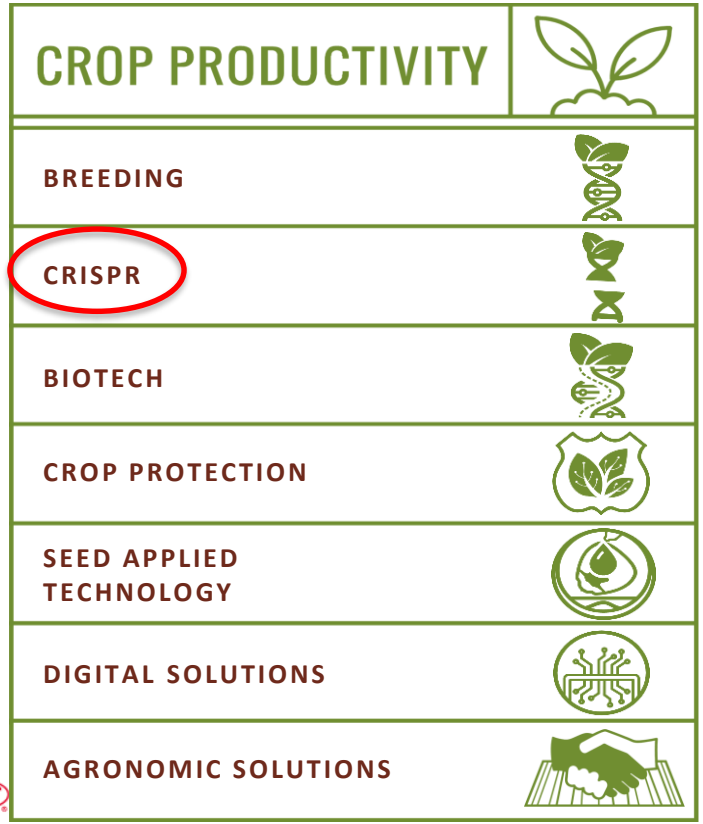
Targeted Breeding Applications of CRISPR-Cas technology for European Markets

Dr. Neal Gutterson, Vice President, R&D

DuPont Ag's key technology platforms



*"We put a great **emphasis in managing our grain on a much more micro level** – so looking not on a field by field basis but more on an **acre by acre and even in some cases on a sub-acre level."***

- DuPont Customer

Only DuPont can collaborate with growers acre by acre to answer these demands.

Everything we do is about solving one problem – a complex problem that has grown and changed with the needs of our growing world –

CROP PRODUCTIVITY

Required Competencies for Targeted Breeding

Understanding Elite Genetics

- High quality DNA sequencing
- Informatics tools and infrastructure

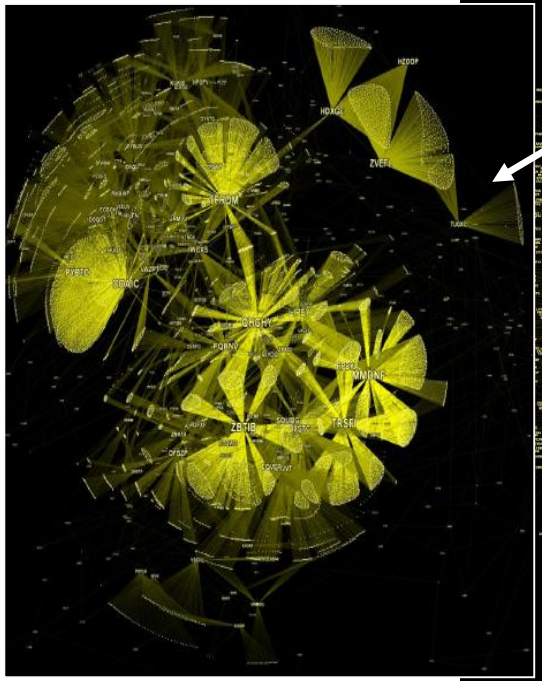
Delivery into Elite Genetics

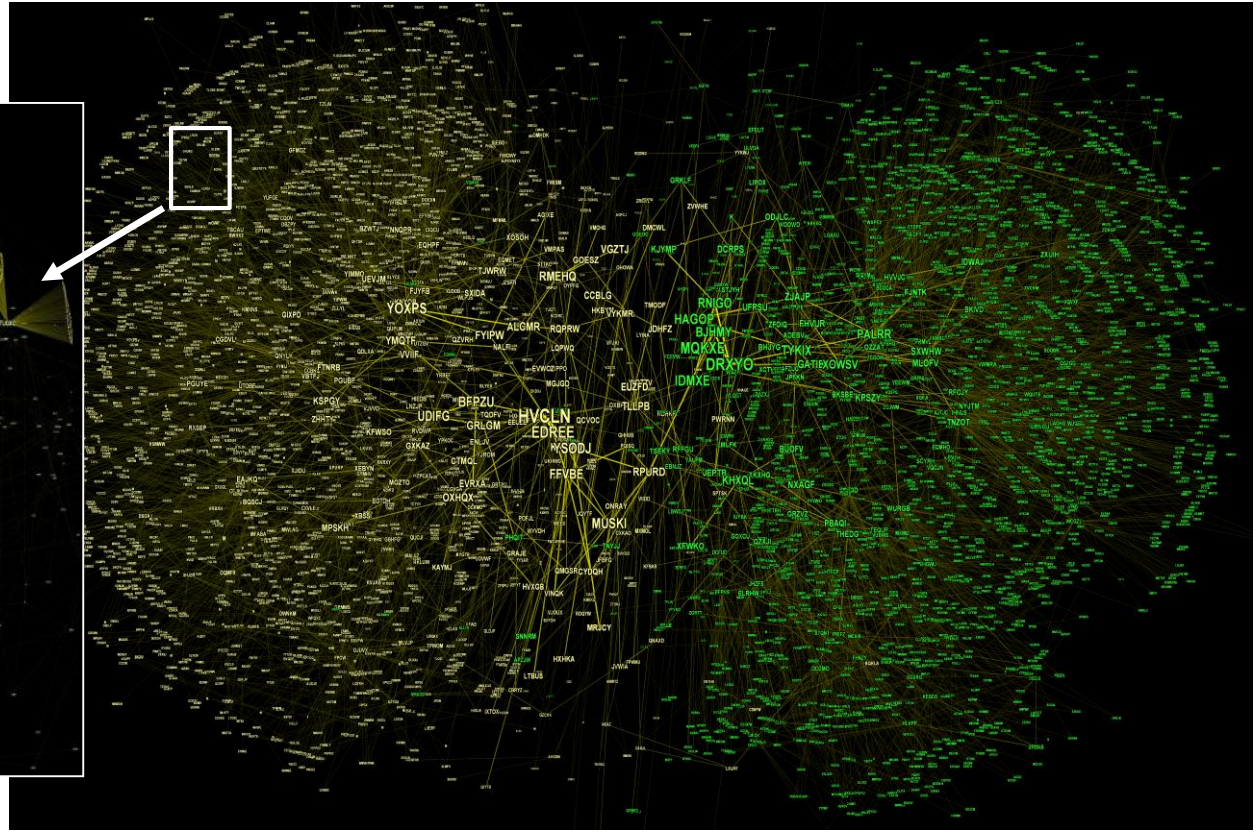
- Ability to directly introduce targeted improvements to already high-quality plants

Advancing CRISPR-Cas Technology

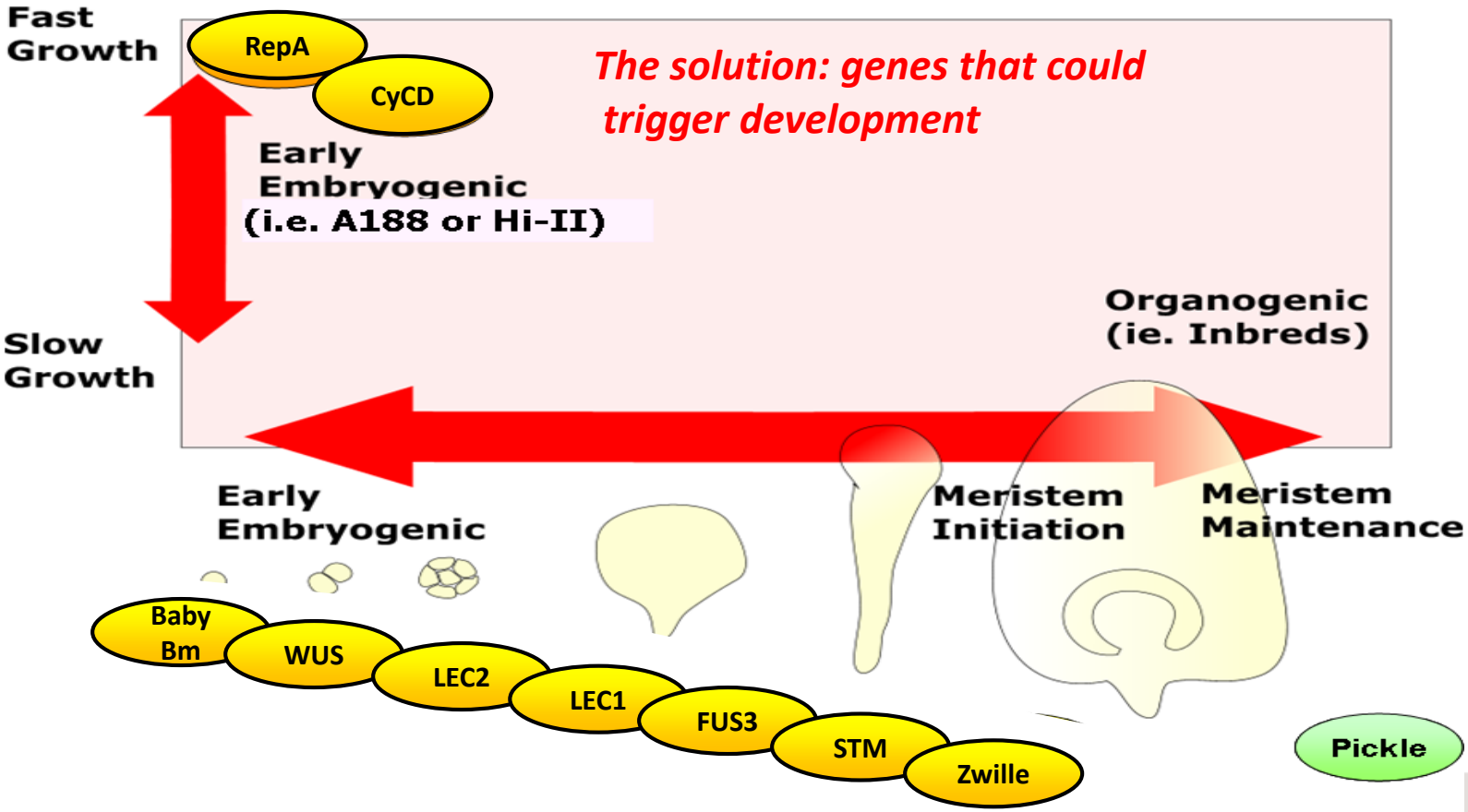
- Tool with superior activity and targeting specificity
- Incorporating in-house & collaborators' expertise

We have deep genomic understanding of products





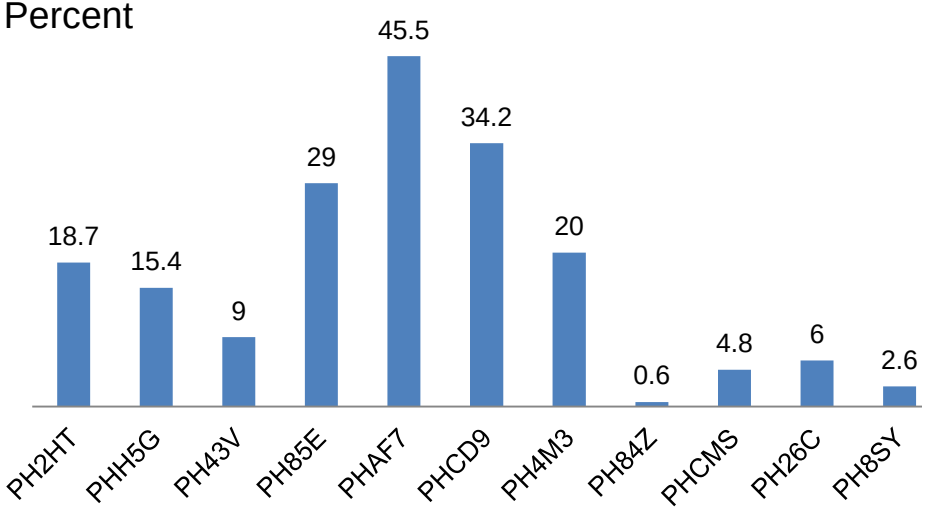
Creating alleles in many inbreds: the problem



Germplasm-independent transformation

Transformation frequency

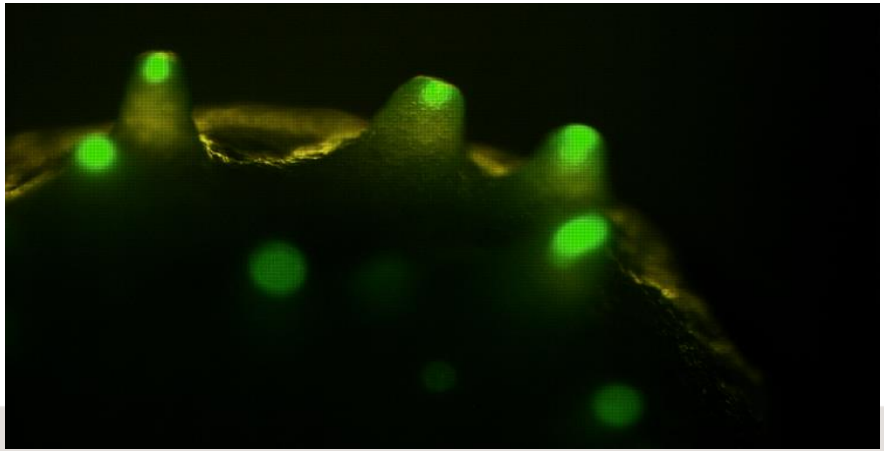
Percent



THE PLANT CELL
AMERICAN SOCIETY OF PLANT BIOLOGISTS

Morphogenic Regulators *Baby boom* and *Wuschel* Improve Monocot Transformation OPEN

Keith Lowe,[a] Emily Wu,[a] Ning Wang,[a] George Hoerster,[a] Craig Hastings,[a] Myeong-Je Cho,[b] Chris Scelonge,[a] Brian Lenderts,[a] Mark Chamberlin,[a] Josh Cushatt,[a] Lijuan Wang,[a] Larisa Ryan,[a] Tanveer Khan,[c] Julia Chow-Yiu,[a] Wei Hua,[a] Maryanne Yu,[b] Jenny Banh,[b] Zhongmeng Bao,[a] Kent Brink,[d] Elizabeth Igo,[d] Bhojaraja Rudrappa,[e] PM Shamseer,[e] Wes Bruce,[f] Lisa Newman,[a] Bo Shen,[a] Peizhong Zheng,[a] Dennis Bidney,[a] Carl Falco,[a] Jim Register,[a] Zuo-Yu Zhao,[a] Deping Xu,[a] Todd Jones,[a] and William Gordon-Kamm[a,1]



DUPONT PIONEER

Next Generation Waxy Corn

Pioneer's first commercial product through targeted breeding

No. 2 Yellow Dent Corn

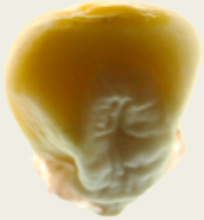
- Functional *wx1*
- Translucent appearance
- Feed / ethanol / food

Starch

75% Amylopectin

25% Amylose

Waxy Corn

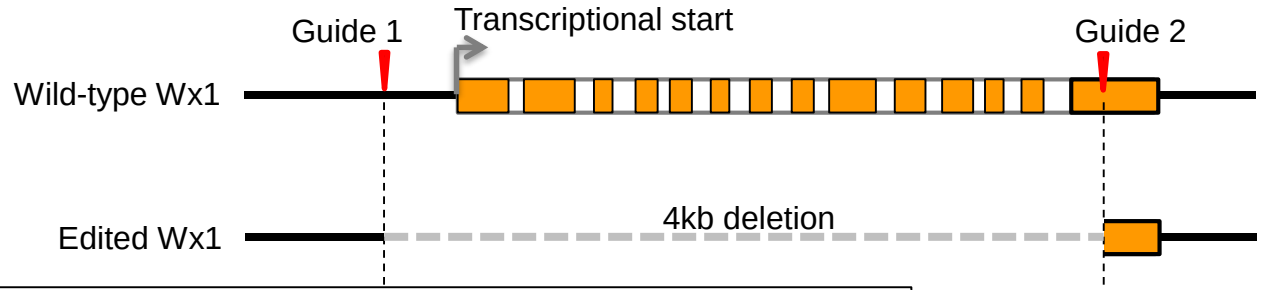


- Mutation in *wx1*
- Candlewax-like appearance
- Food / industrial

Starch

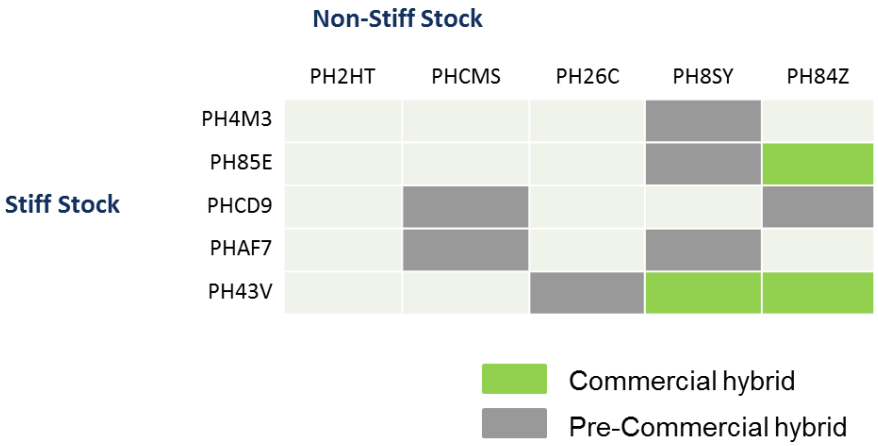
>97% Amylopectin

Waxy corn – edited allele strategy

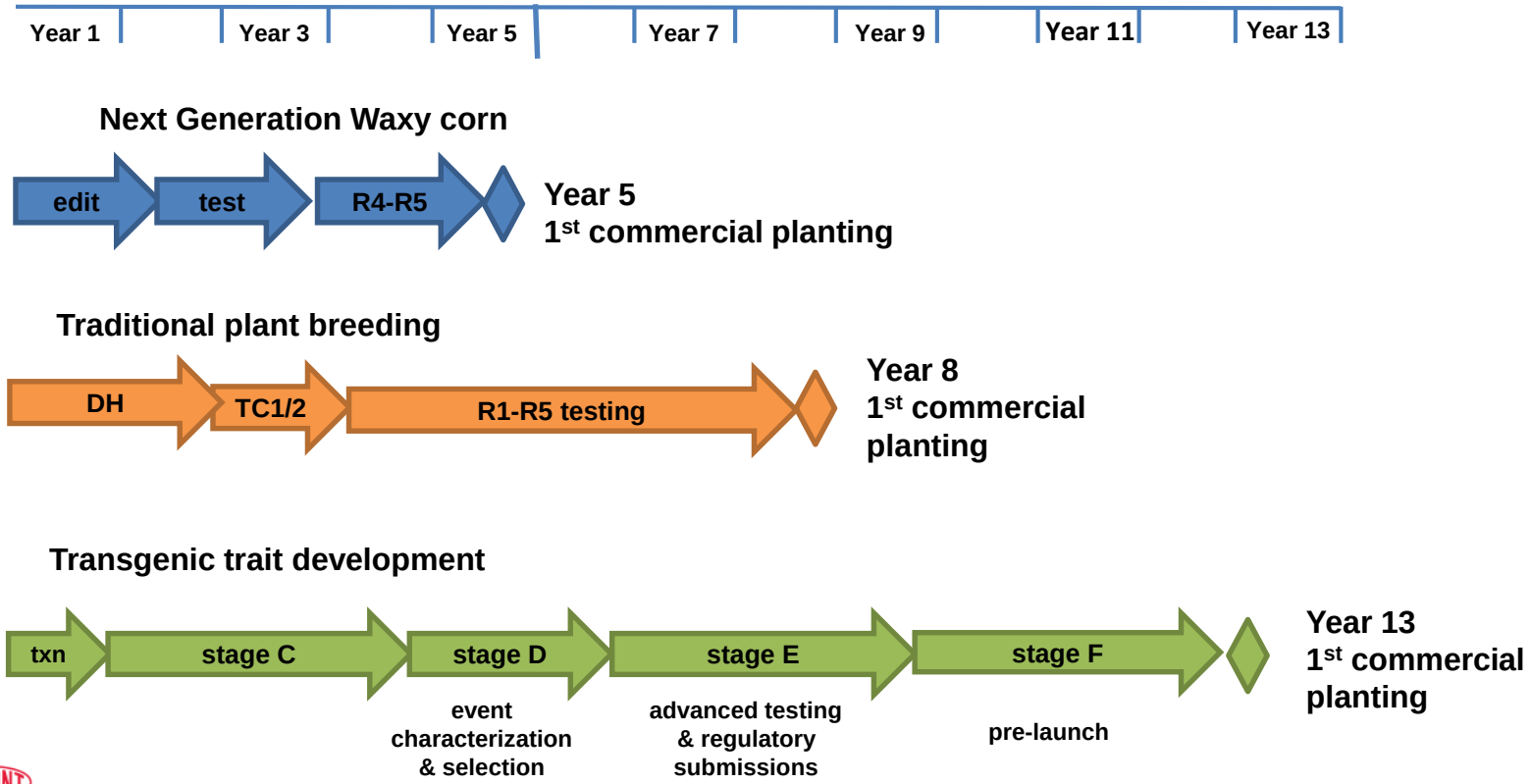


Summary:

- Created alleles directly in multiple commercial inbreds
- Tested in greenhouse and field under standard testing strategy for late stage hybrids
- Sep 2015 initiated transformation of new elite inbreds “on demand”
- Waxy deletions recovered in all 11 inbred lines



Edited variety development can be very efficient



Pioneer will deploy targeted breeding broadly

NEAR-TERM PRODUCTS TO MARKET

WAXY CORN HYBRIDS

- Foundational for future product development
- First commercial agricultural product
- To market by end of current decade

NORTHERN CORN LEAF BLIGHT

- Devastating global disease with potential to cause $1.6B* annual losses in North America alone
- Leveraging germplasm base
- Utilizing native genes, genomic selection, and genome editing
- Providing sustainable grower solutions

BROAD AGRICULTURAL APPLICATIONS OF CRISPR-CAS

	DISEASE RESISTANCE	YIELD & YIELD STABILITY	DROUGHT TOLERANCE	OUTPUT TRAITS	MATURITY
CORN	●	●	●		●
SOY	●			●	●
OSR	●	●		●	
RICE	●	●	●		●
WHEAT	●	●			
SUNFLOWER	●			●	

NORTHERN LEAF BLIGHT READINESS:
First half of next decade

Enabling a wide array of applications

Developing solutions to the toughest agricultural challenges

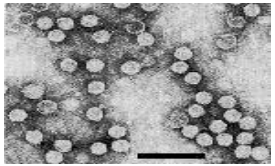
CIMMYT & DuPont Pioneer Public-Private Partnership

Sugarcane Mosaic Virus (SCMV)

+

Maize chlorotic mottle viruses (MCMV)

new to Africa, more severe then SCMV



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Maize Lethal Necrosis Disease

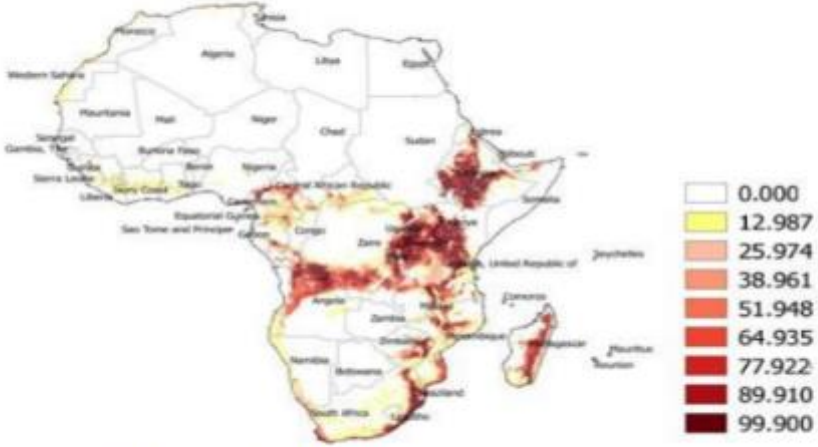
Range of symptoms

- *Vascular discoloration*

Even "clean" plants may show:

- *Sterile tassels*
- *No ears*
- *High cob rot/ predisposed to other challenges*

Projected Suitability of MCMV and potential risk of MLN across Africa by 2020, using Agro-ecological Niche Modelling



Source: Melanie Edwards, BFS/ARP, USAID

*Darker colors (higher Index) indicates higher suitability and risk for MLND

- MLN first observed in Kenya in 2011; spread to neighboring countries in less than five years
- Average reduction in maize production: 3% in drylands; 32% in moist environments; yield reduction at individual farms can be as high as 90% (*de Groot et al., CIMMYT*)
- In Kenya, MLN affects nearly a quarter of total maize production; yearly losses ~$US110 million (*Biosciences for Framing In Africa, 2016*)

Listening to Full Range of Stakeholders

- Recognize that all new technologies require a “social license”
- Engaging with traditional and non-traditional
- Applying insights to our own plans as well as sharing with others in the industry
- On-going discussion

CRISPR-Cas[1] Advanced Plant Breeding Guiding Principles[2]

CRISPR-Cas is one of many tools DuPont uses to deliver improved products and value to customers.

Safety and product stewardship are foundational to any DuPont product offering. See more here

When using **CRISPR-Cas for advanced plant breeding,** DuPont will only work with **genetic material from the target plant/crop.**

DuPont is committed to **open, transparent, and timely communications** about its use of CRISPR-Cas.

DuPont is committed to **responsible development and application** of CRISPR-Cas to help ensure **consumer confidence.**

- **DuPont supports appropriate, science-based regulatory oversight** for plants developed with CRISPR-Cas advanced plant breeding, consistent with plants developed through other plant breeding methods.
- **DuPont intends to enable others** wanting to develop agricultural products using CRISPR-Cas through access to intellectual property (IP), technology capabilities, infrastructure and scientific expertise.
- **DuPont will consider diverse viewpoints** in its decision-making process for products developed with CRISPR-Cas advanced plant breeding.

1"CRISPR-Cas" is derived from naturally occurring "CRISPR" found in many bacteria that naturally protect themselves against bacteriophage. DuPont has used this natural CRISPR for many years to improve dairy product manufacturing and to make food safe and last longer.

2 These principles refer to the DuPont use of "CRISPR-Cas" as a plant breeding technique. If CRISPR-Cas is used to more efficiently develop GMOs, DuPont will follow all applicable GMO regulations and the DuPont Biotechnology Guiding Principles. See more here