Gene editing for accelerated breeding in cereals

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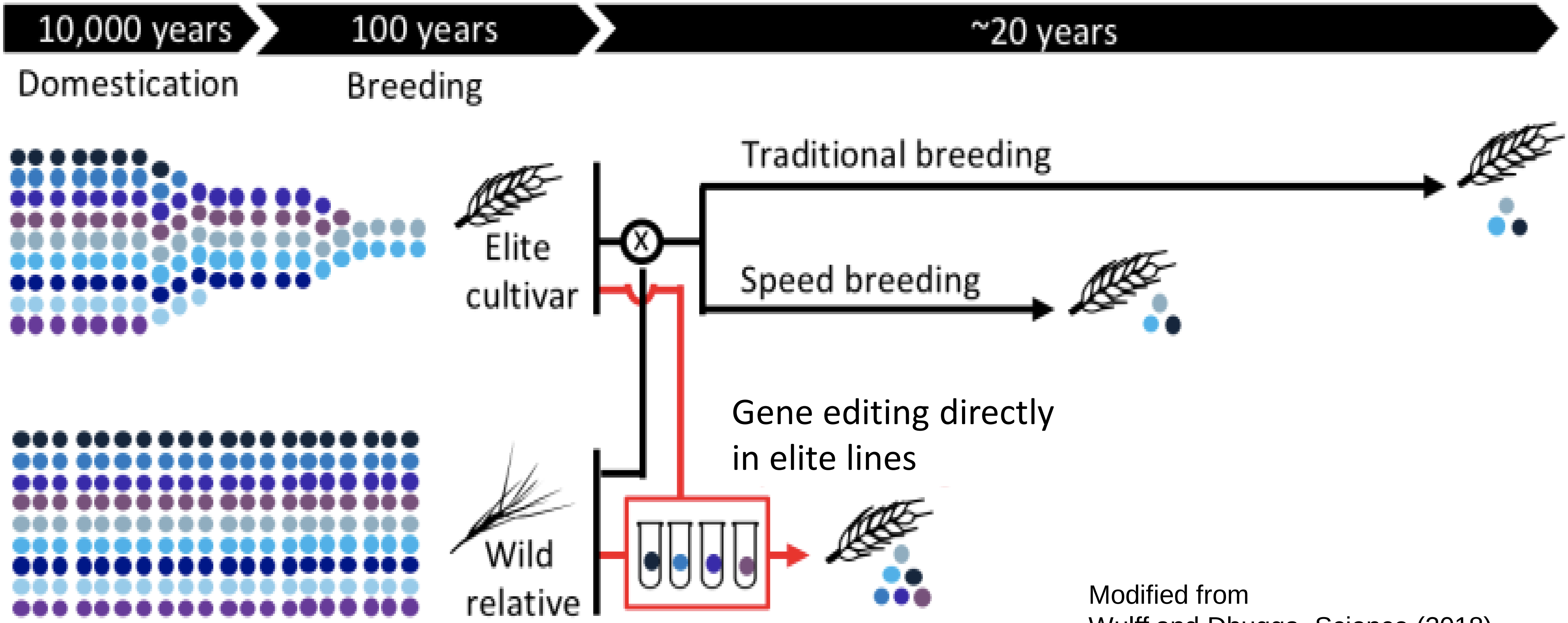
Gene editing for accelerated breeding



Modified from
Wulff and Dhugga, Science (2018)

Challenge in recovering elite genetic background via backcrossing

it is not only the time

Generation	Genome (%) recurrent parent	Genome (%) donor parent	Donor genes (maize)	Donor genes (wheat)
F1	50.0	50.0	20,000	50,000
BC1	75.0	25.0	10,000	25,000
BC2	87.5	12.5	5,000	12,500
BC3	93.8	6.2	2,500	6,250
BC4	96.9	3.1	1,250	3,125

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

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Three scenarios for gene editing: SDN1, SDN2, and SDN3

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

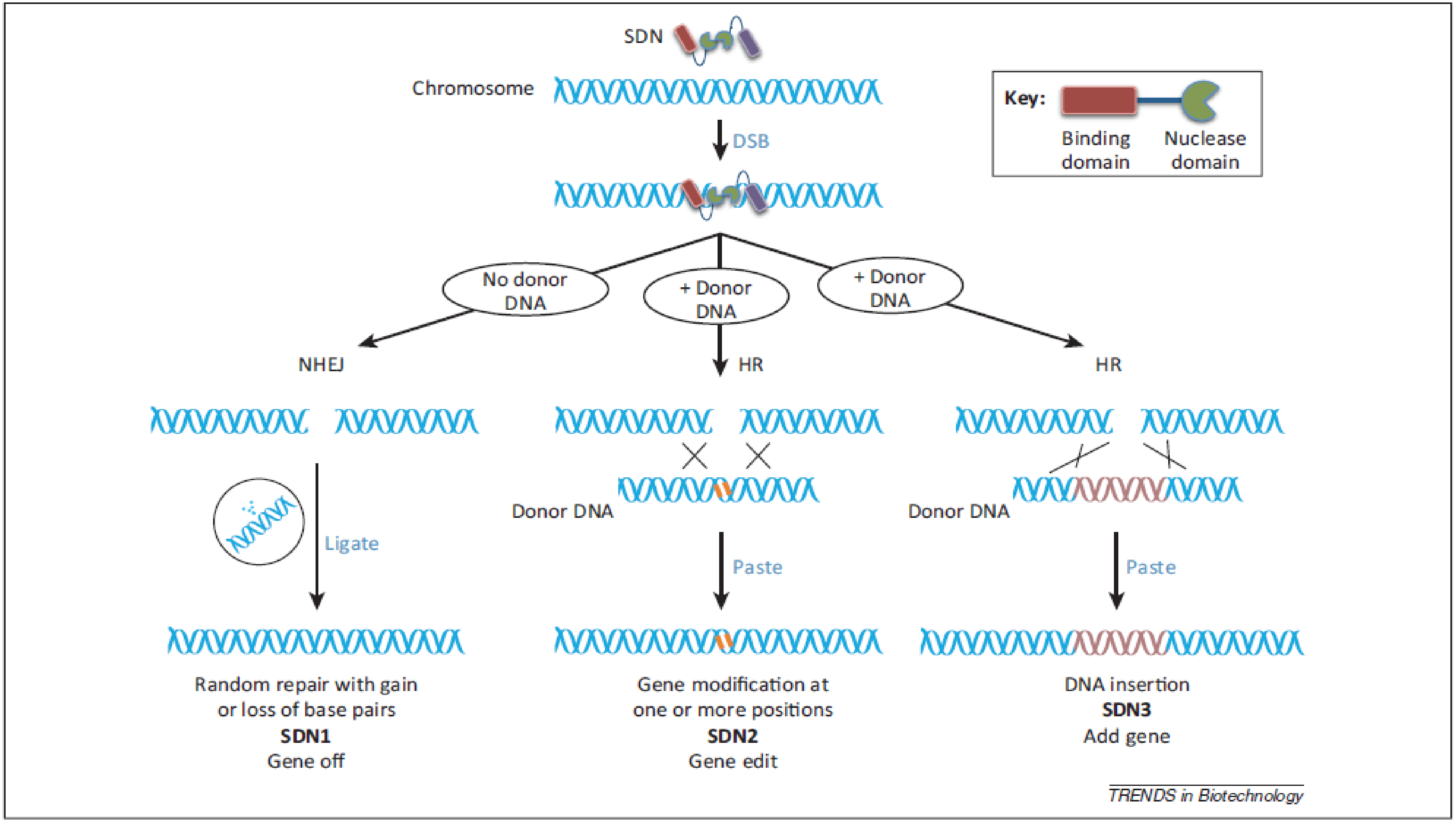
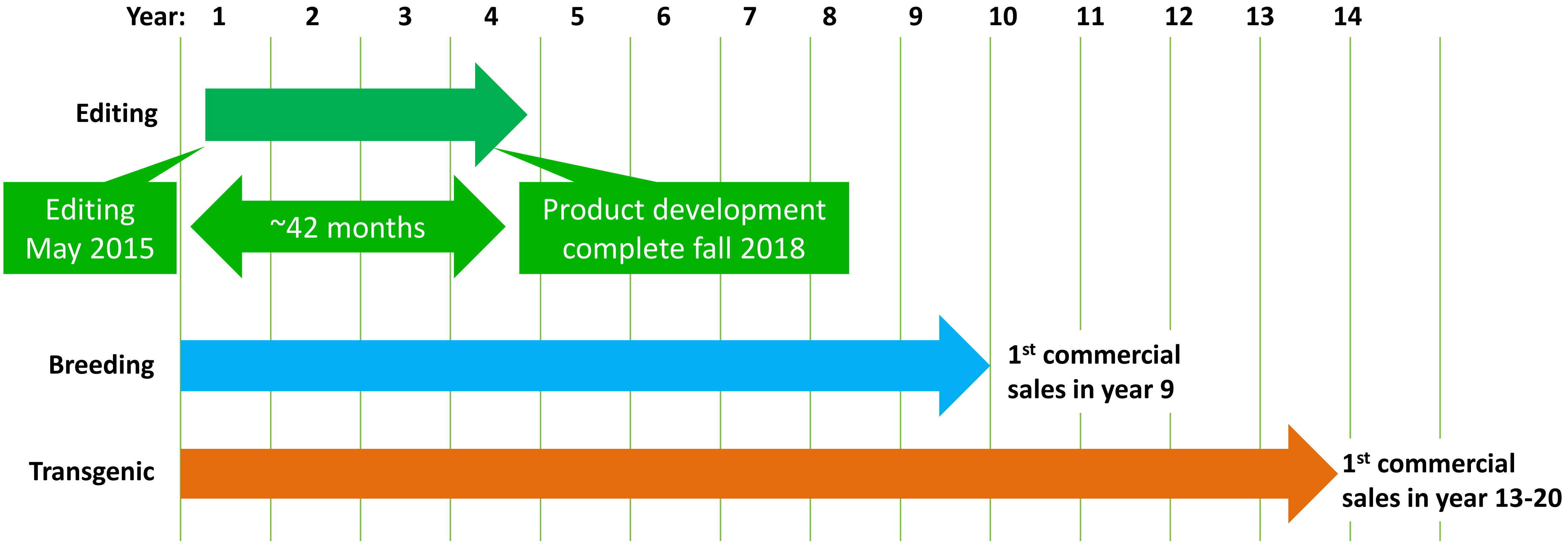


Figure 1. Different site-directed nuclease (SDN) techniques (SDN-1, 2, and 3). An SDN complex is shown at the top in association with the target sequence. The repair can take place via nonhomologous end-joining (NHEJ) or homologous recombination (HR) using the donor DNA. SDN-1 can result in site-specific 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.

CRISPR-edited waxy trait demonstrates rapid product development (Corteva Agriscience)



Traits for gene alteration at CIMMYT

- **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
    - Leaf rust (*Lr34, Lr67*)
    - Powdery mildew (*MLO*)
  - Plant height reduction by alternative mechanisms from Rht genes
  - Biofortification
    - Phytate downregulation for increased Fe and Zn availability

Genotypes resistant (L) or susceptible (R) to MLN

Naivasha, Kenya

CIMMYT

When a drought-tolerant commercial hybrid becomes susceptible to MLN

- Commercial hybrid grown in Kenya and Uganda
- High yielding under drought and optimal conditions
- Turned out to be susceptible to MLN after the disease emerged

Kiboko: No MLN pressure

Naivasha: Artificial MLN inoculation

CML312/CML395//CML566

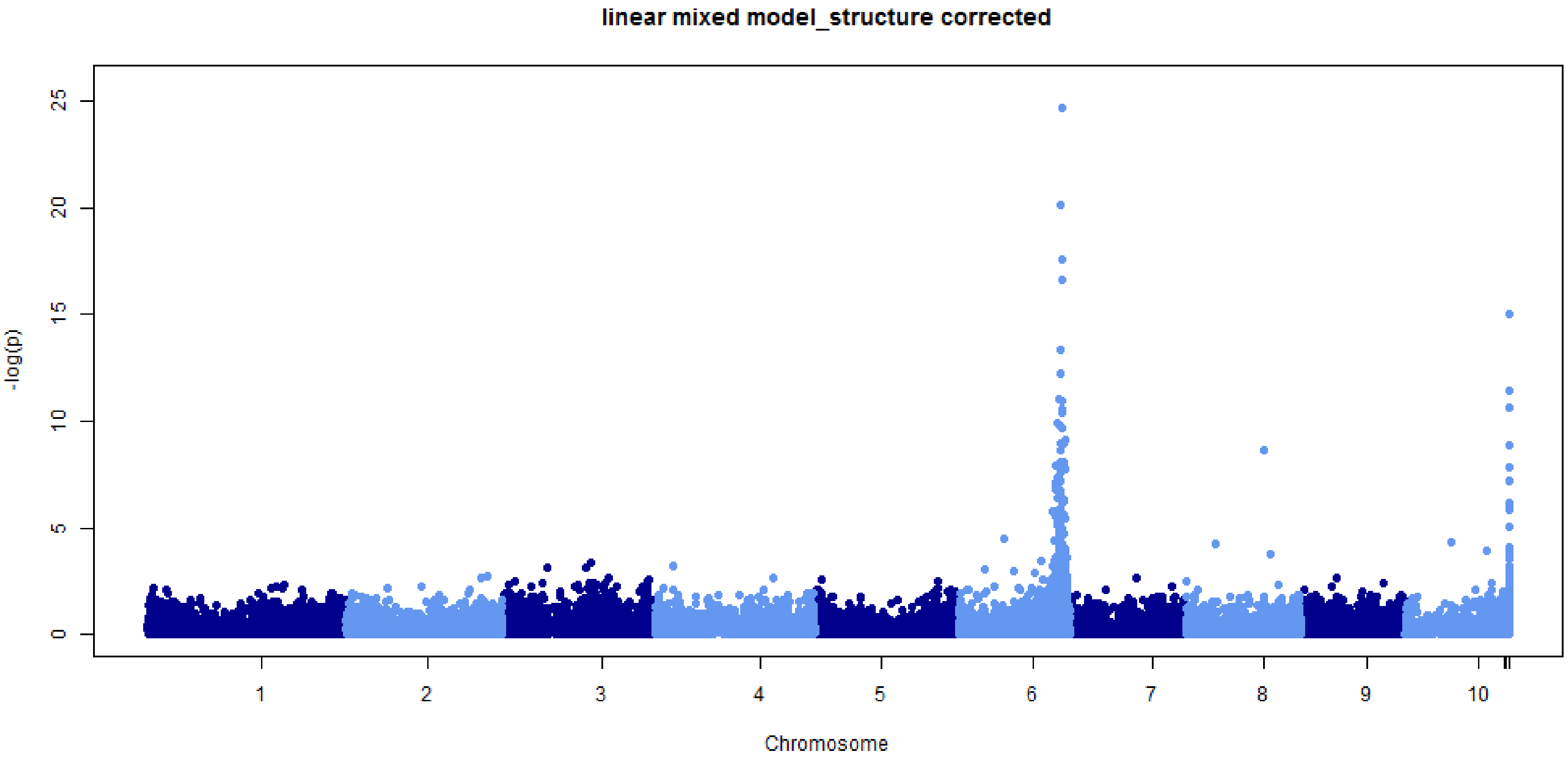
Beyene, Olsen

An exotic line is resistant to MLN

CML395 **KS-23-6** **CML444**

MLN resistance (MLN_R) maps to a single QTL

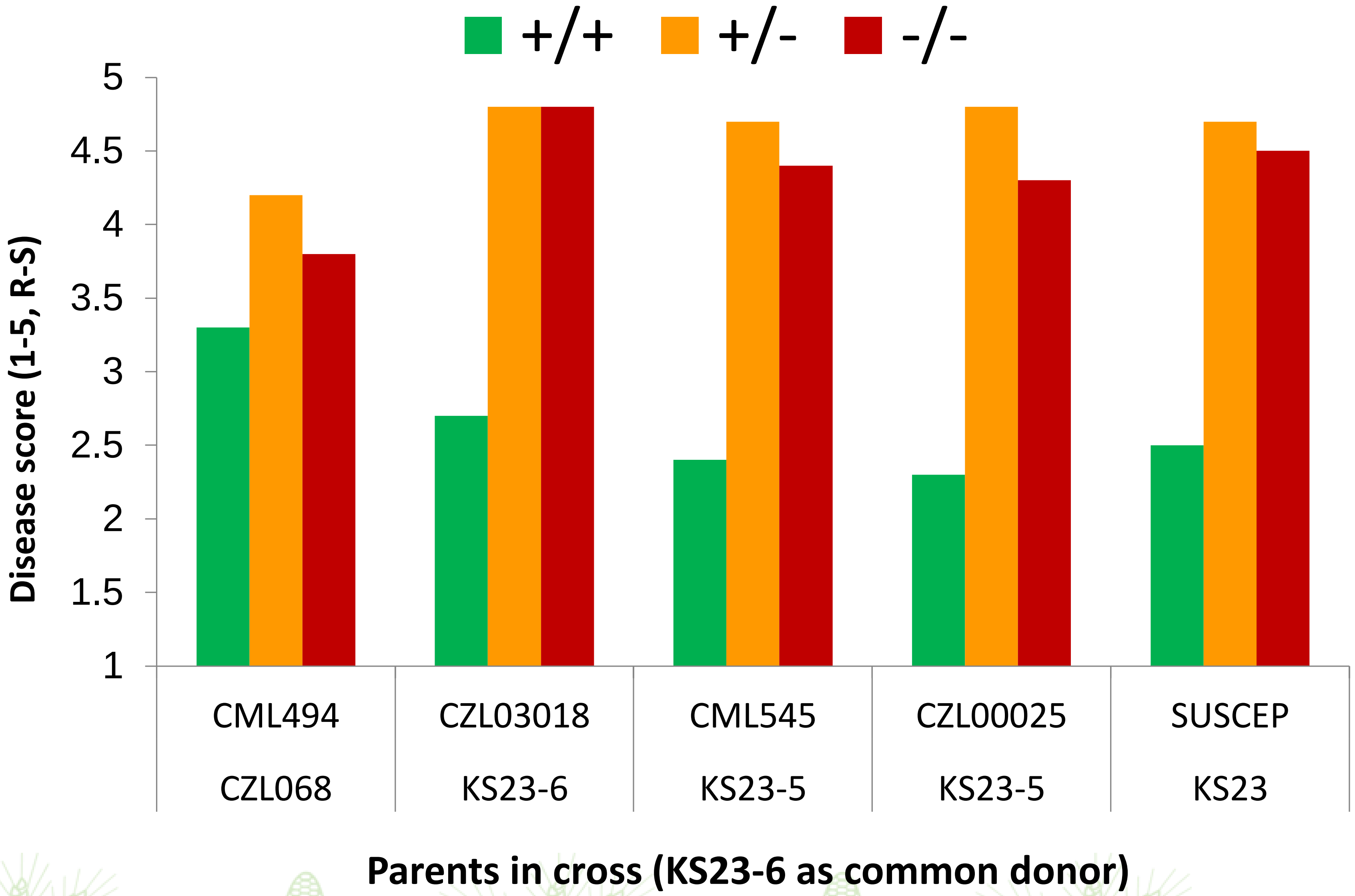
Kenya



Three populations under field conditions

Mike Olsen

MLN_R locus alone explains half of the variation for MLN resistance



Olsen

Effect of MLN_R allele (KS23-6) on resistance

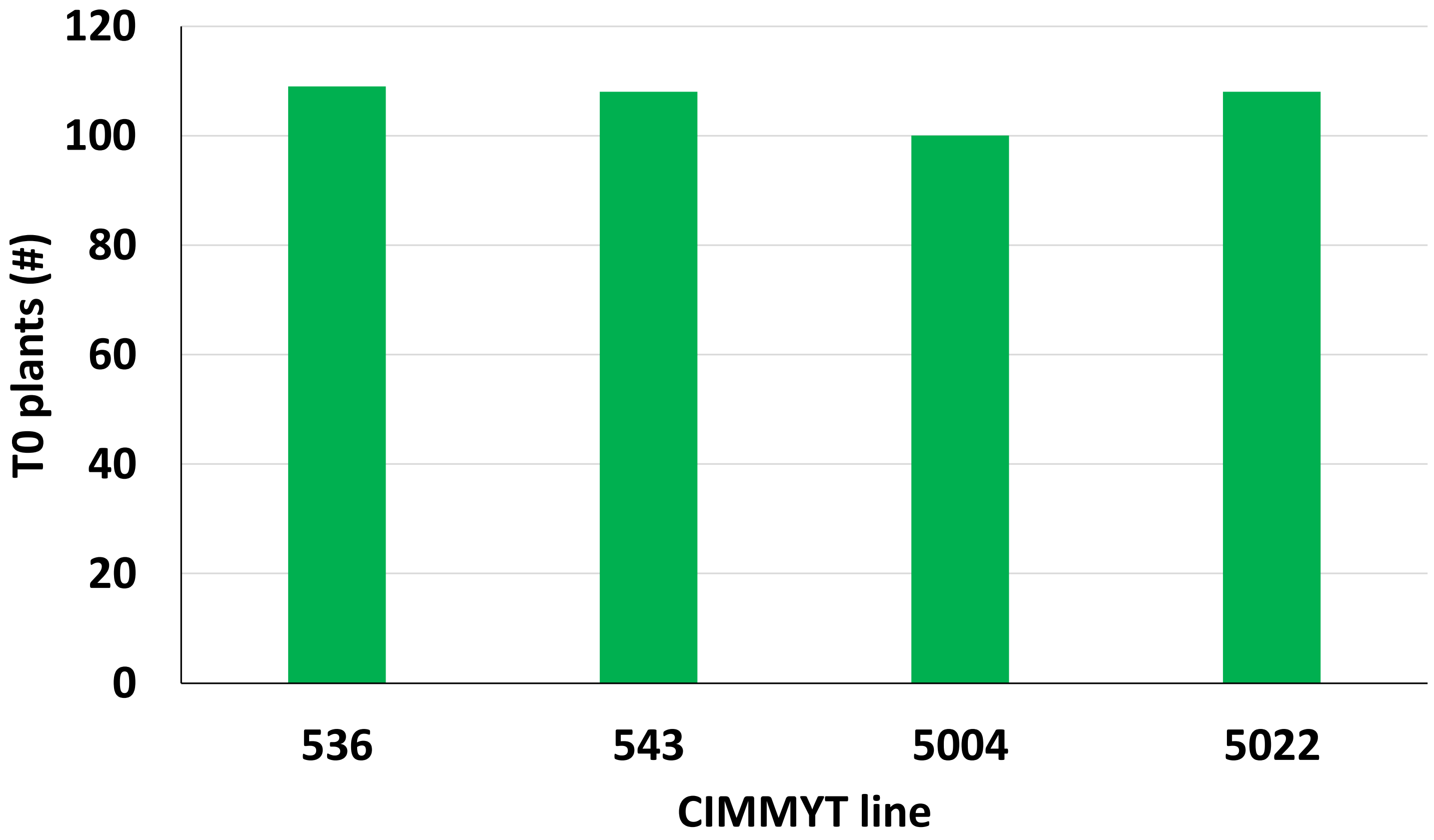
(CKDHL0186*3/KS23-6):B>1026>1106>1042-1011-1009- C:C G:G
(CKDHL0186*3/KS23-6):B>1026>1106>1042-1011-1016- C:C G:G
(CKDHL0186*3/KS23-6):B>1026>1106>1054-2006-1004- T:T T:T
(CKDHL0186*3/KS23-6):B>1026>1106>1054-2006-1006- T:T T:T

- KS23-6 allele + KS23-6 allele

Mike Olsen

Transformation of CIMMYT lines

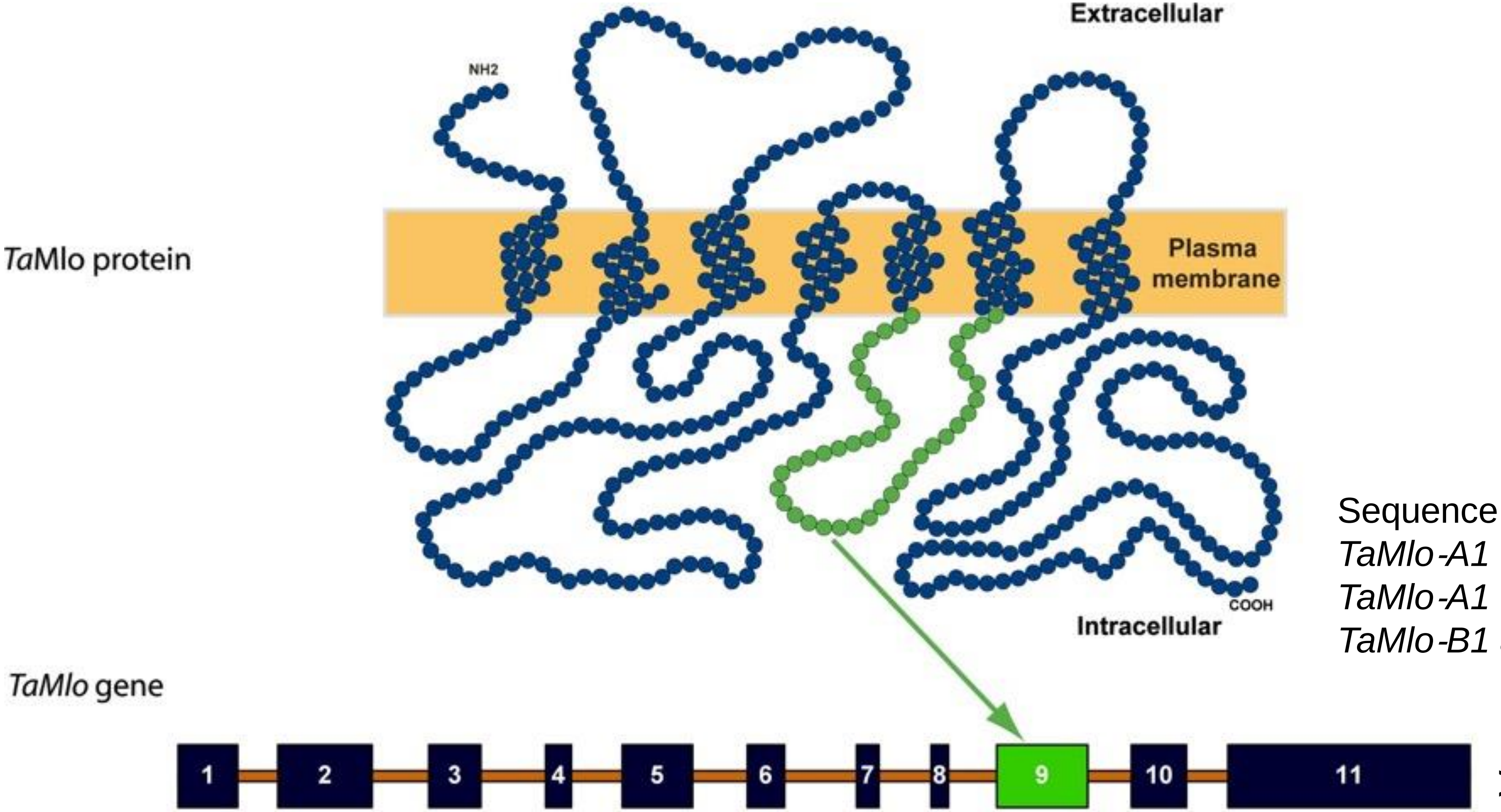
These four lines form two, 3-way cross commercial hybrids



- Obtained more than 100 T0 plants for each line
- Transformation frequency: 100%

Jeff Farrell, Emily Wu, Kay Snopek (Corteva Agriscience)

MLO resistance: inactivate all three copies (A, B, and D)



>3 kb

Sequence identity of:
TaMlo-A1 and *B1* = 95%
TaMlo-A1 and *D1* = 96%
TaMlo-B1 and D1 = 97%

Plant Biotechnol J. 2017 Mar; 15(3): 367–378.

CIMMYT

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Accelerated breeding

- Reconstitution of original genetic background after backcrossing is time consuming yet suffers from linkage drag.
- Maize hybrids in Africa have long lifespans, lasting decades. When popular hybrids go out of production, for example, because of disease susceptibility, smallholder farmers encounter major disruptions.
- Corteva has <u>revolutionized</u> genetic transformation so the tropical maize lines from Africa can be directly edited.
- Edit target gene directly in elite lines.
- Future edits could be stacked onto the previous one.
- These steps will save years worth of time and eliminate linkage drag.
- Significantly contribute toward alleviating poverty and hunger.