Progress in Breeding for MLN Tolerance/Resistance

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Presentation to EAC-CIMMYT Regional Stakeholders Forum on Maize Lethal Necrosis Disease 22-24 May, 2018, Nairobi , Kenya

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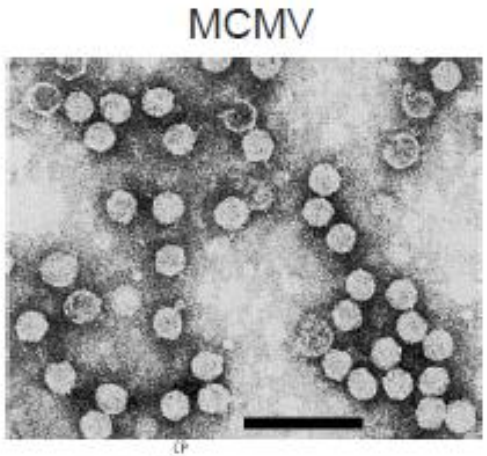
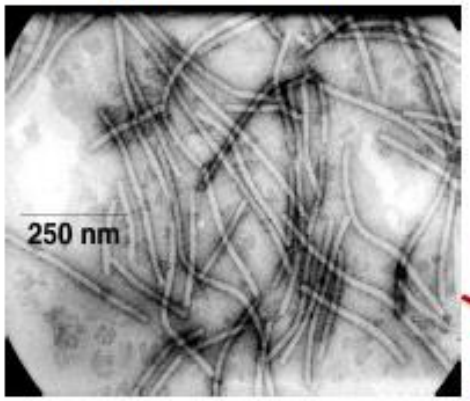
Outline

- Introduction
- Why is MLN devastating in EA?
- What has the CIMMYT-GMP done
- Breeding strategies for MLN tolerance/resistance
- Results of MLN breeding so far:
  - Variety release
  - Genetic study of inheritance
  - Use of DH technology and molecular markers

MLN in eastern Africa is caused by coinfection of maize plants by MCMV and SCMV





CIMMYT-KALRO MLN Screening Facility at Naivasha

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Responses of some commercial maize hybrids against MLN

Why is MLN devastating in EA?

- MCMV is new to the region
- Widespread cultivation of susceptible germplasm that has never been screened for MLN
- Continuous cropping of maize
- Widespread existence of vectors and alternate hosts in EA
- Widespread movement of contaminated seed

What has the CIMMYT-GMP done?

1. Rapid evaluation of existing commercial hybrids and over 98% found to be extremely susceptible
2. Artificial inoculation and screening protocols established in collaboration with Ohio State University
3. MLN screening facility established in Naivasha-Kenya in collaboration with KALRO
4. Nearly 120,000 maize accessions screened for MLN by public and private sector
5. Promising lines hybrids have been identified
6. Some MLN tolerant hybrids released in in Kenya and Uganda

Artificial Inoculation and Scoring System

MCMV and SCMV greenhouse amplification

Blending of viral mixture

Inoculation with motorised knapsack sprayer

Responses of the Inbred Lines: the “Good” and the “Bad”

CML442

CML443

CML448

CML395

CML444

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Results of screening DH lines under AI

Entry	Pedigree			
113	CKDHL120664			
76	CKDHL120312			
181	CKDHL120918			
305	CML312-SR			
30		3.2	4.3	4.7
30		3.7	4.3	4.7
30		3.7	4.3	4.8
31		4.1	5.0	5.1
31		4.5	5.0	4.9
31		3.5	4.8	4.7
31		3.4	3.5	4.1
31		3.4	4.0	4.7
31		4.0	4.5	4.7
316	CML509	3.2	4.0	3.7
	Heritability	0.7	0.1	0.7

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Genetic analysis of tropical maize inbred lines for resistance to MLN

No	Pedigree	GY(t/ha	MLN-early	MLN-late
13	CKDHL120918	1.21***	-0.47**	-0.54**
27	CML550	0.64*	-0.11*	-0.1
12	CKLTI0227	0.61*	-0.13	-0.18*
24	CML494	0.5	-0.15*	-0.22
18	CKLMARSI0022	0.41	-0.03	-0.01
8	CKDHL0500	0.35	-0.14*	-0.21*
15	CKLTI0137	0.29	-0.2*	-0.25*
23	CKLTI0136	0.21	-0.04	-0.07
14	CKLTI0138	0.16	-0.19*	-0.19*

These lines could be distributed to partners for making new breeding starts and hybrids make ups

Beyene et al 2017: Euphytica

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The top 10 new SC hybrids under MLN AI evaluated for 2 yrs at Naivasha

No	Hybrids	GY (t/ha)	% increase over the mean of checks	MLN-early (1-5)	MLN-late (1-5)
1	12 x 27	6.6	1008.3	2.3	2.5
2	13 x 14	6.5	990.4	2.0	2.3
3	13 x 23	6.0	896.9	1.9	1.9
4	12 x 13	5.4	792.4	1.6	1.8
5	12 x 27	5.0	733.9	2.3	2.4
6	18 x 24	4.9	713.2	2.5	2.6
7	4 x 13	4.8	698.5	2.1	2.1
8	11 x 13	4.8	696.2	1.9	2.1
9	13 x 24	4.8	693.7	2.3	2.3
10	6 x 23	4.7	684.4	2.3	2.4
Mean of the top 10 hybrids		5.3	790.8	2.1	2.3
41	WE1101	0.9	54.8	2.7	2.7
42	DK8031	0.8	32.5	3.2	3.7
43	H516	0.5	-22.2	2.9	3.2
44	Duma43	0.2	-72.4	3.4	4.0

- The top 10 hybrids gave 684 to 1008 % more yield that the mean of commercial checks
- These hybrids currently being used as as singe cross females to make three way MLN tolerant hybrids

CKMLN150084
(2nd generation MLN-resistant hybrid)
CKMLN146069
(1st generation MLN-tolerant hybrid)
DK8031
(MLN-susceptible commercial check)

CKMLN150077
Commercial check: DUMA 43

Evaluation of MLN tolerant SC hybrids for yield and adaptation

SC trial evaluation in KYUC under optimal condition in 2015

Naivasha under MLN artificial inoculation in 2014

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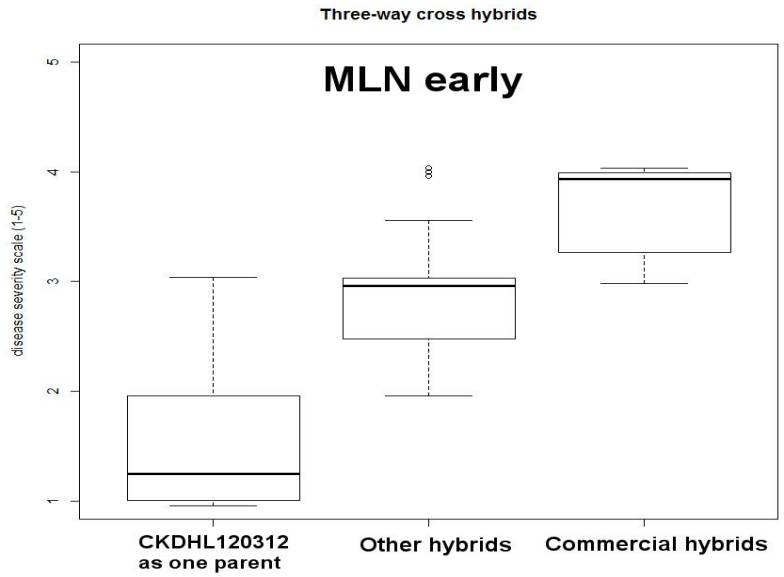
Susceptible commercial checks

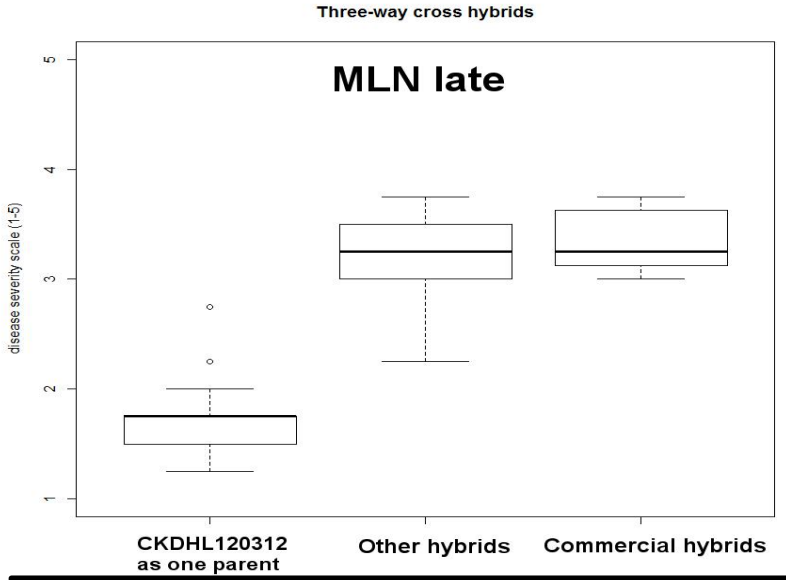
MLN tolerant experimental hybrid

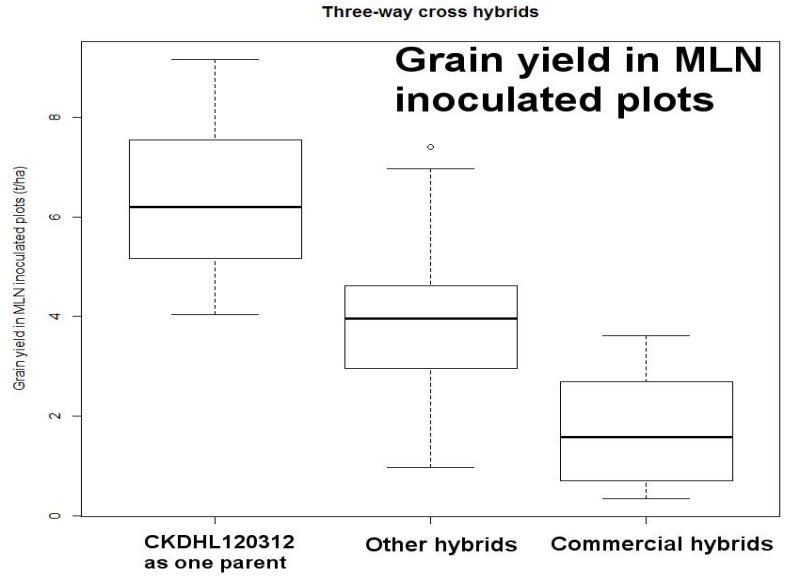
Targeted breeding for MLN tolerance: Development of three way hybrids

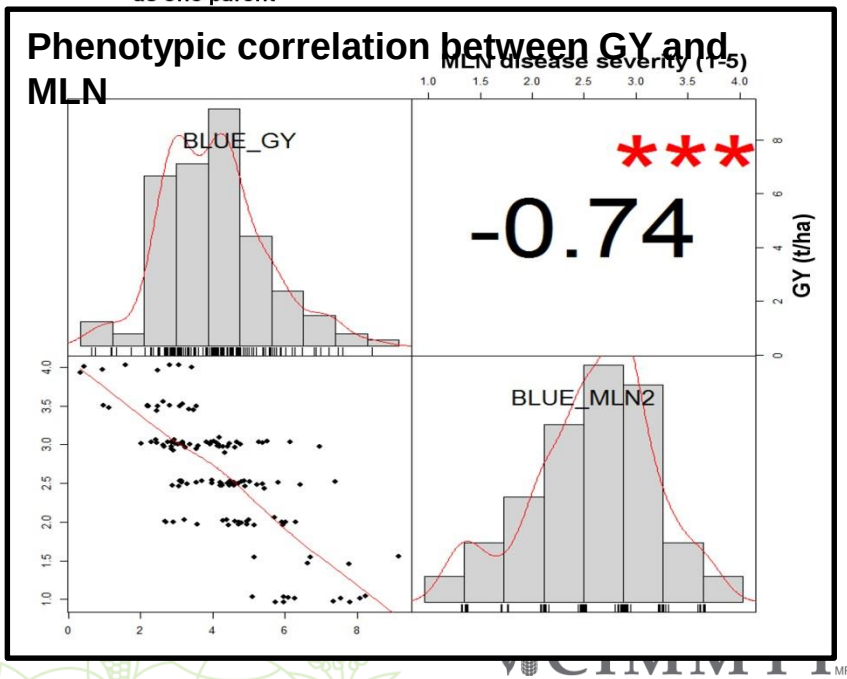
Ears of a MLN tolerant experimental hybrid at maturity

Forward breeding for MLN tolerance









Performance of new MLN tolerant hybrids under different management conditions

Entry	Name	GY t/ha-MLN	% increase	GY t/ha_ OP	MLN_4
16	CKHMLN170060	7.2	380	7.4	3
56	CKHMLN170241	6.7	347	7.5	4
59	CKHMLN170223	6.7	347	6.9	3.5
55	CKHMLN170195	6.6	340	6.2	3
65	CKHMLN170209	6.1	307	6.9	3
10	CKHMLN170034	6.1	307	7.0	3.5
51	CKHMLN170191	6.0	300	5.4	2.5
6	CKHMLN170030	5.9	293	6.6	3.5
2	CKHMLN170016	5.8	287	6.7	4.5
11	CKHMLN170045	5.8	287	7.5	2.5
60	CKHMLN170243	5.6	273	7.8	4
1	CKHMLN170015	5.5	267	7.9	2.5
21	CKHMLN170075	5.2	247	7.0	3.5
9	CKHMLN170033	5.1	240	6.5	4
64	Duma 43	0.3		5.3	8
29	Duma 43	0.3		5.5	8
12	Duma 43	0.2		5.5	8
67	WE5135	5.2		7.5	3.5
68	H517	NA		3.8	8
69	Pioneer 3253	1.5		6.7	4.5
70	WH505	1.5		6.7	4.5
	Heritability	0.7		0.5	0.9
	Grand Mean	3.5		6.4	4.5
	LSD	2.5		1.4	1.2
	CV	0.7		0.5	0.9
	N Locations	1		3	1

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MLN tolerant Hybrid1

Commercial check1

MLN tolerant Hybrid 2

Commercial check1

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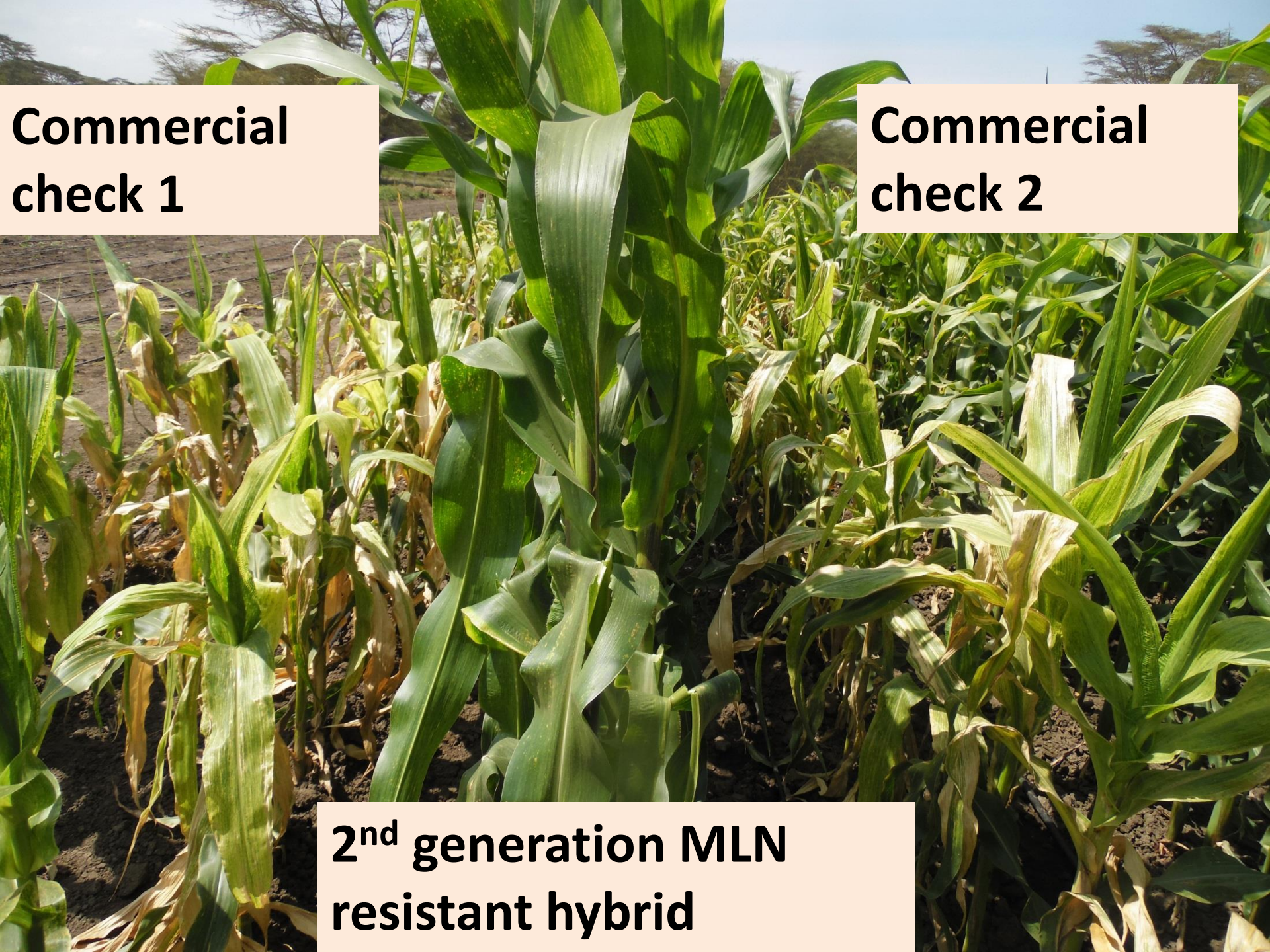
Performance of commercial hybrids under MLN AI at Naivasha

CZH0616

Pioneer 30G19

H517


Commercial check 1
Commercial check 2
2nd generation MLN resistant hybrid

Performance of MLN resistant hybrids under MLN AI at Naivasha

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Performance of selected 2nd generation MLN tolerant hybrids under MLN AI at Naivasha 2016

Entry	Pedigree	MLN _Artificial inoculation Trial 1 Naivasha		MLN _Artificial inoculation Trial 2 Naivasha		MLN _Artificial inoculation Trial 3 Naivasha		Mean Yield across 3 trials	Mean MLN3 across 3 trials	% increase over mean of checks
		Yield t/ha	MLN_3	Yield t/ha	MLN_3	Yield t/ha	MLN_3			
1	CKMLN150073	7.81	1.8	6.30	2.0	7.82	2.3	7.3	2.1	943.8
2	CKMLN150074	7.62	1.5	5.92	2.7	4.63	2.3	6.1	2.5	764.9
3	CKMLN150075	7.15	1.5	5.18	2.8	5.41	2.3	5.9	1.8	744.6
4	CKMLN150076	6.65	1.5	5.34	2.5	4.30	2.5	5.4	2.0	675.8
5	CKMLN150082	5.90	1.8	3.32	2.7	.	.	4.6	2.3	558.4
6	CKMLN150083	7.62	1.5	6.18	2.8	5.34	2.4	6.4	2.0	810.9
7	CKMLN150084	5.02	1.8	4.02	2.3	4.87	2.5	4.6	2.3	562.2
8	WE1101	1.45	3.8	1.75	4.0	0.15	3.9	1.1	3.7	
9	H517	.	.	0.62	3.8	0.57	3.8	0.6	4.0	
10	WH505	1.18	3.8	1.39	4.0	0.39	3.9	1.0	3.9	
11	Duma 43	.	.	0.06	3.8	0.09	4.0	0.1	4.0	
12	DK8031	.	.	0.71	4.2	.	4.0	0.7	4.1	
	Mean	1.31	3.75	0.91	3.96	0.30	3.93	0.70	3.94	
Heritability		**0.80**	**0.89**	**0.78**	**0.77**	**0.93**	**0.95**			
Grand Mean		**4.16**	**3.0**	**4.11**	**2.9**	**3.32**	**3.2**			
LSD		**1.97**	**0.58**	**2.51**	**0.72**	**1.45**	**0.37**			
CV		**24**	**10**	**30**	**12**	**22**	**6**			
n Replicates		2	2	2	2	3	3			

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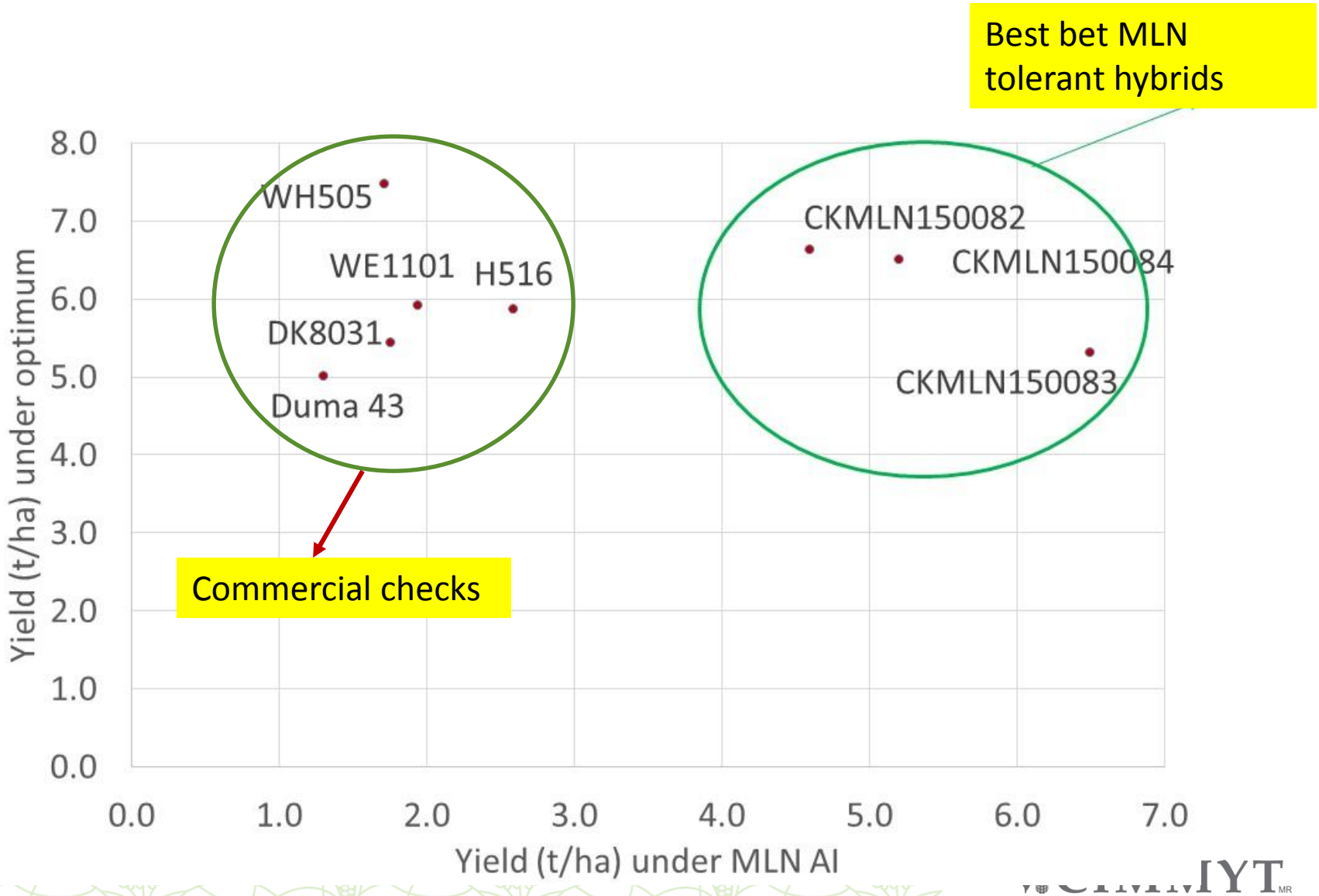
Yield comparison under optimum across locations and artificial MLN inoculation in Naivasha between MLN tolerant hybrids and commercial checks



Performance of MLN-tolerant & Comm. Check Under AI MLN; Without MLN; & Optimum Condition

CKHMLN1500084

Naivasha under AI MLN

Naivasha without MLN

Kiboko-Optimum

DK8031

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Results of NPT testing in Kenya in 2017

				VARIETY STATUS		YIELDt/ha				DISEASES				LODGING %		MATURITY DAYS	
NPT YRS	EXP_NAME	TYPE	SOURCE	TEST STATUS	DUS YRS	MEAN	% ABOVE BEST CHECK	% ABOVE MEAN OF CHECKS	CON-TRAST	BLT	GLS	RST	MNLD 1-5	BS	RL	DPS	SLK
2	WE6109	HYBRID	KALRO KATUMANI	CANDIDATE	1	6.78	35.62	43.16	0	1.9	2.33	1.67	2.67	2.31	1.75	70.05	72.47
2	WE6108	HYBRID	KALRO KATUMANI	CANDIDATE	1	6.06	21.21	27.95	0	1.83	2.67	2	3.17	0.59	0	73.76	75.95
2	WE6110	HYBRID	KALRO KATUMANI	CANDIDATE	1	5.33	6.6	12.54	0.05	2	2.33	1.33	2.83	0.14	0	76.43	79.29
2	WE6111	HYBRID	KALRO KATUMANI	CANDIDATE	0	4.83	-3.55	1.82	0.77	2.1	2	1.67	2.67	0.44	0.3	73.67	75.71
2	MU14-509	HYBRID	KALRO MUGUGA	CANDIDATE	1												
2	EASH1220	HYBRID	EAST AFRICA SEED CO. LTD	CANDIDATE	0												
2	SC DUMA 43	HYBRID	AGRISEED Co. LTD	CHECK	0	5				1.98	2.33	2.33		0.91	0.31	69.86	72.5
2	DH04	HYBRID	KENYA SEED Co. LTD	CHECK	2	4.87				2	1.33	1.33		3.09	0.29	72.81	75.19
2	DK8031	HYBRID	MONSANTO KENYA LTD	CHECK	2	4.35				1.93	2.67	1.33	3.5	0.3	0	72.14	74.67
2	ZZ1MEAN	MEAN				5.29				1.94	2.26	1.67	2.97	2.38	0.54	73.53	74.56
2	ZZ2PV	P-VALUE				0				0.36				0.35	0.41	0	0.67
2	ZZ3CV	CV%				12.76				9.65				293.7	375.9	3.56	13.43
2	ZZ4R2	R^2				0.92				0.83	1	1	1	0.41	0.33	0.92	0.49
2	ZZ5LSD	LSD5%				0.68				0.24				7.03	2.03	2.63	10.07

- 35.62 % higher grain yield than the best commercial check DUMA 43 and 43.16 % higher than the mean of the checks after two year of testing across locations
- It has similar maturity date with DUMA 43
- MLN score of 2.67 with 1-5 scale

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MLN tolerant hybrid at Naivasha Demo plots in 2017

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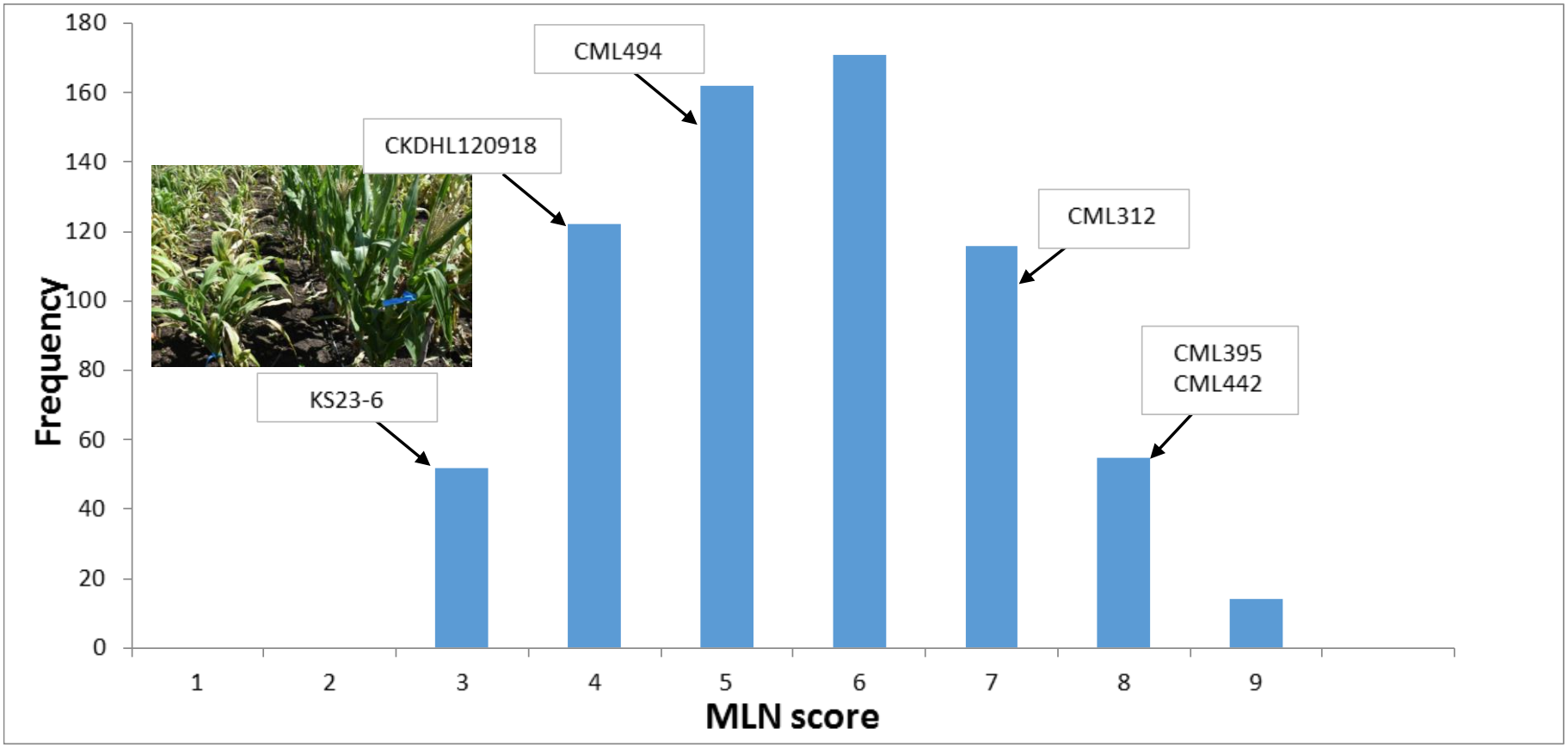
MLN tolerant hybrids at Babati under natural MLN infestation in 2017

- MLN tolerant experiment hybrids at Babati under MLN natural pressure
- WE7117, WE7118, WE7119, WE5135, WE5141, WE7133

Distribution of 704 lines for MLN tolerance



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New MLN tolerant DH lines , 2017

CKDHL164090

CKDHL163627

KS23-6

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New MLN tolerant hybrids: Naivasha in 2018

Performance of MLN tolerant hybrids under natural MLN

Bapati (Tanzanian)

Wondogent (Ethiopia)

Yield at Bapati (Tanzanian)

Kiboko (Kenya)-Optimum

Development MLN resistant / tolerant hybrids

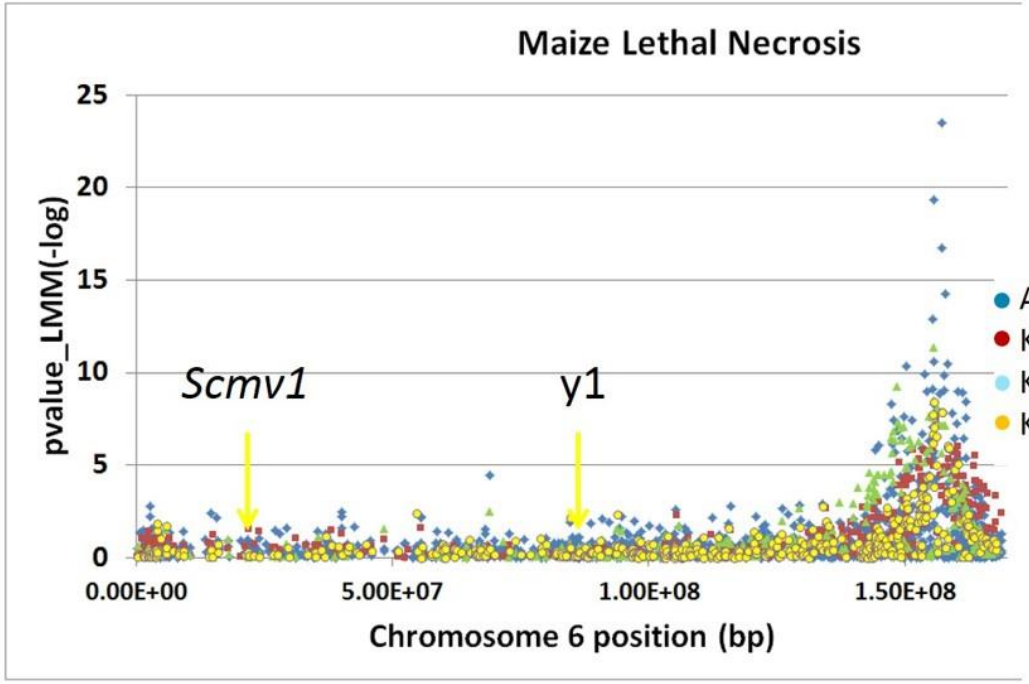
MLN resistant hybrid

MLN tolerant hybrids are also good for yield under optimum condition and early maturing

MLN tolerant hybrid at Kiboko Demo, Sep 2017

Commercial checks, susceptible to MLN

Fine-mapping of a major QTL for MLN resistance



- The major QTL on Chr. 6 is being fine-mapped by DuPont-Pioneer team, in collaboration with CIMMYT
- Target for **gene editing** for MLN resistance in collaboration with DuPont-Pioneer

KS23-6: MLN resistant; MSV-susceptible; Yellow kernels

CML395: MLN-susceptible; Drought-tolerant; MSV-resistant; White kernels

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List of F2 populations developed for introgression MLN resistance genes from KS23

Entry	Pedigree	% White Kernels	# DH lines returned
1	CML539/KS23-6	32	38
2	CKDHL0186/KS23-6	25	123
3	CKDHL0221/KS23-6	31	63
4	CML442/KS23-6	32	136
5	CML537/KS523-5	28	132
6	CML312/KS23-6	30	201

Susceptible

Resistant- DH lines - Tropical x KS23-6

CML312/KS23-6-B-DH4

CML312S23-6-B-DH4

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List of F2 populations developed for introgression MLN resistance genes from KS23

CKDHL0221

CKDHL0221/KS23

CML312/KS23

CML312

CML442

CML539

CKDHL0186

Results of NPT testing in Kenya in 2018

			YIELD(t/ha)						
		VARIETY STATUS				DISEASES			MATURITY (DAYS)
NPT YRS	EXP_NAME	TEST STATUS	MEAN	% ABOVE BEST CHECK	% ABOVE MEAN OF CHECKS	BLT	GLS	DPS	
2	WE7118	CANDIDATE	4.82	14.13	23.94	1.48	1.02	61.43	
2	WE7117	CANDIDATE	4.56	8.03	17.32	1.28	1.02	67.37	
2	WE7119	CANDIDATE	4.53	7.26	16.48	1.35	1.02	63.60	
2	DK8031	CHECK	4.22			1.38	1.07	65.87	
2	SC DUMA 43	CHECK	3.97			1.42	1.08	64.63	
2	DKC80-33	CHECK	3.74			1.44	1.00	66.02	
2	DH04	CHECK	3.63			1.48	1.08	64.83	
2	MEAN		4.12			1.40	1.06	65.47	
2	P-VALUE		0.00			0.07	0.04	0.00	
2	CV(%)		18.03			12.01	9.76	8.58	
2	R^2		0.90			0.58	0.45	0.64	
2	LSD(5%)		0.63			0.14	0.09	4.76	

- 23.942 % higher grain yield than the mean of best commercial checks after two year of testing across locations
- It has similar maturity date with DUMA 43
- MLN resistance

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MLN tolerant hybrid at Kiboko Demo, Sep 2017

MLN tolerant hybrid at Kiboko Demo, Sep 2017

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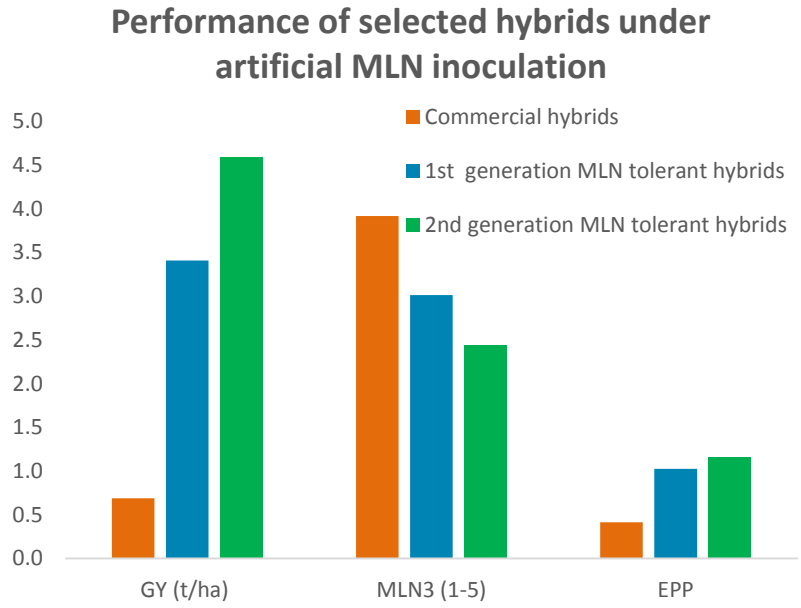
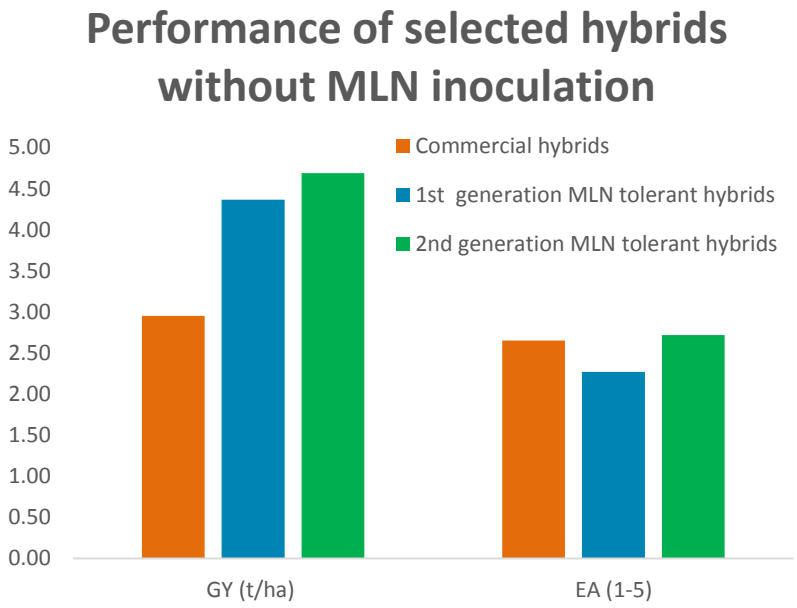
Results of Yield loss study due to MLN





	GY t/ha	EPP	MLN31-5
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/hawithout 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

Evaluation of lines and hybrids at Naivasha under Artificial MLN inoculation in 2013 vs 2017

2013; Naivasha

2017; Naivasha

2015

Babati under natural MLN infestation

2017

Best MLN lines nominated for CML release

No	Code	HG	Comments
1	CKLMARSI0022	B	MLN tolerant
2	CKDHL120312	A	MLN tolerant
3	CKDHL120918	B	MLN tolerant

CKLMARSI0022 –MLN tolerant

CKDHL120918–MLN tolerant

MLN tolerant hybrids released in EA

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

Potential Impacts of MLN tolerant Hybrids

Potential multiplier (economy wide) effects of having MLN-tolerant hybrids

US$245–756 million
in Ethiopia

US$195–678 million
in Kenya

Combined economic benefits

$440 million-1.4 billion
depending on severity of the disease

The area covered by MLN hybrids estimated

25-75%

of total maize area

Social impacts

No of people potentially protected from falling below the poverty line

2.1 million
in Ethiopia

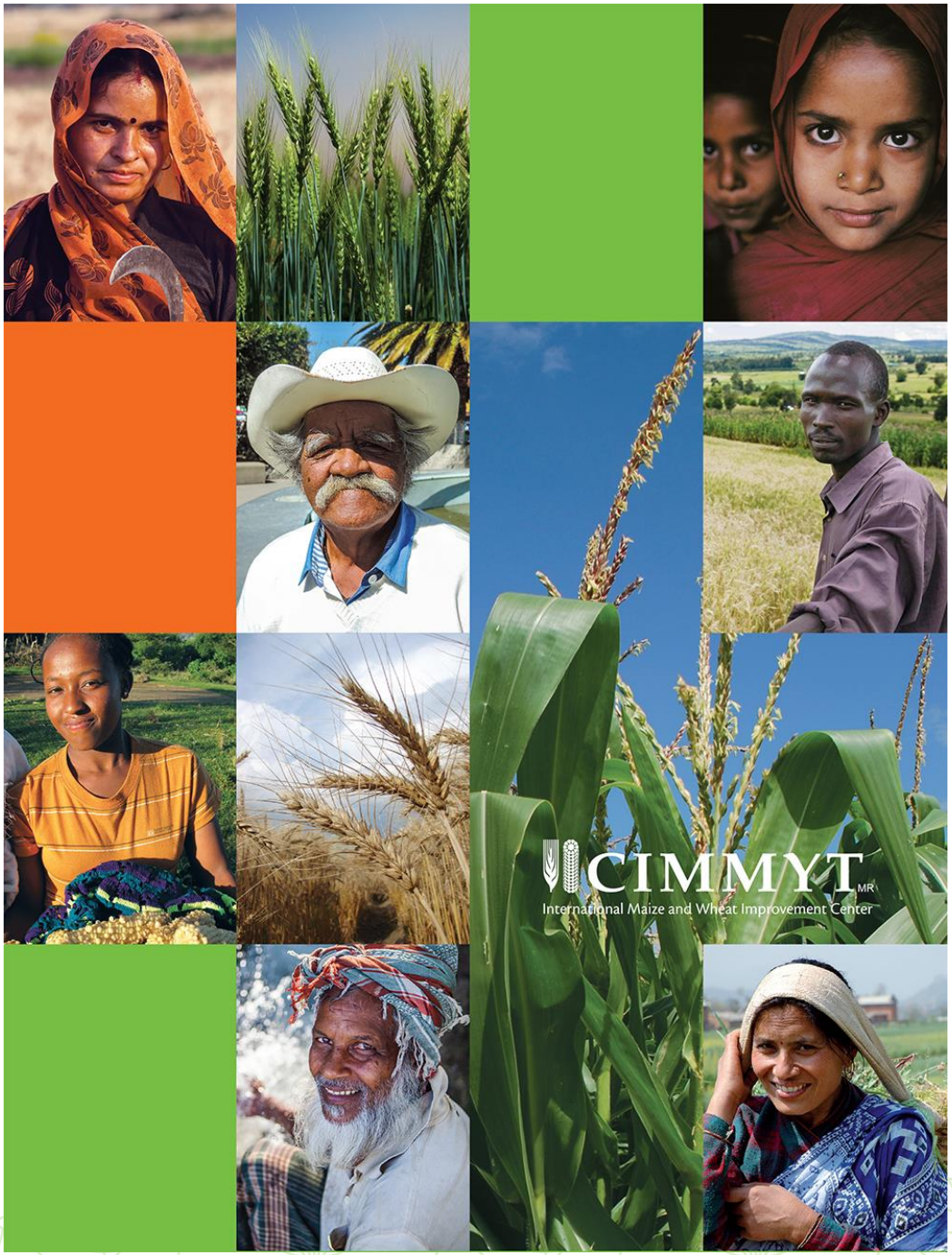
1.2 million
in Kenya

Ex ante: estimated benefits that would be forgone *without* MLN-tolerant hybrids: case for action!

Marenya et al. (2018) Ag Systems 162, 220-228

Thanks to…

- Bill & Melinda Gates Foundation, USAID, SFSA and CRP MAIZE, for supporting the work on MLN
- KALRO, NARES and Private sector partners
- USDA/Ohio State University, Univ. of Minnesota & ICIPE
- CIMMYT colleagues, for their dedicated work



Thank you for your interest!

CIMMYT MR