Part I. Case examples showing contribution of genome editing

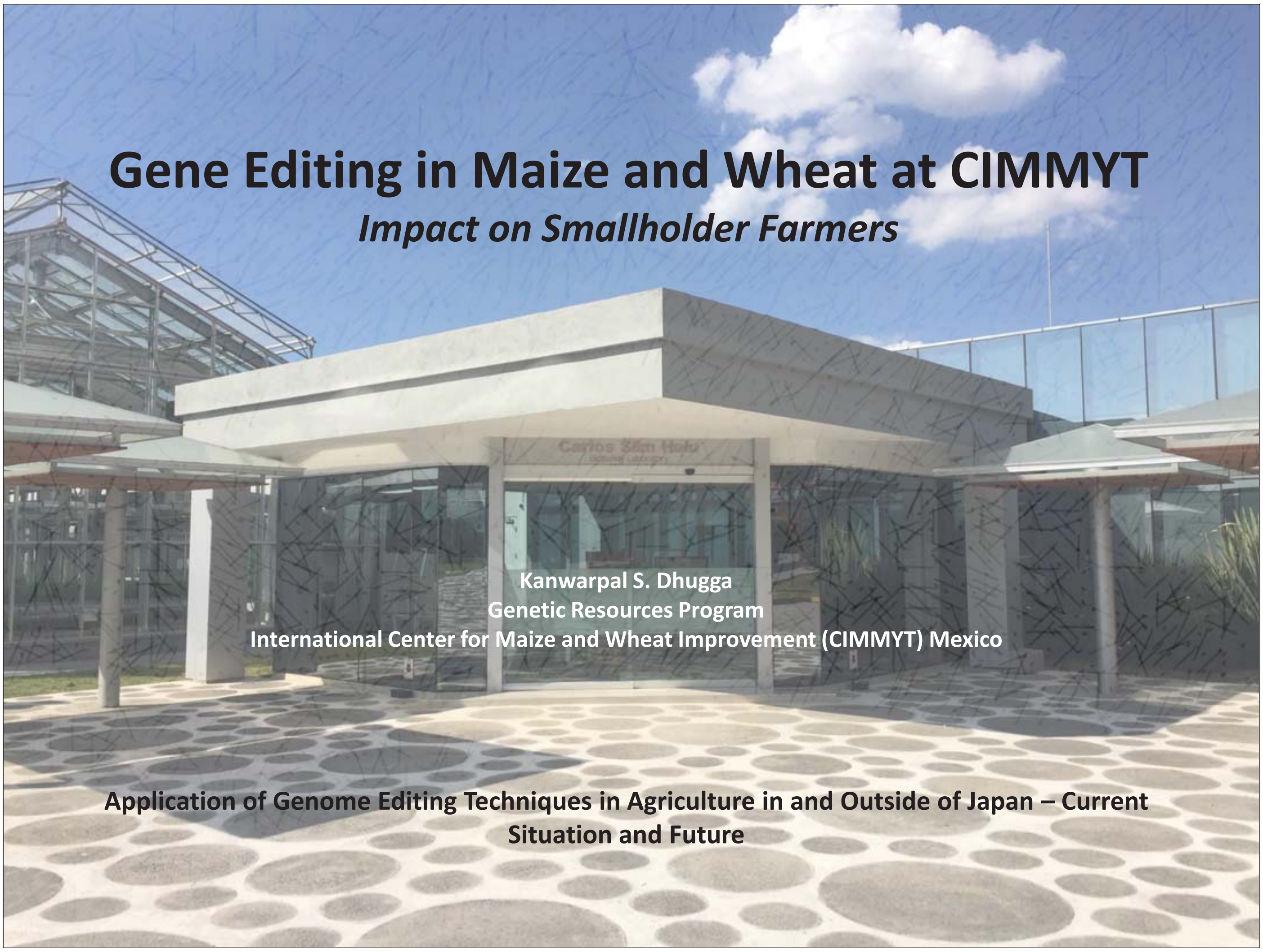
10:20-10:50 Gene Editing in Maize and Wheat at CIMMYT: *Impact on Smallholder Farmers*

Dr. Kanwarpal Dhugga, Principal Scientist, Head, Biotechnology for Agricultural Development, International Maize and Wheat Improvement Center (CIMMYT), Mexico

Gene Editing in Maize and Wheat at CIMMYT: *Impact on Smallholder Farmers*

Kanwarpal S. Dhugga, Principal Scientist and Head, Biotechnology for Agricultural development, CIMMYT

Abstract:
International Maize and Wheat Improvement Center (CIMMYT) is best known for introducing semi-dwarf wheat lines half a century ago, which led the way to Green Revolution. The focus of research continues to be to provide improved germplasm for maize and wheat with the goal of sustainably stabilizing crop production at small, marginal farms. CIMMYT also strives to extend the benefits of modern technology to smallholder farmers to help further alleviate poverty. CIMMYT and DuPont Pioneer have joined hands to exploit the gene editing (CRISPR-Cas) technology to improve maize and wheat germplasm. A specific example where this technology will be employed in the short-term is maize lethal necrosis (MLN), a devastating viral disease that has spread in many countries of East Africa in a short span of five years since it was first detected in Kenya. We identified a strong source of resistance against MLN, have fine-mapped it to a 1 MB region of chromosome 6, and expect to isolate the gene that confers resistance in the next 4-6 months. Our first targets will be the parents of long-standing commercial hybrids in East Africa that were developed before the appearance of MLN and have since become susceptible to this disease. DuPont Pioneer has developed a technology whereby any maize line can be transformed independent of its genetic background. As compared to conventional backcrossing to introgress a resistant locus from an exotic source into an elite genetic background, gene editing offers considerable benefit of accelerated breeding, which expedites product development while at the same time minimizes yield drag caused by the undesirable donor alleles. The susceptible form of the gene against MLN will be edited to its resistant version directly in the parents of two widely grown maize hybrids in East Africa. This will help deploy the resistant forms of popular hybrids in a much shorter period than conventional breeding. Several additional examples of future gene editing targets in wheat and maize will be discussed.


Gene Editing in Maize and Wheat at CIMMYT
Impact on Smallholder Farmers
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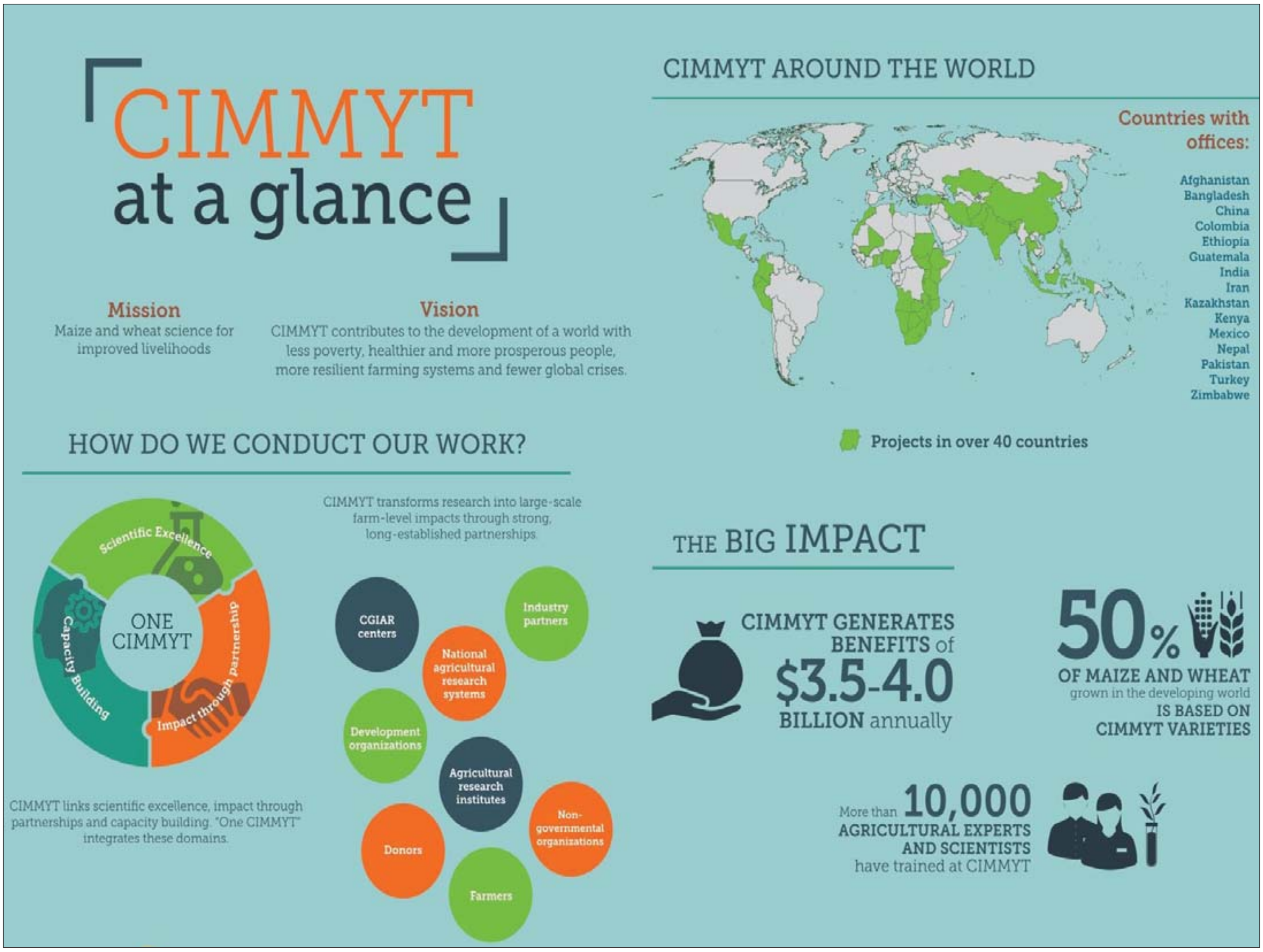
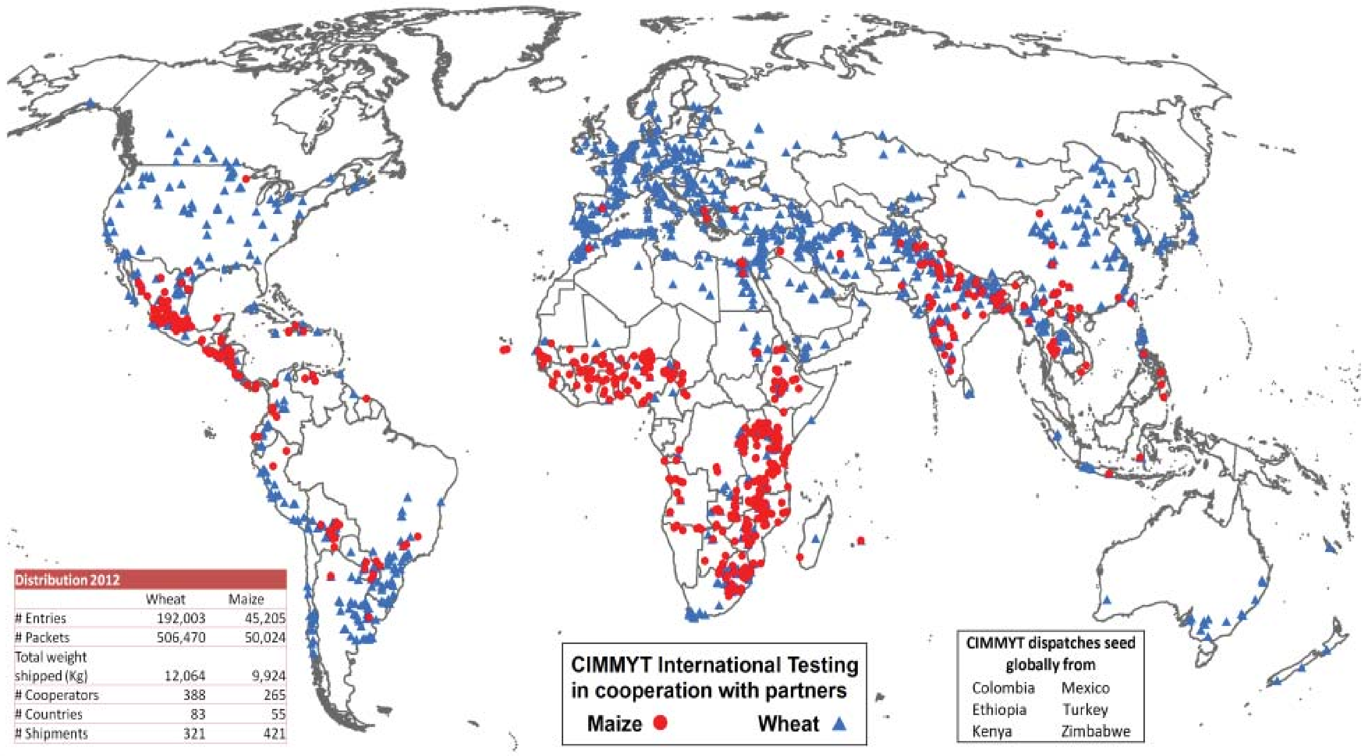
Application of Genome Editing Techniques in Agriculture in and Outside of Japan – Current Situation and Future


CIMMYT
at a glance
Mission
Maize and wheat science for improved livelihoods
Vision
CIMMYT contributes to the development of a world with less poverty, healthier and more prosperous people, more resilient farming systems and fewer global crises.
CIMMYT AROUND THE WORLD
Countries with offices:
Afghanistan
Bangladesh
China
Colombia
Ethiopia
Guatemala
India
Iran
Kazakhstan
Kenya
Mexico
Nepal
Pakistan
Turkey
Zimbabwe
Projects in over 40 countries
HOW DO WE CONDUCT OUR WORK?
Scientific Excellence
Impact through Partnership
Capacity Building
ONE CIMMYT
CIMMYT links scientific excellence, impact through partnerships and capacity building. "One CIMMYT" integrates these domains.
CIMMYT transforms research into large-scale farm-level impacts through strong, long-established partnerships.
CGIAR centers
Industry partners
National agricultural research systems
Development organizations
Agricultural research institutes
Non-governmental organizations
Donors
Farmers
THE BIG IMPACT
CIMMYT GENERATES BENEFITS of
$3.5-4.0
BILLION annually
50%
OF MAIZE AND WHEAT
grown in the developing world
IS BASED ON CIMMYT VARIETIES
More than 10,000
AGRICULTURAL EXPERTS AND SCIENTISTS
have trained at CIMMYT

CIMMYT Varieties Have Global Presence



Distribution 2012

	Wheat	Maize
# Entries	192,003	45,205
# Packets	506,470	50,024
Total weight shipped (Kg)	12,064	9,924
# Cooperators	388	265
# Countries	83	55
# Shipments	321	421

Reasons for CIMMYT to Develop In-house Gene Editing Capabilities

- To extend the benefits of modern technologies to small-holder farmers.
- A recent technological breakthrough has made it possible to precisely alter gene function in a native genetic background.
- CIMMYT possesses state-of-the-art laboratories and expertise to carry out gene editing with the goal to produce novel products that complement conventional breeding.
- DuPont Pioneer has pioneered and streamlined the gene editing system in plants.
- CIMMYT and DuPont Pioneer have signed an agreement to join hands in utilizing gene editing to improve maize and wheat, particularly for small-holder farmers of developing countries.

Principles Guiding Genetic Engineering at CIMMYT

- Recognize and respect the sovereignty of each and every country if and when considering the use of crops produced by alternative means (to plant breeding) in their territories.
- Provide technical support, as requested and required in this process.
- CIMMYT does not support the work of institutions that do not comply with state biosafety laws and procedures.
- CIMMYT is a member of Excellence Through Stewardship.

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Traits for Gene Alteration - Examples

- **Maize**
  - Resistance to maize lethal necrosis (MLN)
  - Biofortification
    - Increase provitamin A by down-regulating CCD genes
    - Fe and Zn availability via phytate downregulation
- **Wheat**
  - Disease resistance
    - Rust (Lr34 and Lr67)
    - Powdery mildew
  - Plant height reduction by alternative mechanisms from Rht genes
  - Biofortification
    - Phytate downregulation for increased Fe and Zn availability

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Review
Trends in Biotechnology June 2013, Vol. 31, No. 6

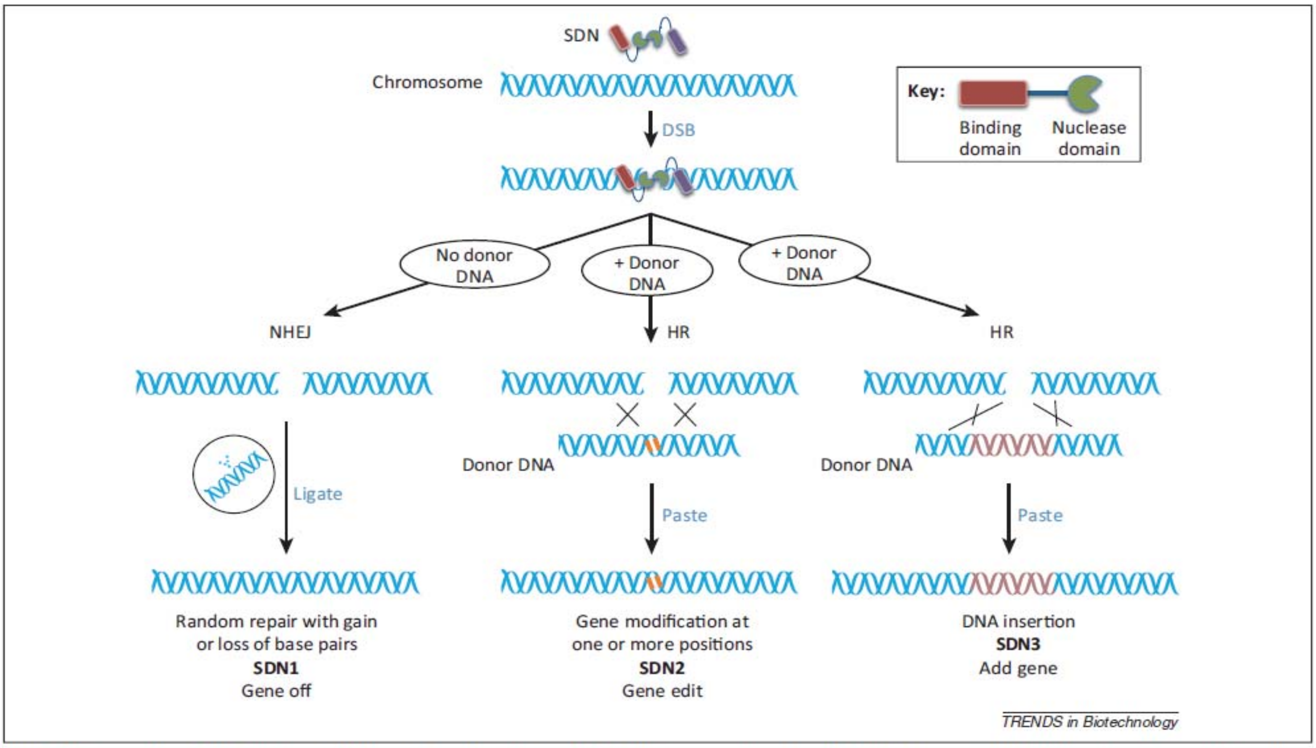

Figure 1. Different site-directed nuclease (SDN) techniques (SDN-1, 2, and 3). An SDN complex is shown at the top in association with the target sequence. The repair can take place via nonhomologous end-joining (NHEJ) or homologous recombination (HR) using the donor DNA. SDN-1 can result in site-specific random mutations by NHEJ. In SDN-2, a homologous donor DNA is used to induce specific nucleotide sequence changes by HR. In SDN-3 DNA is integrated in the plant genome via HR.

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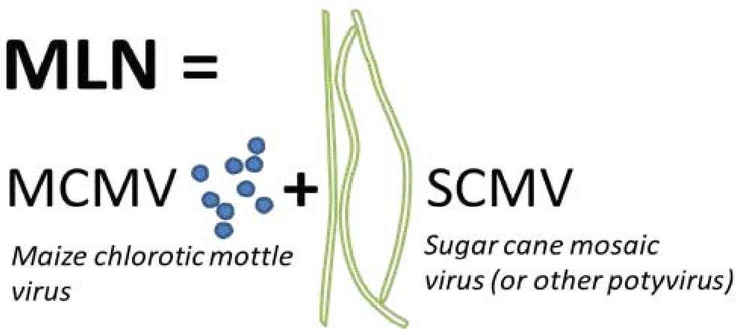
Maize Lethal Necrosis

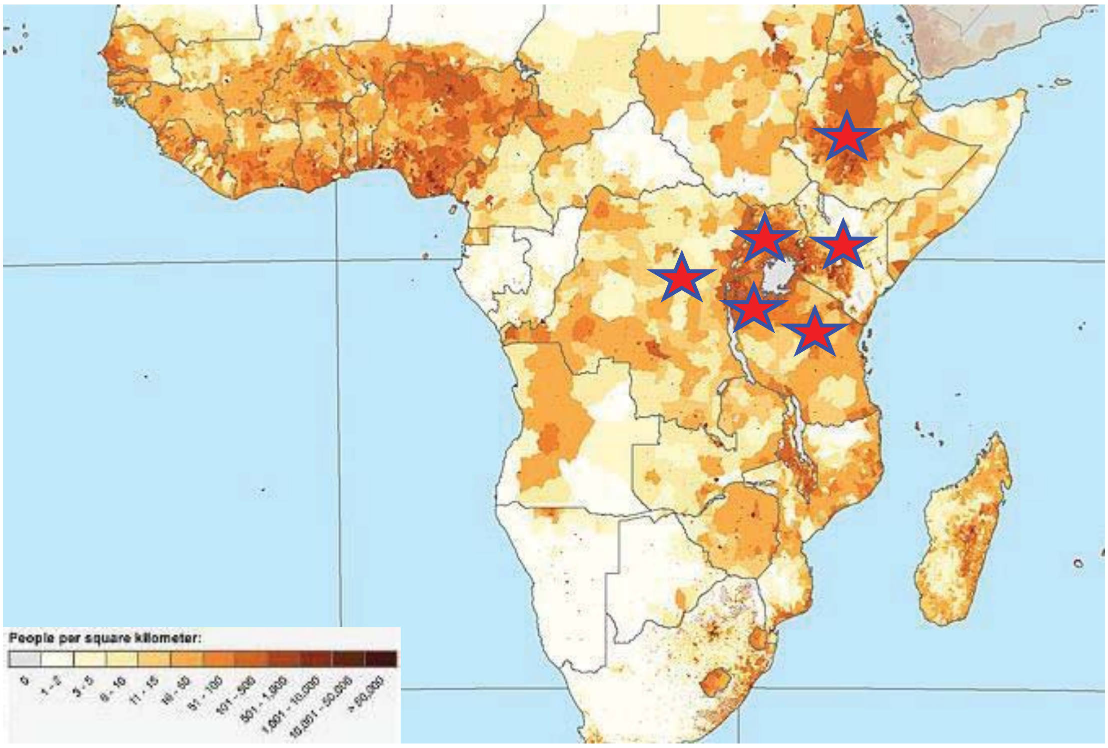
MLN is caused by a combination of Maize Chlorotic Mottle Virus (MCMV) and any of the Potyviruses that infect cereals, especially Sugarcane Mosaic Virus (SCMV)



The disease was first reported in Bomet county of Kenya in Sept 2011, and since then has spread to several countries in eastern Africa.

Prasanna 8 CIMMYT

Countries Affected by MLN



Kenya, Tanzania, Uganda, Rwanda, Ethiopia, D.R. Congo
Possibly South Sudan and Burundi

Prasanna

Maize Production Losses in Kenya to MLN

Agro-ecological zone	Maize production (tons)	Estimated loss (tons)	Average loss (%)
Moist mid-altitude	304,994	96,707	32
Moist transitional West	1,040,794	298,277	29
Highland tropical	583,681	87,750	15
Moist transitional East	49,003	2,649	5
Dry mid-altitude	157,159	5,021	3
Dry transitional	27,409	762	3
Lowland tropical	8,228	1,227	15
< 5% maize	141,579	21,634	15
Total	2,171,268	492,393	23

Hugo de Groote *et al.*

Genotypes Resistant (L) or Susceptible (R) to MLN

Naivasha, Kenya, Screenhouse 2 February 2017

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Resistance Against MLN From an Exotic Genetic Resource

CML395 KS-23-6 CML444

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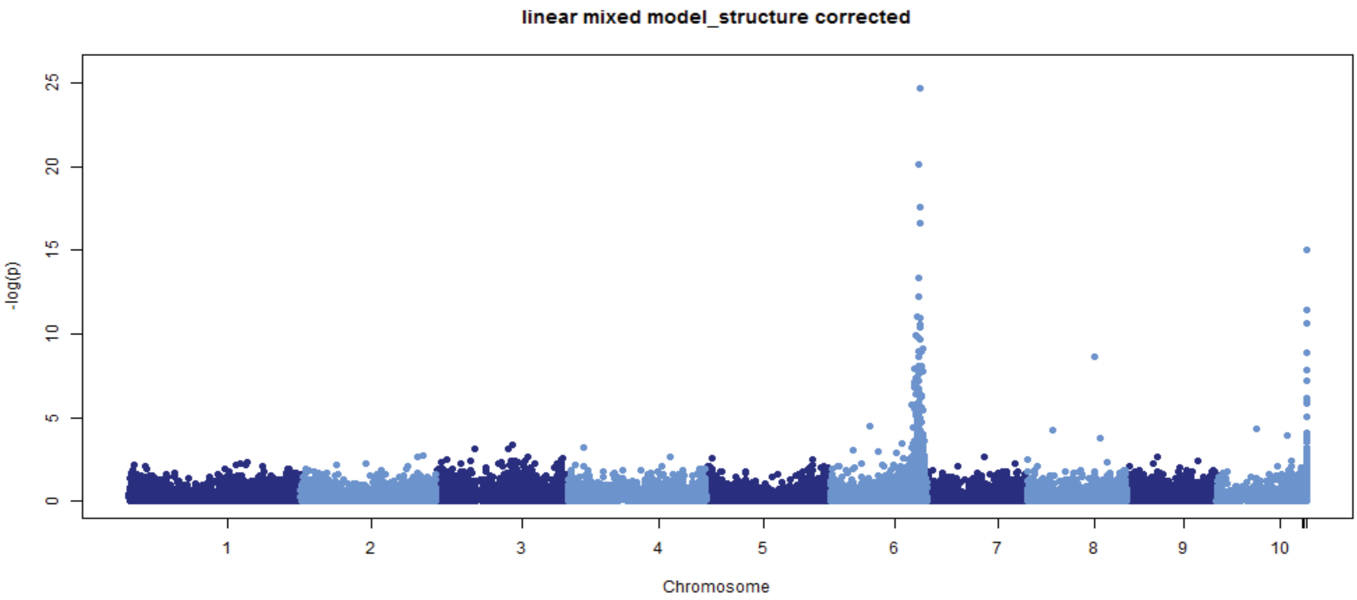
MLN Resistance in Maize

Kenya



Association mapping of MLN in three populations under field conditions.

Olsen

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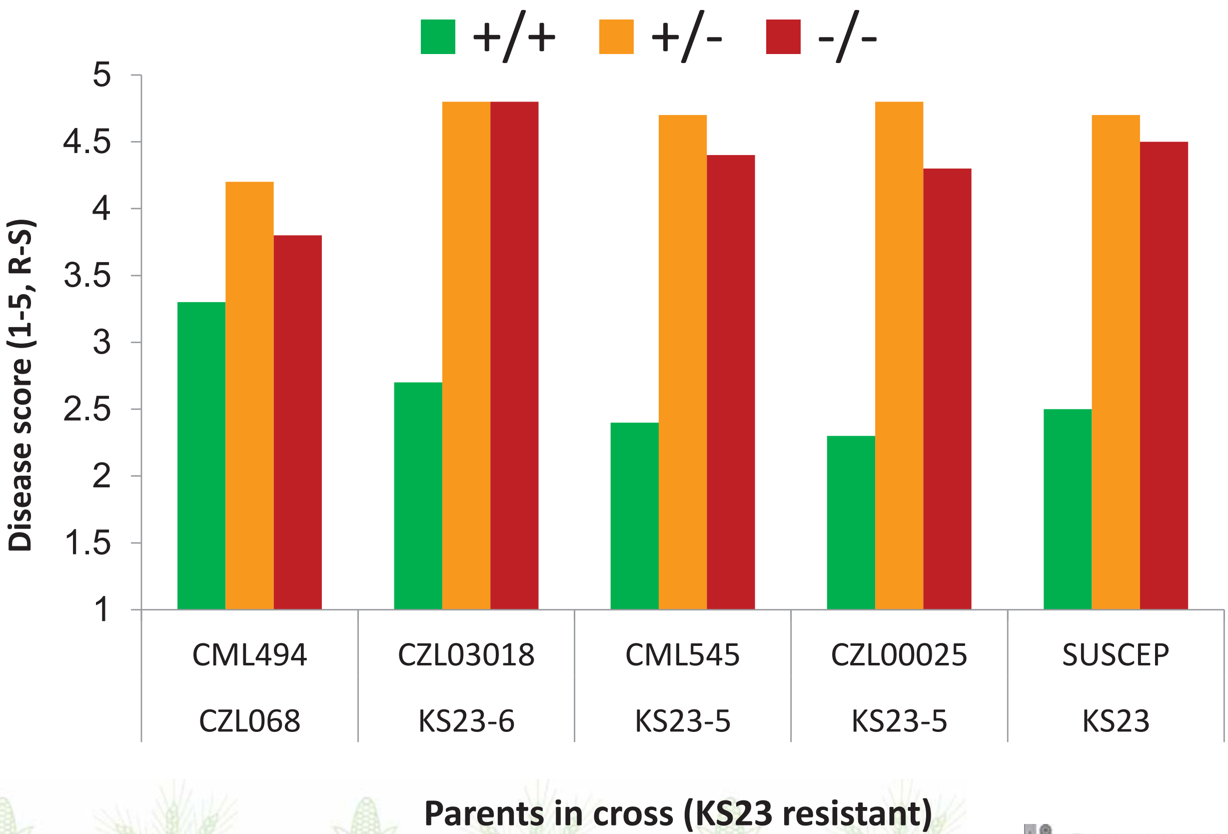
Contribution of MLN_R Locus to Resistance Against MLN



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CML539 X KS23-6: Entries 52 and 53

CML442 X KS23-6: Entries 297 and 298

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MLN$_R$ Locus Fine-mapped by Pioneer Using 20K Markers

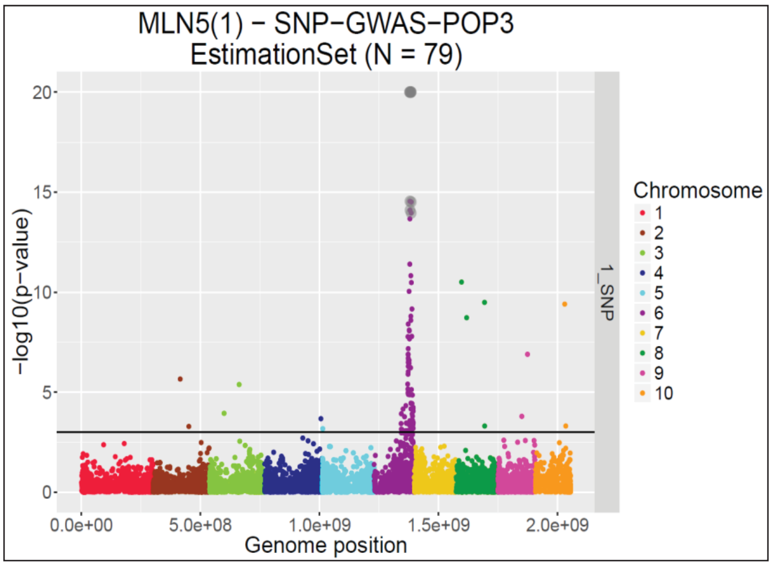
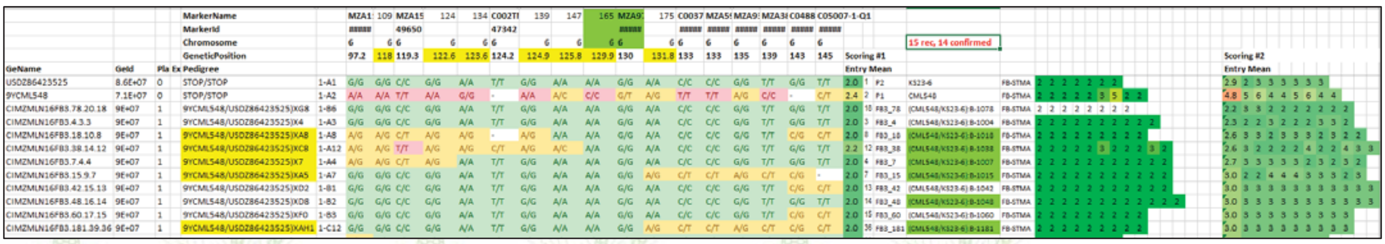


Table 8.2: Statistics of detected signal; -log10(p), effect, and proportion of phenotypic variance explained with the forward-regression model containing only detected signals, minor allele frequency (MAF), number of observations with known marker score (Ne), and smallest possible map interval based on informative flanking markers. Markers with zero effect were not fitted in the final model.

Term	Marker	Chr.	Pos.	-log10(p)	MAF	N_e	Effect	r^2	Interval
1_SNP	4773269-28	1	44.58	0.2	0.39	437	0.03	0.00	44.4-44.6
1_SNP	4582795-46	1	179.86	0.7	0.29	437	0.10	0.00	179.8-179.9
1_SNP	2429956-61	2	38.19	0.0	0.32	437	0.00	0.00	38.1-38.3
1_SNP	2467290-40	3	94.52	0.5	0.25	437	-0.11	0.00	94.0-94.5
1_SNP	4774094-35	4	88.84	0.4	0.35	437	-0.10	0.00	88.3-88.8
1_SNP	4774869-20	4	104.64	0.5	0.40	437	0.12	0.01	104.6-104.7
1_SNP	2382756-40	4	122.78	3.5	0.30	437	0.43	0.03	122.4-123.0
1_SNP	9719493-31	4	138.13	0.7	0.41	437	0.14	0.00	138.1-138.1
1_SNP	4585413-20	6	41.39	0.3	0.19	437	-0.11	0.00	41.2-41.4
1_SNP	100047706-66	6	71.18	0.0	0.18	437	0.00	0.00	71.1-71.2
1_SNP	2607337-59	6	81.63	0.0	0.21	437	0.00	0.00	81.5-81.6
1_SNP	9698459-43	6	93.97	0.8	0.48	437	-0.09	0.00	93.9-94.0
1_SNP	2539050-44	6	106.15	5.7	0.26	437	-0.44	0.08	106.1-106.2
1_SNP	9712316-5	6	117.18	6.2	0.33	437	-0.40	0.05	117.2-117.2
1_SNP	4578964-57	6	128.39	1.4	0.42	437	-0.14	0.01	128.0-128.4
1_SNP	4768653-23	6	140.22	0.4	0.17	437	-0.14	0.00	140.2-140.2
1_SNP	4582450-11	8	74.16	0.7	0.23	437	-0.18	0.01	74.0-74.2
1_SNP	5585631-8	8	86.95	0.8	0.13	437	-0.18	0.00	86.9-87.0
1_SNP	100066975-11	9	79.83	3.1	0.26	437	0.38	0.03	79.8-79.8
1_SNP	100067859-8	10	62.76	0.3	0.22	437	0.08	0.00	62.7-62.8

By Screening F3 Progenies, Interval Narrowed to ~ 1 MB (Chr. 6,



Jung, Olsen

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When a Drought-tolerant Commercial Hybrid Becomes Susceptible to MLN

- Commercialized in Uganda and Kenya
- High yielding under optimal conditions and drought
- Susceptible to MLN

Kiboko: No MLN Pressure

Naivasha: Artificial MLN inoculation

CML312/CML395//CML566

Beyene, Olsen

CIMMYT

Candidate Hybrids for Gene Editing to Confer MLN Tolerance

Kiboko: No MLN Pressure

Naivasha: Artificial MLN inoculation

CML395/CML444//CML539

Beyene, Olsen

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Challenge in Reconstituting Elite Genetic Background Via Conventional Breeding

It is not only the time

Backcross Generation	Recurrent Parent Genome	Donor Parent Genome	Approximate Donor Genes
BC1	75.0	25.0	12500
BC2	87.5	12.5	6250
BC3	93.8	6.2	3125
BC4	96.9	3.1	1563
BC5	98.4	1.6	781
BC6	99.2	0.8	391

Proportion of recurrent genome = $(2^{n+1}-1)/2^{n+1}$

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Accelerated Breeding

- Edit MLN_S gene to its MLN_R form directly in lines that are parents to commercial hybrids in Africa.
- Most hybrids are three-way crosses.
- Reconstitution of original genetic background after backcrossing can be challenging.
- Hybrids have a long lifetime in Africa, sometimes lasting decades.
- DuPont Pioneer has streamlined genetic transformation so the tropical maize lines from Africa can be directly edited.
- Future sources of resistance could be stacked onto the previous one.
- These steps will save years worth of time.
- Significantly contribute toward alleviating poverty and hunger.

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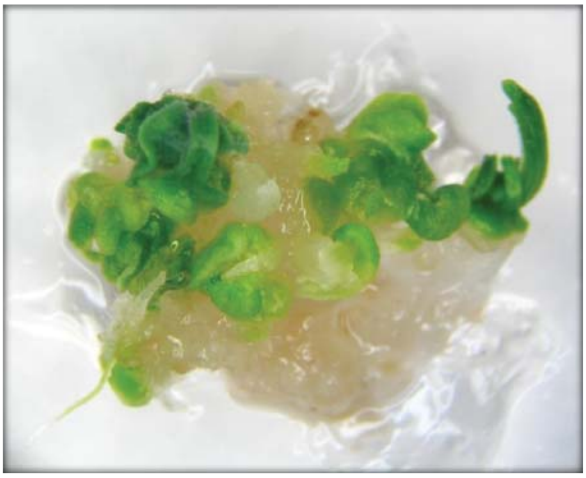
Fielder

CIMMYT

Borlaug 100



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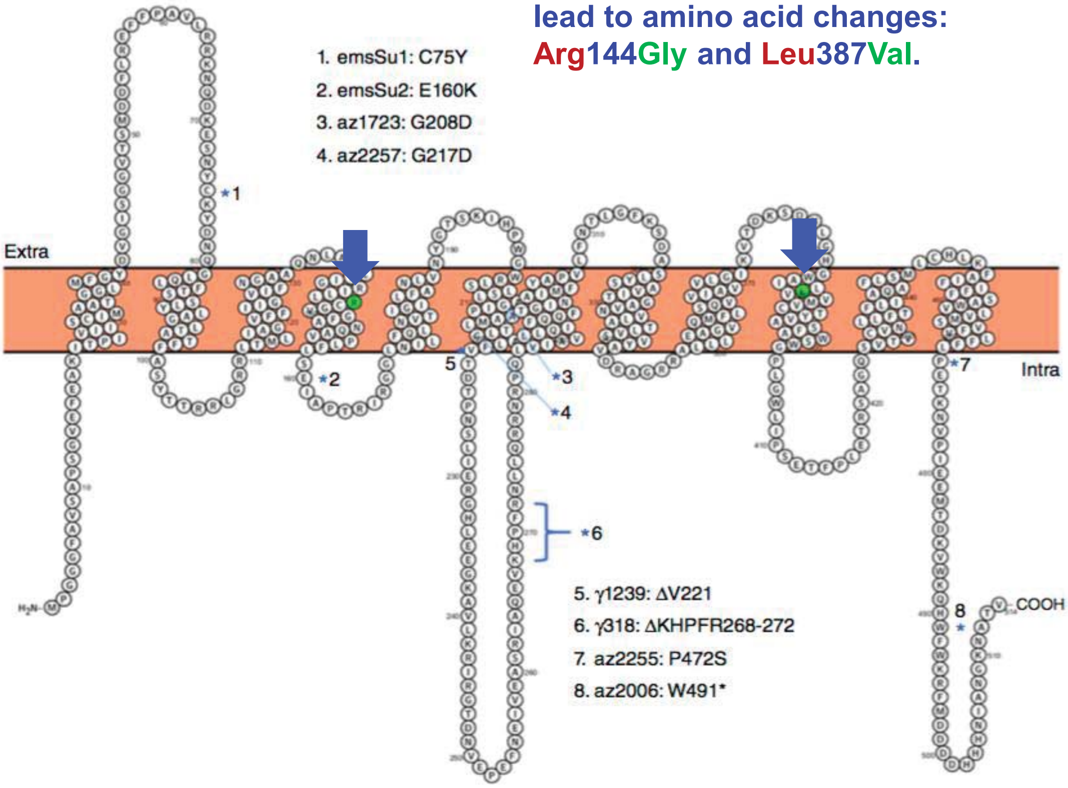
Resistant allele of Lr67 differs from the susceptible by only two nucleotides that lead to amino acid changes: Arg144Gly and Leu387Val.



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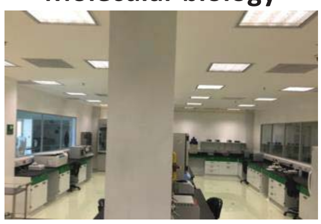
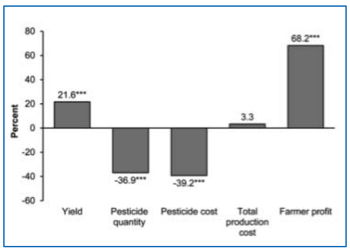
The Gene Editing Platform at CIMMYT

Testing for altered function

Molecular biology





Screening for alterations

Transformation and regeneration

We have world class facilities; we can and must use them fully to benefit our customers

CIMMYT

COI Disclosure Information

Kanwarpal S. Dhugga

I have no financial relationships to disclose.

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