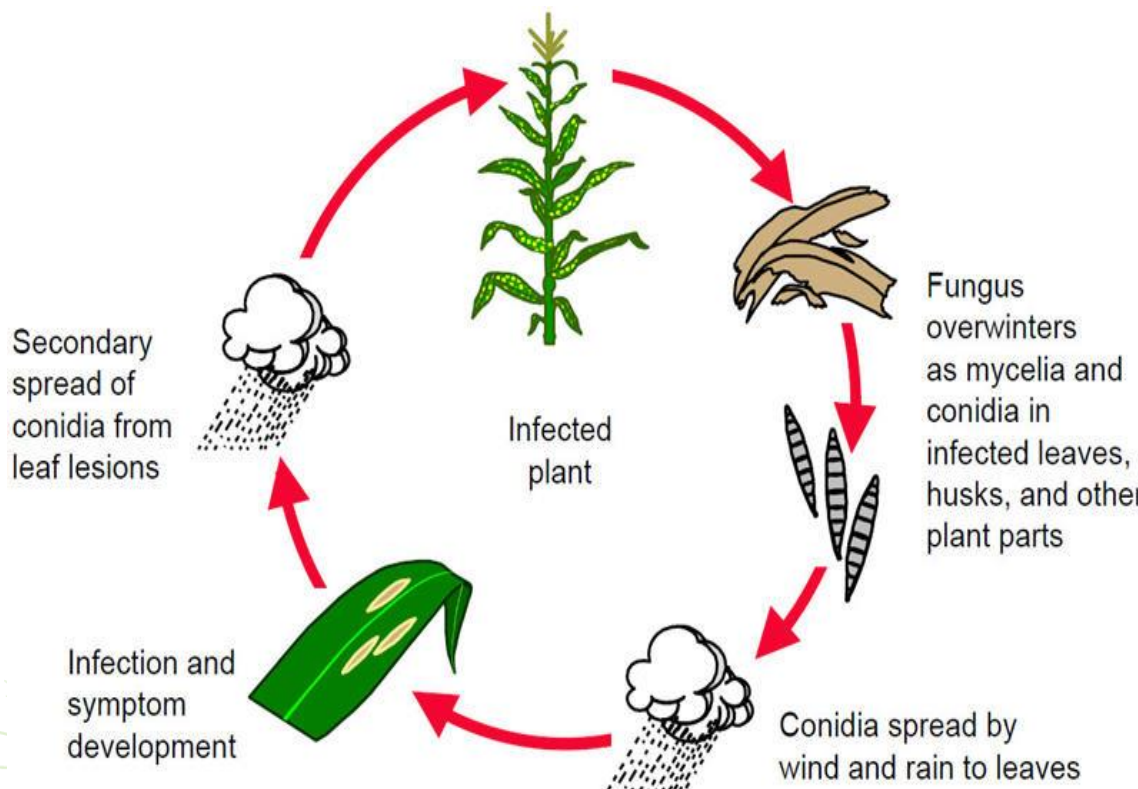
Turcicum Leaf Blight

- Can account for up to 50% yield loss
- Qualitative and dominant R gene available therefore TLB is generally a problem in inbred maintenance and seed production but not in final hybrids
- Global problem and thrives in moderate temps and high temperatures.
- **Qualitative and dominant strain specific R genes**



Disease evaluation

Various degree of TLB infection on different genotypes of maize despite of using same inoculum, same inoculation and observation time. Therefore, it requires standardized disease rating scale.

Rating of Germplasm Responses against Turcicum Leaf Blight (TLB) *Exserohilum turcicum* on a 1-9 Scale

1

No infection to very slight infection

2

Slight infection, a few lesions scattered on two lower leaves affecting 1% of the leaf area

3

Less infection, a moderate number of lesions scattered on four lower leaves, affecting 2% of the leaf area.

4

Less infection, a moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the ear, affecting 3-5% of the leaf area.

5

Moderate infection, an abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the ear, affecting between 6-10% of the leaf area.

6

An abundant number of lesions scattered on lower leaves, moderate infection on middle leaves, and a few lesions on two leaves above the ear affecting 11-20% of the leaf area.

7

Heavy infection, an abundant number of lesions scattered on lower and middle leaves, and a moderate number of lesions on two to four leaves above the ear. Lesions are abundant on leaves affecting between 21-40% of the leaf area.

8

Severe lesions are abundant on leaves affecting between 41-80% of the leaf area.

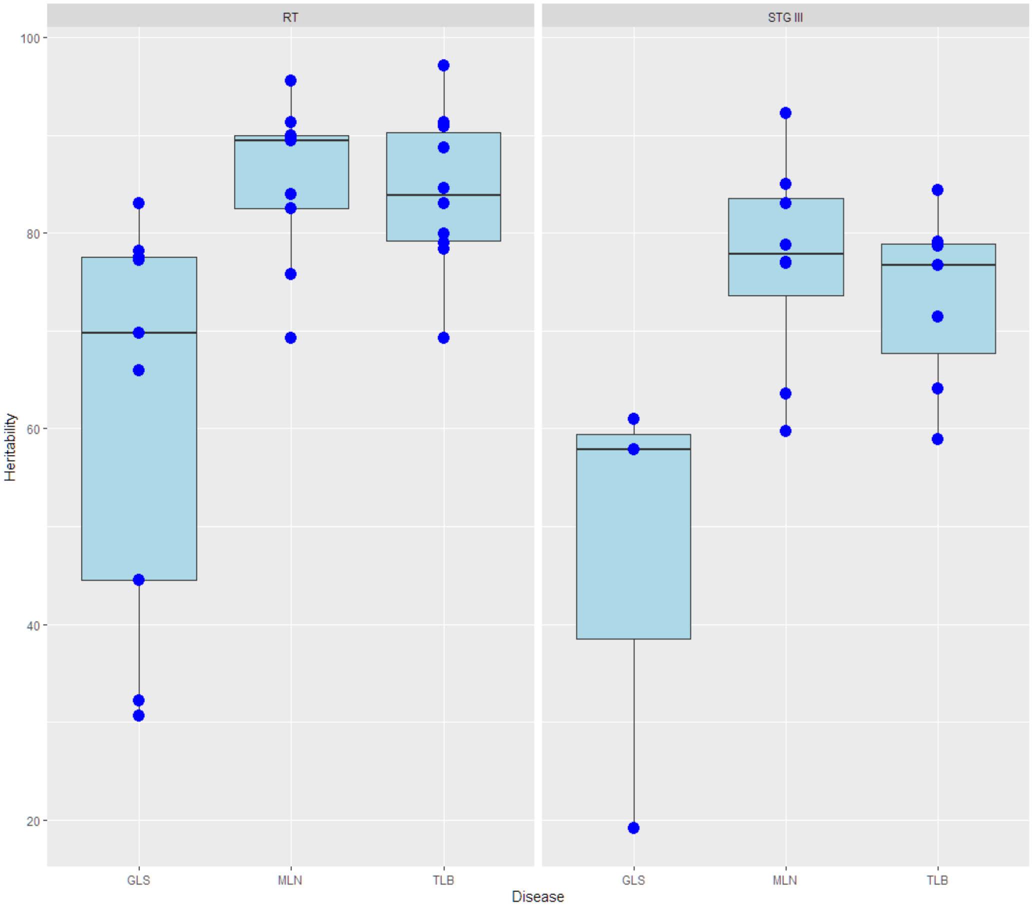
9

Severe lesions abundant on almost all leaves, plants prematurely dried or killed with 86-100% of the leaf area affected.

- The first scoring is done 40 days after 1st inoculation (Reproductive stage-1 or R1)
- The second scoring is done 10-15 days after first scoring (R2 stage)
- The third scoring is done 10-15 days after second scoring (R3 stage)

CHPROA19_TLB		
Heritability		0.70
Genotype Variance		0.41
Residual Variance		0.36
Grand Mean		4.36
LSD		1.23
CV		13.70
n Replicates		2
Genotype significance		0.0
Max		6.50
Min		3.00
Mean		4.36
Check	Trait Category	Score
SC301	S	6.47
SC419	S	4.56
PAN 4M-23	R	4.32
SC513	S	4.17
CZH15543	R	3.86
Local check 1 (SC303)	S	6.19
Local check 2 (SC533)	S	5.64

StageIIINUMPVA217_TLB		
Heritability		0.78
Genotype Variance		0.49
Residual Variance		0.27
Grand Mean		5.72
LSD		1.16
CV		9.05
n Replicates		2.00
Genotype significance		0.00
Max		7.15
Min		3.94
Mean		5.72
Check	Trait category	Score
P3812W	S	4.45
SC653	R	4.51
PHB30G19	R	3.50
PAN53	R	4.15
SC729	R	4.10
SC719	S	4.05
Mukwa MN701	S	4.50
CZH15026	R	3.10
Local check 1 (SC727)	S	4.06



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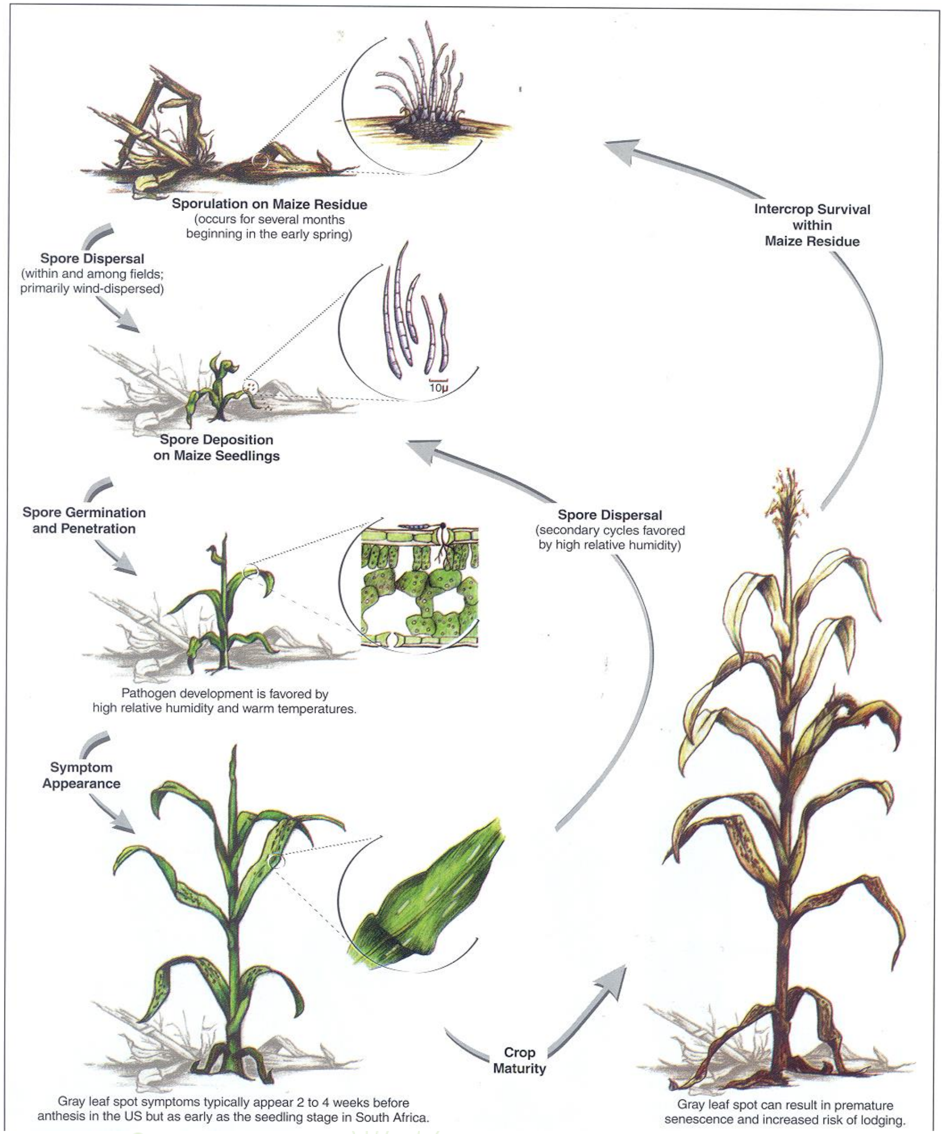
Grey Leaf Spot

- More prevalent under warm, humid conditions and CA
- Sporadic in nature but can account for upto 30% yield loss
- R is additive with minor effect QTLs



Disease cycle of GLS. Source: Ward *et al*. (1999).

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Rating of Germplasm Responses against Grey Leaf Spot (GLS) *Cercospora zeae-maydis* on a 1-9 Scale

1

Clean. No visible symptoms

2

Fine or small lesion, three to five scattered lesions on the leaves. Leaf area with 1% damage.

3

Moderate number of lesions on the leaves affecting less than 2% of the leaf area.

4

Moderate number of lesions on emerging new leaves below the ear, affecting 3-5% of the leaf area.

5

Abundant lesions on the leaves, above the ear, affecting between 6-10% of the leaf area.

6

More lesions throughout the plant up to flag leaf, necrosis of leaf margin, affecting between 11-20% of the leaf area.

7

Lesions are abundant on leaves, throughout the plant, affecting between 21-40% of the leaf area.

8

Lesions are abundant on leaves affecting between 41-80% of the leaf area.

9

Lesions abundant on almost all leaves, plants prematurely dried or killed with 81-100% of the leaf area affected.

- The first scoring is done after 40 days of 1st inoculation (Reproductive stage-1 or R1)
- The second scoring is done after 10-15 days of first scoring (R2 stage)
- The third scoring is done after 10-15 days of second scoring (R3 stage)

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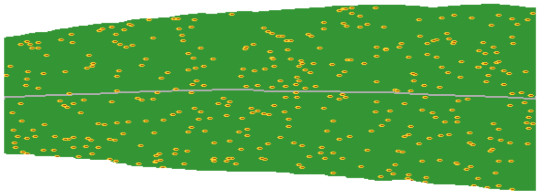
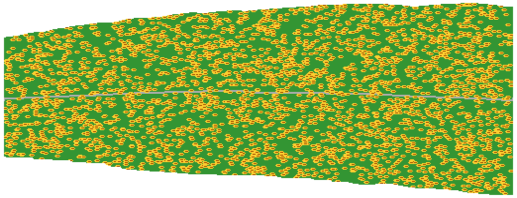
Naturally occurring epidemic of common rust
Puccinia sorghi

Standard Area Diagram to use with the 1-9 Disease Rating Scale for polysora rust (*Puccinia polysora*)

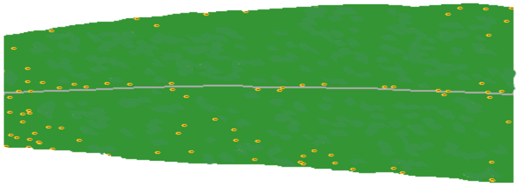
1 = 0%



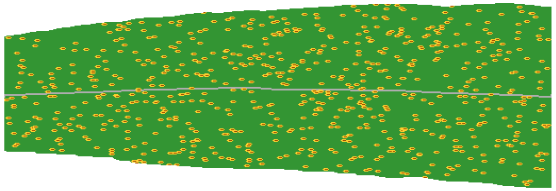
4 = 3-5%



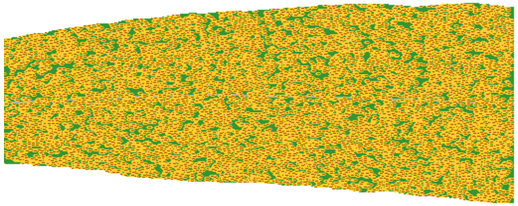
7 = 21-40%



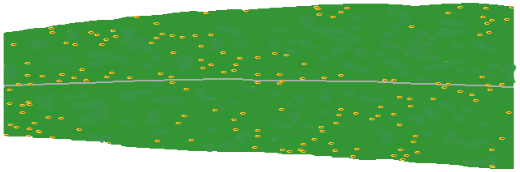
2 = 1%



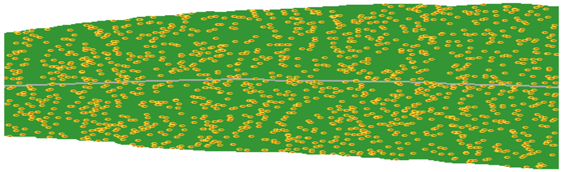
5 = 6-10%



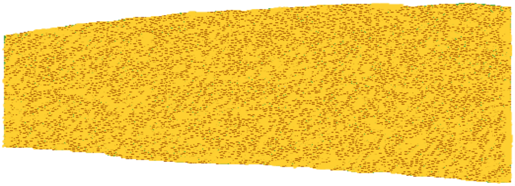
8 = 41-80%



3 = 2%



6 = 11-20%



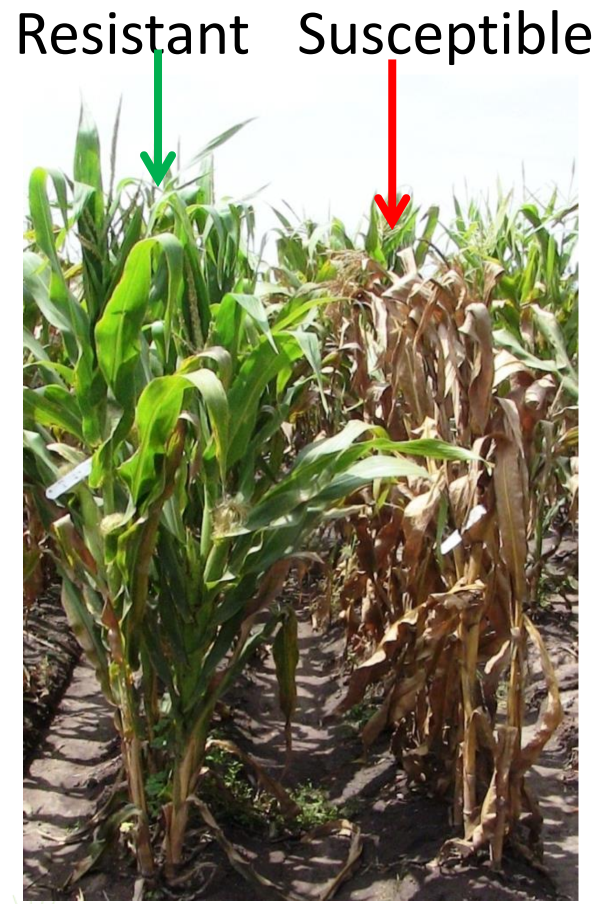
9 = 81-100%

The percent area affected is recorded as the accumulative leaf area with lesions beginning on the leaf below the primary ear. Individual leaf data is not recorded, but the diagrams above assist in estimation of leaf area affected. Data is recorded at four weeks post-flowering.

Image from hdimage.com

Good phenotyping data across locations – Common Rust (*Puccinia sorghi*)

Oxalis sp. – an alternative host for *Puccinia sorghi*



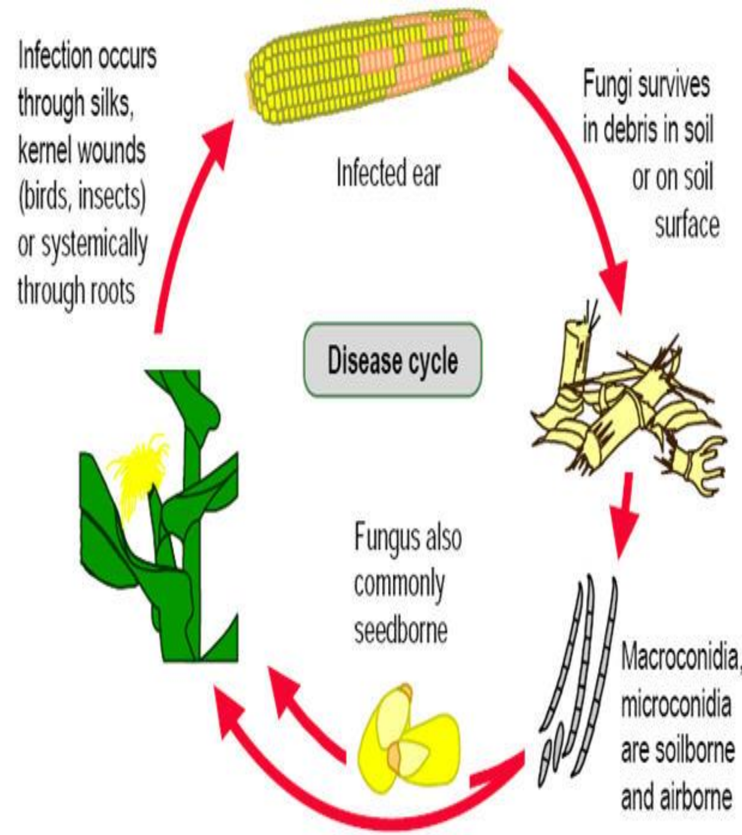
Replications =3

Number of entries = 300 genotypes

Ear Rots

- Significant yield damage
- Mycotoxin risk
- Highly environmentally variable

- Major Ear Rots
  1. Aspergillus ear rot (Aspergillus flavus)
  2. Fusarium Ear Rot (Fusarium moniliforme and other species (Gibberella))
  3. Diplodia Ear Rot (Stenocarpella maydis)



Ear rot Disease

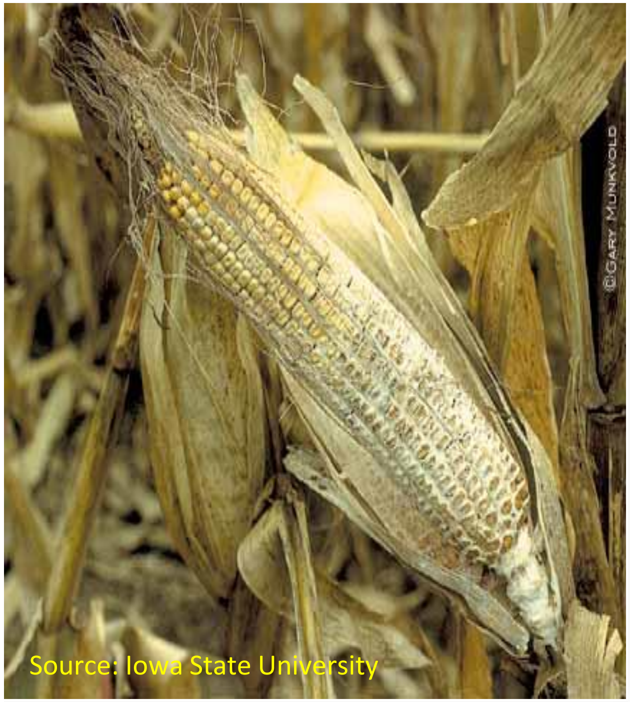
Fusarium Ear rot

Fusarium verticilloides

- Common in all maize growing ecologies
- Hot dry weather after flowering favors Fusarium ear rot
- Symptoms
    - Whitish/pink mycelium on/between kernels
    - Starburst symptoms on kernels
- Fungi overwinters in crop debris and airborne conidia infect silks at flowering
- Control through host resistance and proper storage
- Quantitative Resistance (composition of kernel is important)

Diploidia ear rot

Stenocarpella maydis



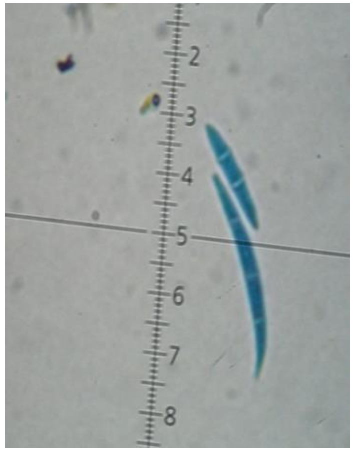
- Prevalent when wet weather follows silking
- White / grey mycelial growth over husks and kernels
- Infected kernels may be very light and appear to be glued together
- Black pycnidia are produced late in the season
- Fungi overwinters in infected stalks

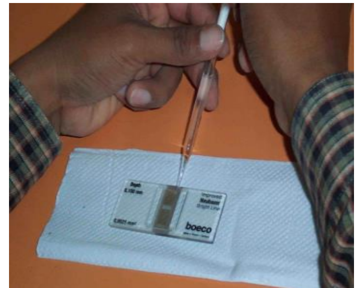
Aspergillus ear rot

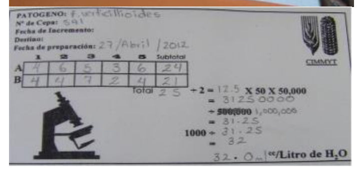
Aspergillus flavus

- Most serious under high temp and drought
- Production of aflatoxins
- Overwinters in crop debris and spores are dispersed by wind or insects
- Host resistance offers best control. Rotation has minimal effect as a lot of alternate hosts
- Difficult to phenotype. R is likely to be quantitative but difficult to phenotype reliably

In the case of Fusarium ear rot - Steps for preparing inoculum







PATOGENO: F. verticillioides
Nº de Cepa: 591
Fecha de Incremento:
Destino:
Fecha de preparación: 27/Abril/2012

	1	2	3	4	5	Subtotal
A	4	6	5	3	6	24
B	4	4	7	2	4	21

Total 25 ÷ 2 = 12.5 X 50 X 50,000
= 31250000
÷ ~~500,000~~ 1,000,000
= 31.25
1000 ÷ 31.25
= 32
32.0 ml cc/Litro de H_2O

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Inoculating with Fusarium ear rot -

Silk channel inoculation technique

Kernel puncher inoculation technique
(pin prick method)

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Rating of Germplasm Response against Fusarium Ear Rot (FER) *Fusarium verticillioides* on a 1-9 Scale

1

Clean, No Infection

2

Less infection on-ear with 1% of the kernels on the ear have visible infection

3

Less infection on-ear with 2-5% of the kernels on the ear have visible infection

4

Moderate infection on-ear with 6-10% of the kernels on the ear have visible infection

5

More infection on-ear with 11-20% of the kernels on the ear have visible infection

6

More infection on-ear with 21-40% of the kernels on the ear have visible infection

7

Abundant infection on-ear with 41-60% of the kernels on the ear have visible infection

8

Severe infection on ear with 61-80% of the kernels on the ear have visible infection

9

Severe infection on ear with 81-100% of the kernels on the ear have visible infection

- Evaluations are done at harvesting time. Symptoms include a white streak on the pericarp or the development of purple mycelium on the infected kernels.
- Individual ears are scored on a 1 to 9 rating scale and the total number of ears are used to calculate the % ear loss with the formula given below.
- % Ear loss= ((#ears in score2*0.01)+(=((#ears in score3*0.05)+(=((#ears in score4*0.1)+(#ears in score5*0.2)+(#ears in score6*0.4)+(#ears in score7*0.6)+(#ears in score8*0.8)+(#ears in score9*1))/total # ears*100

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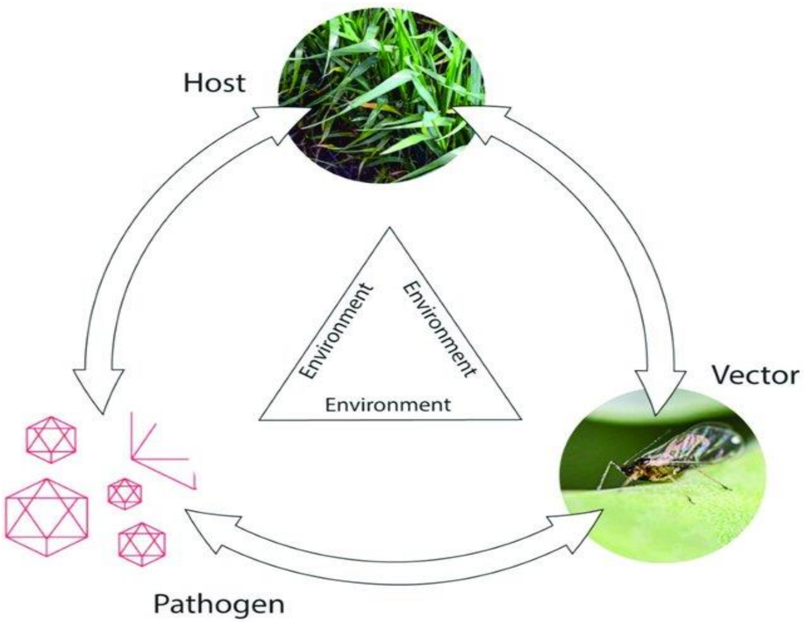
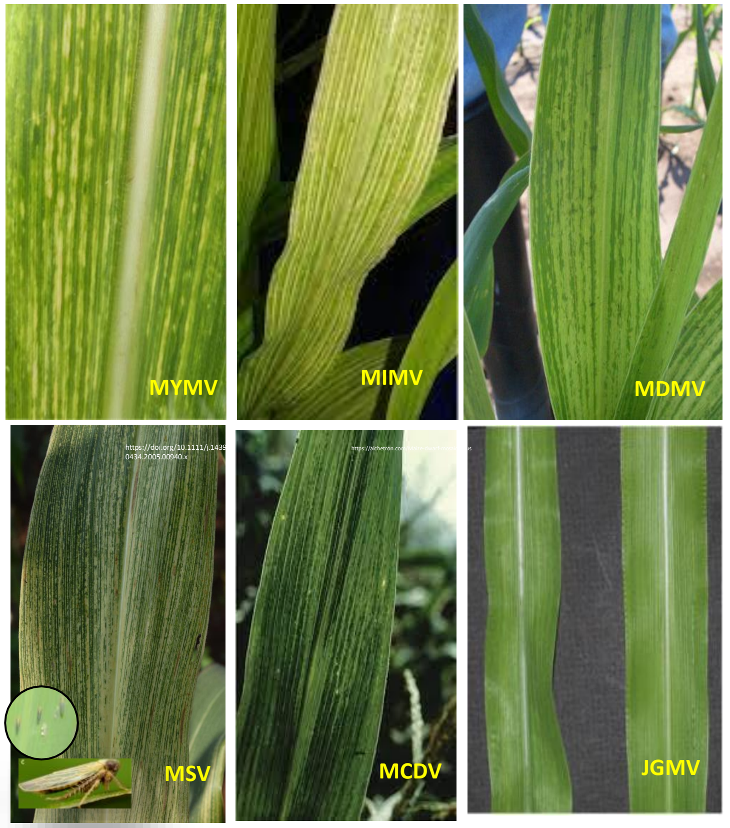
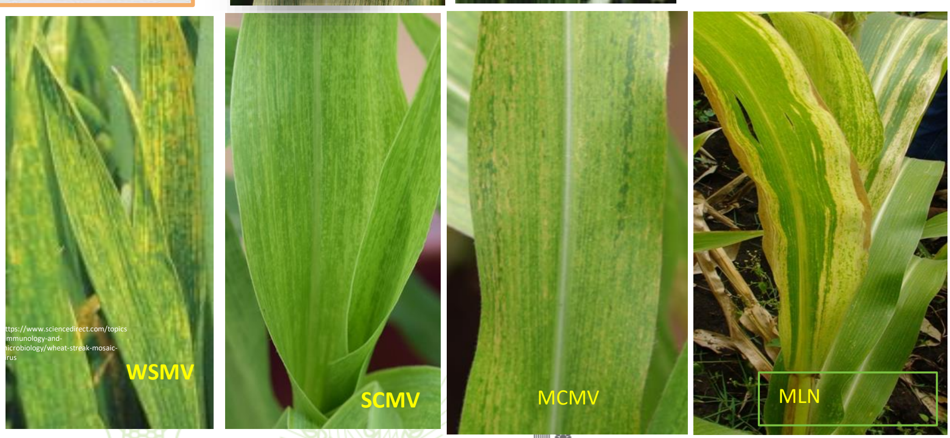
Few viruses affecting maize

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.,





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Maize Lethal Necrosis (MLN)

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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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.

MLN - 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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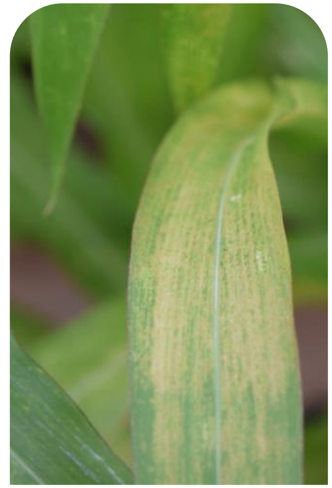
MCMV symptoms

Mottling

Chlorotic stripes

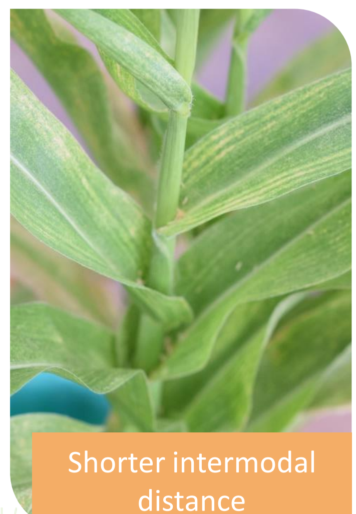


Inter-veinal necrosis / severe chlorosis

Coalition of chlorotic spots forms chlorotic stripes

Mild chlorotic stripes

Mosaic symptoms with chlorosis



Shorter intermodal distance

MLN Symptoms

Mild mosaic and mottling

Mild mosaic and mottling

Chlorosis and Mottling

Diffuse mottling and chlorosis

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MLN Screening Facility at KALRO-Naivasha, Kenya

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

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MLN Phenotyping facility – Center of Excellence

Inoculation Protocol





Facilities




Process



Phytosanitation




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MLN Screening protocol, calendar and Capacity - Naivasha

Cycle	capacity	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
1st	12000	P	P	I	E	E							
2nd	25000				P	P	I	E	E				
3rd	12000							P	P	I	E	E	
4th	25000	E								P	P	I	E
5th	12000	I	E	E								P	P

Planting	**P**
Inoculation	I
Evaluation	E

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Maize Lethal Necrosis (MLN) Disease Rating on a 1-9 Scale

Lines

Rating of Germplasm Responses against MLN

1. Completely clean plants with no visible MLN disease symptoms
2. Fine or no chlorotic specks, but no loss of plant vigor
3. Mild chlorotic streaks on emerging leaves
4. Moderate chlorotic streaks on emerging leaves
5. Chlorotic streaks and mottling throughout the plant
6. Intense chlorotic mottling throughout the plant, with necrosis of leaf margins
7. Severe chlorotic mottling, mosaic, and leaf necrosis all through the plant
8. Severe chlorotic mottling, leaf necrosis, dead heart, and premature death of plants.
9. Complete plant necrosis, and dead plants

- Beginning two weeks after the second inoculation, plants are scored for the MLN severity on a weekly (inbred lines) or bi-weekly (hybrids) basis.
- Disease Incidence: Number of plants out of a total number of plants in each plot displaying MLN symptoms.
- Note: The score is given on a plot basis; however, for some high-precision experiments like fine-mapping or marker validation trials, a similar scale is followed but on an individual plant basis.

Hybrids

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Rating of Germplasm Responses against Maize Chlorotic Mottle Virus (MCMV) on a 1-9 Scale

No visible MCMV symptoms

Fine or no chlorotic specks, but no loss of plant vigor

Mild chlorotic streaks on emerging leaves

Moderate chlorotic streaks on emerging new leaves

Chlorotic streaks and mottling throughout the plant

Intense chlorotic mottling throughout the plant, with necrosis of leaf margins

Severe chlorotic mottling, mosaic, and leaf necrosis all through the plant

Severe chlorotic mottling, leaf necrosis, and sometimes premature death of plants.

Plant death

- Evaluate plants weekly for MCMV symptoms after the second inoculation and repeat this weekly for inbred lines and bi-weekly for hybrids.
- Disease incidence: Number of plants out of a total number of plants in each plot displaying MCMV symptoms.

Note: The score is given on a row basis; however, for specific high precision experiments like fine mapping or marker validation trials, a similar scale is followed but on an individual plant basis.

Maize Steak Virus (MSV)

Severity depends on genotype

R gene identified on Chr 2 – molecular markers available

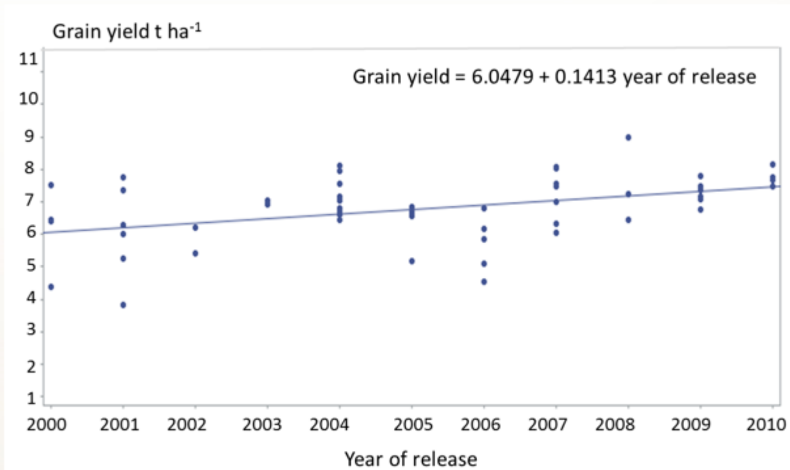
Transmitted by leafhoppers

Epidemics are sporadic but devastating

Symptoms: chlorotic spots that coalesce into stripes occupying the entire leaf

Host resistance is the most suitable means of control

Estimated gains in tolerance to MSV: 141.3 kg ha^{-1} yr^{-1} (2.2% yr^{-1})



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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.

Maize streak virus – Artificial screening

Inoculum

Leaf hopper

Leaf hopper rearing

Leaf hopper rearing

Viruliferous Leaf hopper acquisition

- MSV-A strain – economic importance among 6 MSV strains
- It can infect 80 other species in the family Poaceae
- *Cicadulina mbila Naude* and *C. Storeyi* – Vectors

- Acquisition : 24 hrs.
- 3-4 hoppers plants
- 3-4 weeks for symptom expression
- Disease rating 1-9 scale

Rating of Germplasm Responses against Maize Streak Virus (MSV)

Disease severity scoring: Plot level visual scores recorded on a 1 to 9 scale

No visible symptoms, clean

Few, mild chlorotic streaks on emerging leaves

More chlorotic streaks throughout the leaves in the entire plants

Severe streaks, with more chlorosis mosaic, all through the plant

Severe chlorotic leaf streaks; severe leaf necrosis on the entire plant

- Disease Incidence data: Number of plants out of the total number of plants in each plot displaying MSV symptoms.
- Evaluate plants for MSV symptoms three weeks after inoculation, in weekly intervals, for a total of four MSV scores during crop growth.
- Note: The score should be given on a row basis; however, for specific high precision experiments like fine mapping or marker validation trials, a similar scale should be followed but on an individual plant basis.

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Conclusions

- A large number of inbred lines, open-pollinated varieties, hybrids, and source population with resistance to the major diseases are available for use
  - As sources of alleles to breed maize for resistance to the major diseases
- Other disease for which artificial inoculations are conducted / protocols available
  - Ear rots (Fusarium), Stalk Rots, turcicum blight, southern corn leaf blight, common rust.

Conclusions

- Information on sources of disease resistance on CIMMYT website & available to collaborators

- Establishing diseases phenotyping network comprised of different institutions and seed companies etc.

- Build capacity of collaborators on use of harmonized disease evaluation protocols

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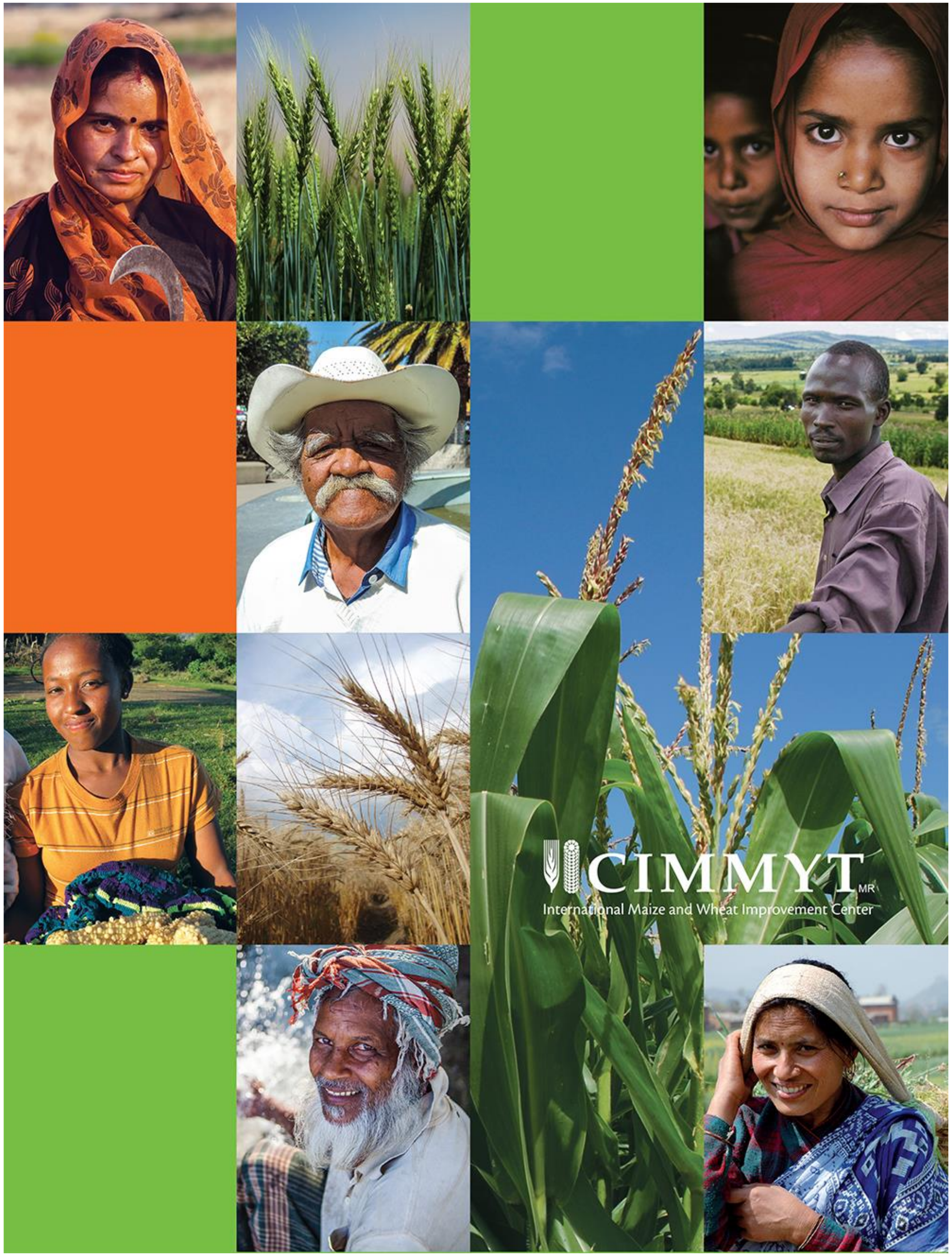

Thank you for your interest!

Continuous Improvement

CIMEAPP1-Early: Mean grain yield, agronomic t

EntNo	Name	Entry description	MLN_AI	GLS_AI	TLB_AI
			al and artificial innoculation		
			1-9 scale	1-9 scale	1-9 scale
21	SAWA(DSLH103)	CC, GLS&TLB R	5.99	4.02	4.15
22	DUMA43	CC, MLN S	8.00	4.19	3.86
23	CKHMLN181140	MLN resistant	4.01	3.93	4.40
24	DK777	Cross cutting check	4.99	4.43	4.66
25	CKHMLN181066	Rolling check, MLN R	4.00	3.34	5.03
	Heritability		0.98	0.86	0.87
	Genotype Variance		1.94	0.73	0.72
	GenxLoc Variance		NA	NA	NA
	Residual Variance		0.11	0.21	0.21
	Grand Mean		4.26	4.09	4.36
	LSD		0.75	1.07	1.03
	CV		7.71	11.07	10.50
	n Replicates		2	2	2
	n Environments		1	1	1
	Genotype significance		0.00	0.00	0.00
	GenxEnv significance		NA	NA	NA

CIMEAPP1-Intermediate: Mean grain yield,

EntNo	Name	Entry description	MLN_AI	GLS_AI	TLB_AI
			al and artificial innoculatio		
			1-9 scale	1-9 scale	1-9 scale
28	WE3106	IGG	7.00	4.22	5.61
29	PHB3253	MLN,GLS,TLB S	6.97	6.31	6.47
30	PHB30G19	CC; GLS & TLB R	6.95	3.90	4.16
31	DK777	Commercial check	5.00	5.01	4.11
32	CKDHH190064	Rolling check	7.00	3.95	3.98
33	WE7118 (MLN); H	MLN R	3.95	4.35	4.05
	Heritability		0.96	0.67	0.62
	Genotype Variance		1.60	0.25	0.33
	GenxLoc Variance		NA	NA	NA
	Residual Variance		0.15771	0.25	0.44
	Grand Mean		5.47	4.36	4.51
	LSD		0.91	1.14	1.49
	CV		7.26	11.56	14.68
	n Replicates		2.00	2	2
	n Environments		1	1	1
	Genotype significance		0.00	0.01	0.02
	GenxEnv significance		NA	NA	NA

CIM22EAPP2-Late: Mean grain yield, a

Ent No	Name	Entry description	MLN_AI	GLS_AI	TLB_AI
			al and artificial innoculati		
			1-9 scale	1-9 scale	1-9 scale
32	UH5354	CC, GLS&TLB R	6.50	3.36	3.20
33	H6213	Commercial and IGG	6.99	4.94	4.89
34	PHB3253	MLN S	7.02	5.01	5.81
35	CKH155902	IGG check	6.52	4.00	4.11
36	DK777	Cross cutting check	5.49	4.15	5.45
37	CKH182952	Rolling check	5.99	3.01	5.40
38	WE7119	GLS & TLB S; MLN R	4.01	5.42	6.80
	Heritability		0.87	0.68	0.84
	Genotype Variance		0.42	0.38	0.51
	GenxLoc Variance		NA	NA	NA
	Residual Variance		0.13178	0.35	0.19
	Grand Mean		6.60	3.66	4.74
	LSD		0.79	1.31	1.03
	CV		5.50	16.14	9.15
	n Replicates		2	2	2
	n Environments		1	1	1
	Genotype significance		0.00	0.00	0.00
	GenxEnv significance		NA	NA	NA

CIMSTG322EAPP2-Late: Mean grain yield,

Ent No	Pedigree	Entry description	MLN_AI	GLS_AI	TLB_AI
			al and artificial innoculatio		
			1-9 scale	1-9 scale	1-9 scale
119	UH5354	CC, GLS&TLB R	5.50	3.24	4.55
120	H6213	CC	6.50	4.00	5.44
121	PHB3253	MLN, GLS, TLB S	6.99	5.65	6.95
122	CKH155902	IGG check	6.00	3.18	3.54
123	DK777	Cross cutting che	5.00	4.95	5.62
124	CKH182952 (CIM19EAPP2	Rolling check	6.00	3.10	5.02
125	WE7119	MLN R	5.00	5.37	4.50
	Heritability		0.84	0.61	0.79
	Genotype Variance		0.39	0.30	0.47
	GenxLoc Variance		NA	NA	NA
	Residual Variance		0.16	0.40	0.26
	Grand Mean		6.00	3.80	4.88
	LSD		0.80	1.38	1.09
	CV		6.62	16.69	10.50
	n Replicates		2	2	2
	n Environments		NA	NA	NA
	Genotype significance		8E-15	2E-04	1E-09
	GenxEnv significance		NA	NA	NA

CIMSTG322EAPP1-Intermediate: Mean grain yield, a

Ent No	Pedigree	Entry description	MLN_AI	GLS_AI	TLB_AI
			al and artificial innoculation		
			1-9 scale	1-9 scale	1-9 scale
112	PHB30G19	CC, TLB R	6.57	4.47	4.40
113	DK777	Commercial checl	4.84	4.91	6.04
114	WE3106	IGG, GLS R	6.95	3.89	4.98
115	PHB3253	GLS & TLB S	6.95	5.45	6.50
116	CKDHH190064	Rolling check	6.00	4.37	5.00
	Heritability		0.95	0.49	0.60
	Genotype Variance		1.58	0.15	0.27
	GenxLoc Variance		NA	NA	NA
	Residual Variance		0.17	0.32	0.34
	Grand Mean		4.46	4.26	5.34
	LSD		0.86	1.19	1.21
	CV		9.26	13.33	10.89
	n Replicates		2	2	2
	n Environments		1	1	1
	Genotype significance		0E+00	0.00	0.00
	GenxEnv significance		NA	NA	NA

CIMSTG322EAPP1-Early: Mean grain yield, ag

Ent No	Pedigree	Entry descriptio	MLN_AI	GLS_AI	TLB_AI
			d artificial innoculation		
			1-9 scale	1-9 scale	1-9 scale
110	SAWA(DSLH103)	CC, GLS R	6.08	3.40	4.87
111	DUMA43	CC, MLN S	8.04	3.94	4.64
112	CKHMLN181140	GG, MLN R	4.58	3.09	4.13
113	DK777	Cross cutting	5.05	3.56	3.81
114	CKHMLN181066	Rolling check	4.55	3.67	4.92
115	Local check	PAN4M19 (MLN)	6.96	4.46	3.44
116	WE6101	TLB R		4.51	3.53
	Heritability		0.93	0.49	0.57
	Genotype Variance		1.29	0.15	0.35
	GenxLoc Variance		NA	NA	NA
	Residual Variance		0.19	0.31	0.55
	Grand Mean		4.60	3.70	4.36
	LSD		0.94	1.21	1.57
	CV		9.45	15.00	16.94
	n Replicates		2	2	2
	n Environments		1	1	1
	Genotype significance		0E+00	2E-03	2E-04
	GenxEnv significance		NA	NA	NA

Identification of TLB and GLS trait donors

- 494 elite lines were evaluated under artificial inoculated conditions in Kakamega, Kenya.
- Best lines for TLB and GLS are identified as donors or resistant checks.

TLB

GLS

Genotype	GLS (1-9scale)	AUDPC
CKDHL142989	2.55	34.85
([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-1-B-B-B-B	2.89	35.46
CML559	3.01	38.38
CKDHL0089	3.14	48.80
DL14501	3.16	45.47
((BRAZIL1546)DH4/CML395)-B-1-2-1-B-B	3.35	49.92
(CML543/LaPostaSeqC7-F71-1-2-1-2-B-B-B-B)-B-168-1-1-B-B	3.50	44.59
CKDHL120423	3.58	43.24
CKLMARS1C3S50196	3.67	49.35
CML540	3.68	49.71
CML536	3.75	59.91
CLRCY039	3.87	45.46
ECAVL30-16-2-2-1-3-2-B-B-B-B	3.88	45.30
CML172	4.01	45.08
((DTPWC9:@.F16.1.1.1.1.@.@.)*2/S3512Z)-B-6-1-1-3-B-B	7.47	99.80
((DTPWC9:@.F115.1.4.1.1.@.@.)*2/S3512Z)-B-88-1-1-1-BBB	7.55	89.36
(CKL05017/[CML442/CML197//[TUXPSEQ]C1F2/P49-SR]F2-45-7-3-2-BBB]-2-1-1-2-2-BBB-B-B)DH11-B-B-B	7.59	93.51
h2	0.51	0.60
Mean	5.67	71.95

Genotype	TLB (1-9 scale)	AUDPC
CLWN211	2.36	32.02
CKL131049	2.73	31.68
ML-1A	2.99	41.32
CLMLND1067	3.06	31.23
(CKL05003/[CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-2-2-BB-B-B)DH148-B-B-B	3.24	35.26
CML536	3.29	38.35
((KU1403x1368)-7-2-1-1-B-B/CML444)-B-3-2-3-2-2-1-3-B-B-B-B	3.31	42.30
CKL131305	3.41	43.17
ML-16	3.41	32.13
CKL13870	3.50	37.57
DL12110-B	3.51	39.94
(CML488*2/S1920Z)-B-11-1-6-1-B-B-B	3.53	43.49
CKL131056	3.54	41.78
CKL131280	3.59	43.42
CKL13731	8.52	104.77
CKL05017	8.57	78.13
CKDHL0364	8.68	99.05
h2	0.75	0.78
Mean	5.54	65.94

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