Part I. Case examples showing contribution of genome editing

11:50-12:20 Targeted breeding applications of CRISPR-Cas technology

Dr. Neal Gutterson, Vice President, Research and Development, DuPont Pioneer, USA

# Targeted breeding applications of CRISPR-Cas technology

Neal Gutterson, Vice President, Research & Development, DuPont Pioneer

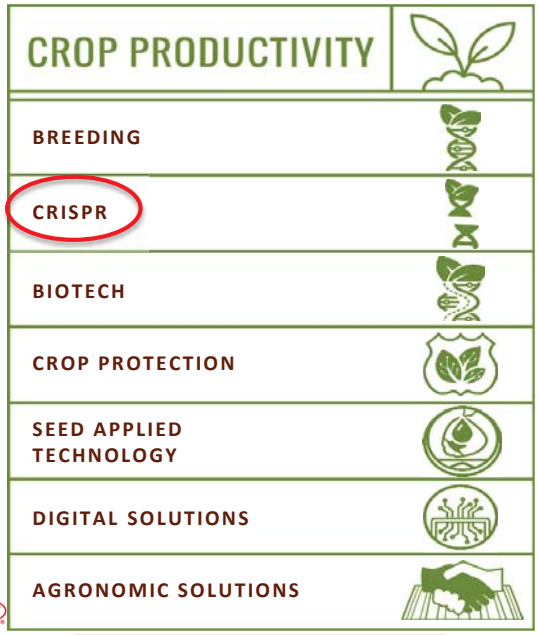
Abstract:

CRISPR-Cas as an advanced plant breeding tool is a more efficient way to improve plants and help farmers produce more and better food, with fewer resources**.** The superior properties of CRISPR-Cas allow scientists to develop innovative and sustainable seed products for growers similar to those realized through conventional plant breeding, but with even greater efficiency, accuracy and quality. DuPont Pioneer is leading the application of this tool to develop customized agriculture solutions.

In this talk, Pioneer's next generation of waxy maize hybrids as the first agricultural product of CRISPR-Cas scheduled for commercialization will be discussed. In addition, potential product targets of this promising technology will be explored, including rice improvement based on a rich understanding of the rice genome. Approaches to fostering social license and developing an open innovation model for CRISPR-Cas will also be reviewed.


## Abundant Natural Variation

# 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**

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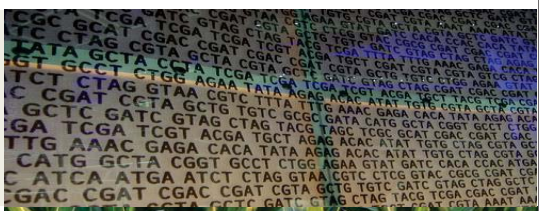
# Required Competencies for Targeted Breeding

## 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

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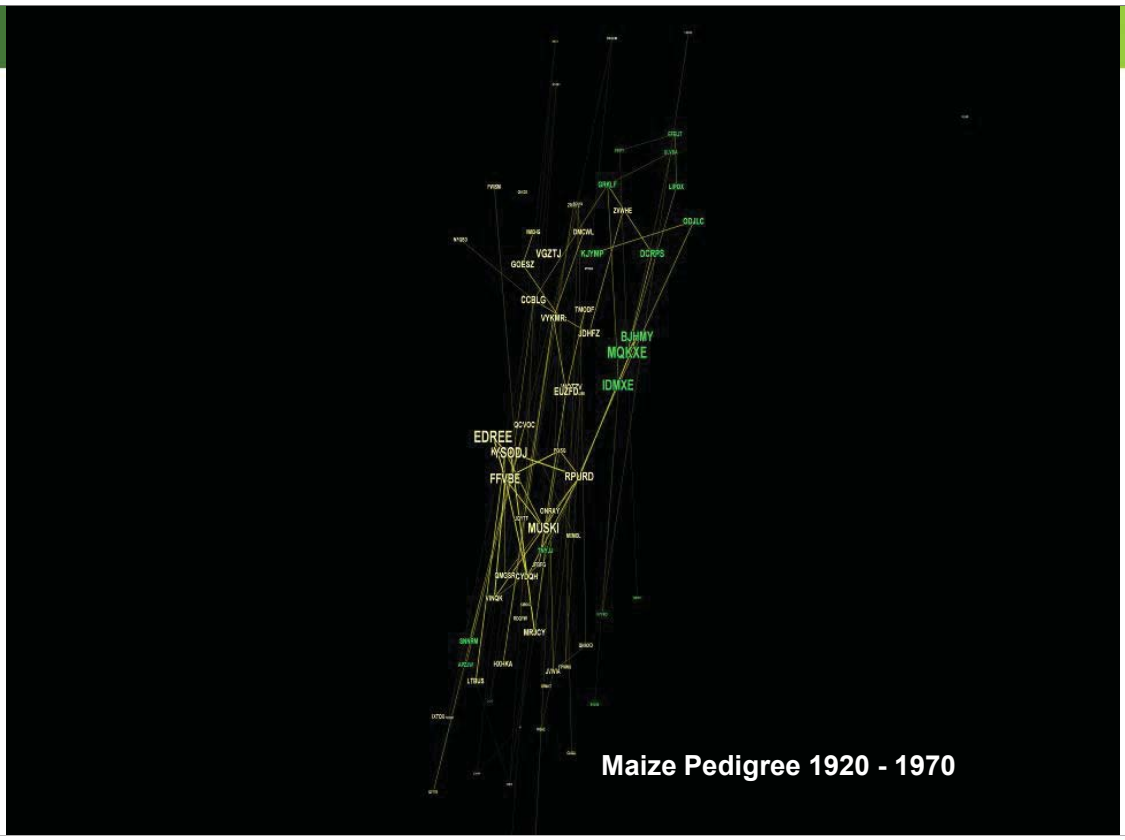
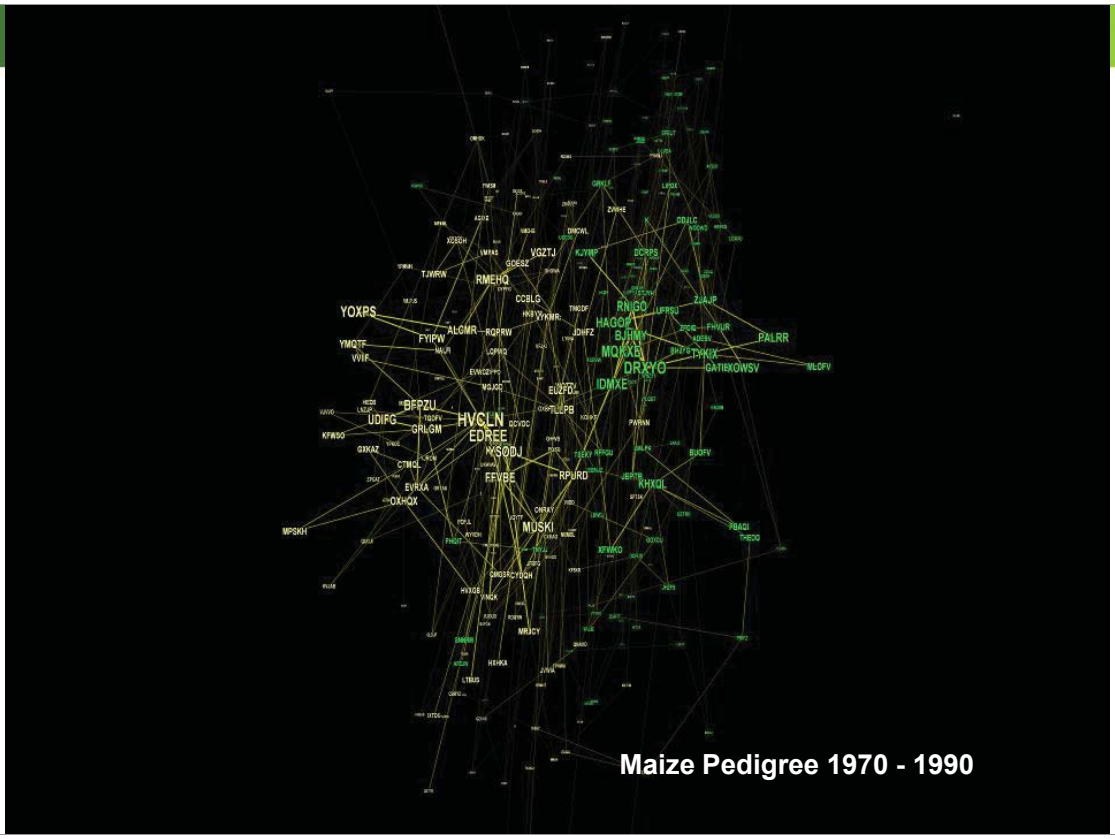
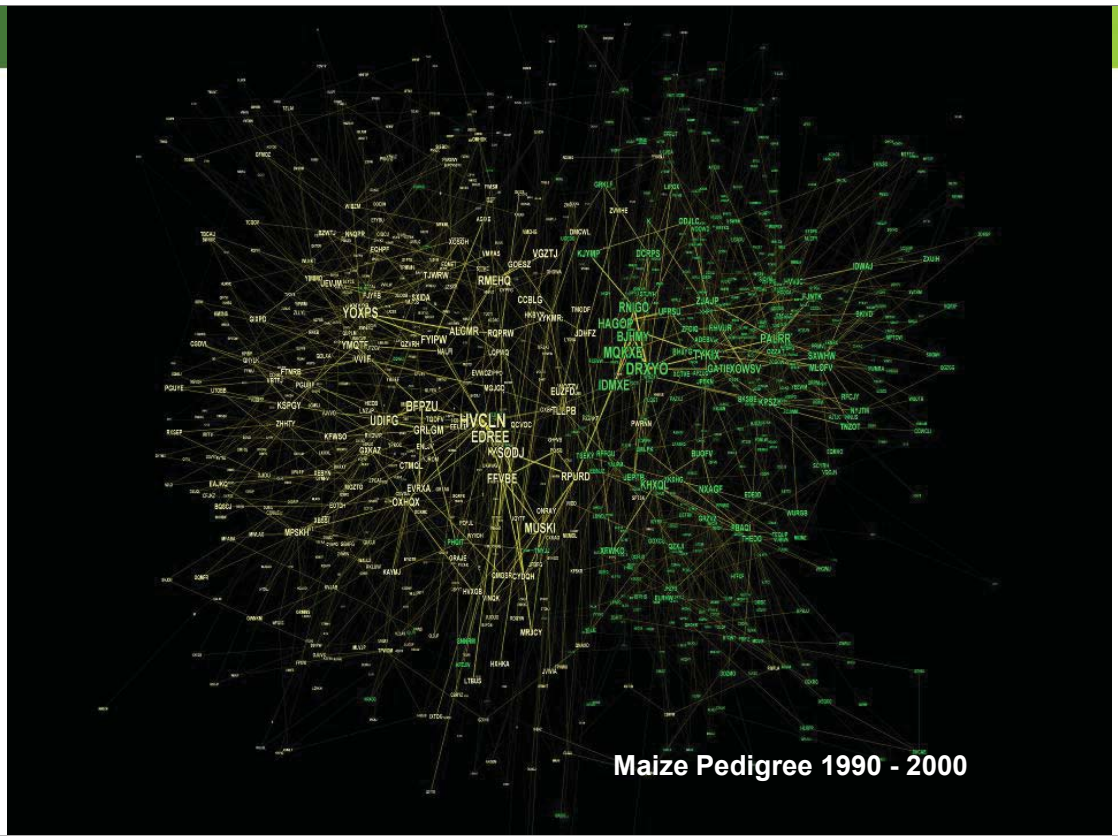
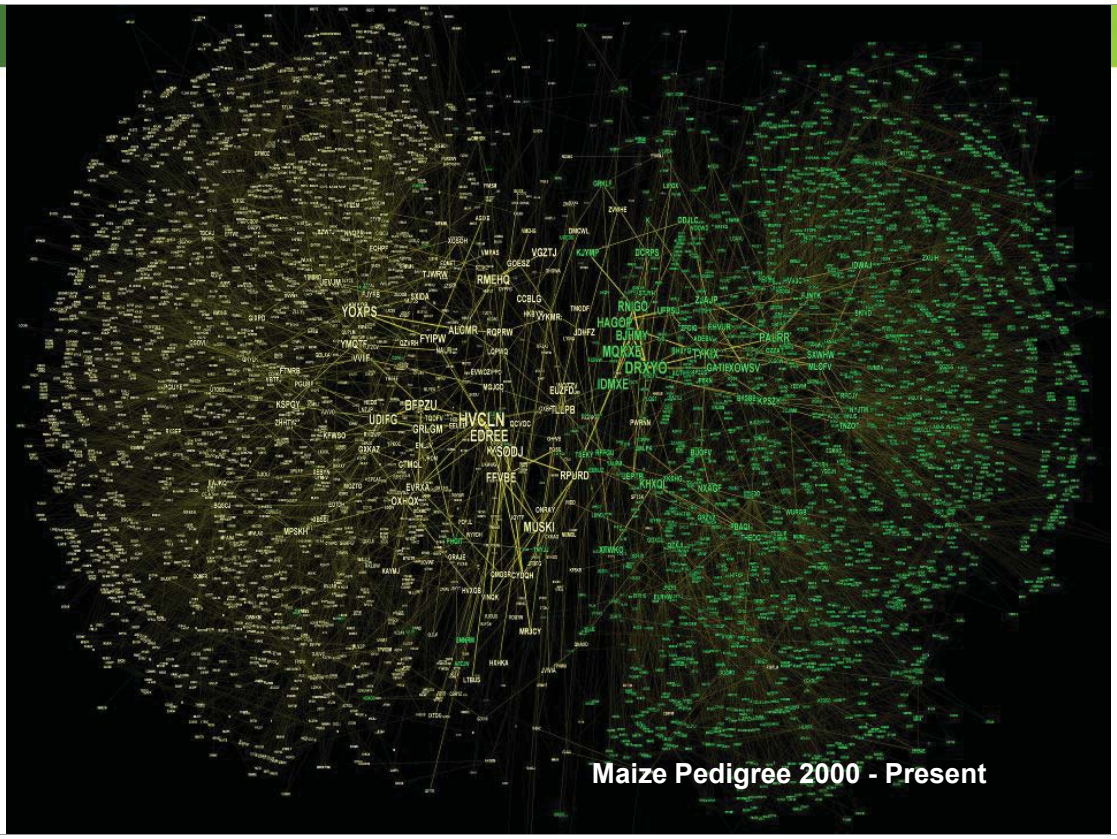
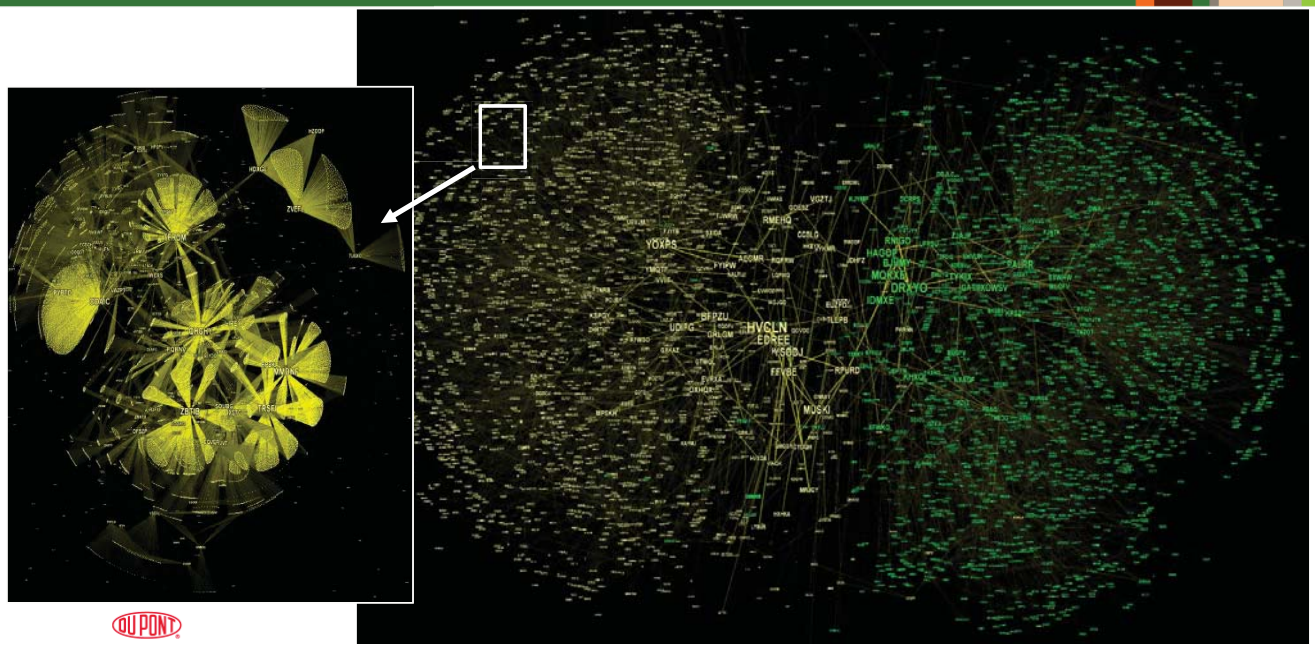

EDREE
MQKXE
IDMXE
MUSKI
Maize Pedigree 1920 - 1970


YOXPS
HVCLN
EDREE
MQKXE
IDMXE
MUSKI
Maize Pedigree 1970 - 1990


Maize Pedigree 1990 - 2000


Maize Pedigree 2000 - Present

## We have deep genomic understanding of products



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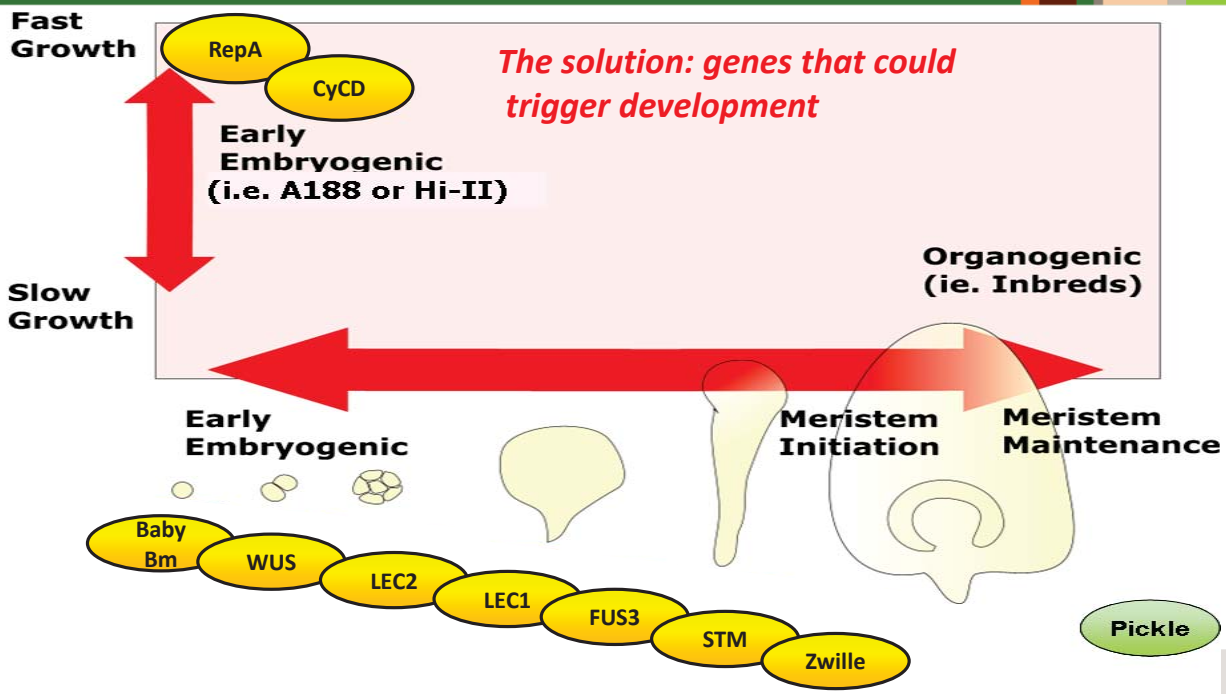
## Creating alleles in many inbreds: the problem

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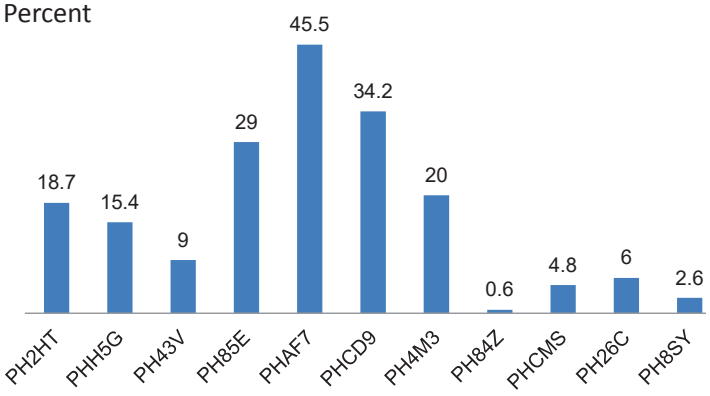
# Germplasm-independent transformation

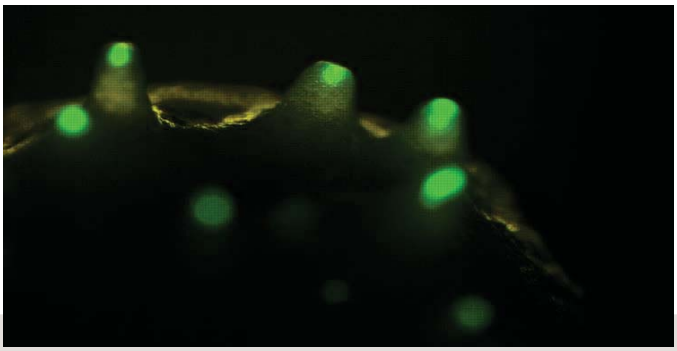
**Transformation frequency**
Percent




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,[g] 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]



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# 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

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*Products, benefits and concepts described herein will not be offered for sale or distribution until completion of field testing and applicable regulatory reviews.*
** Source: Internal analysis and USDA.*

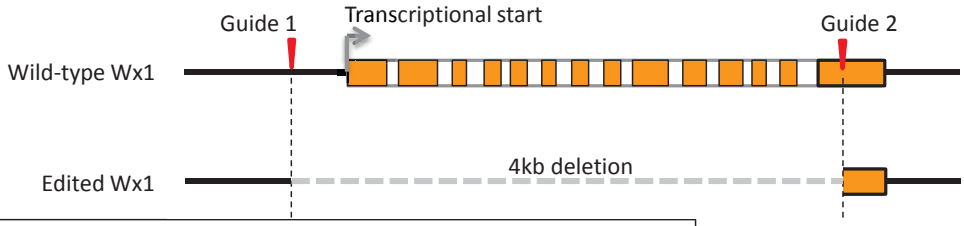
# Next Generation Waxy Corn

**Pioneer's first commercial product through targeted breeding**

| No. 2 Yellow Dent Corn | Waxy Corn |
|---|---|
|  |  |
| • Functional *wx1*<br>• Translucent appearance<br>• Feed / ethanol / food | • Non-functional *wx1*<br>• Candlewax-like appearance<br>• Food / industrial |
| Starch: 75% Amylopectin, 25% Amylose | Starch: >97% Amylopectin |

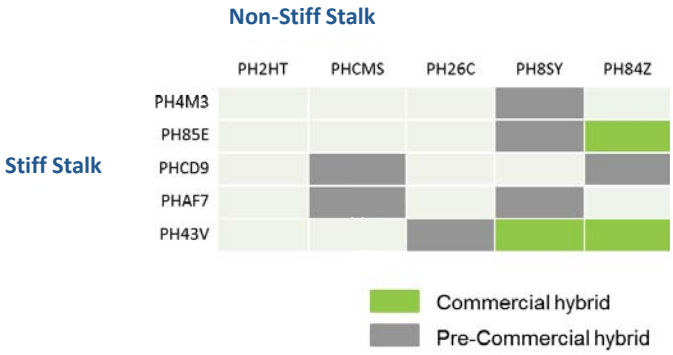


# Waxy corn – edited allele strategy

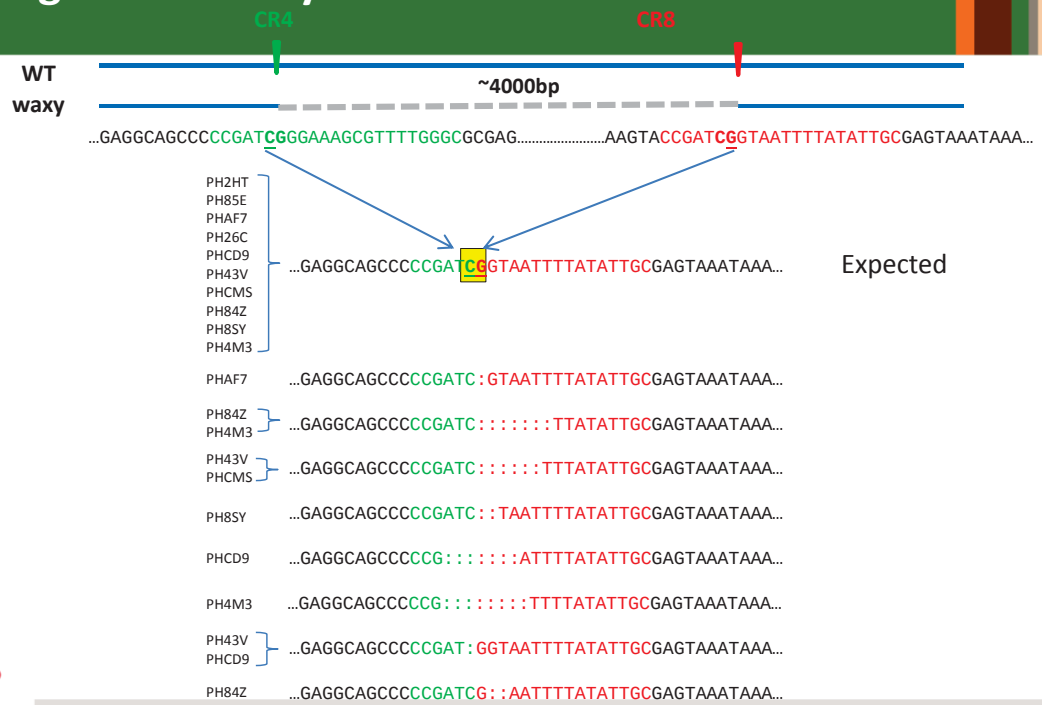



**Summary:**

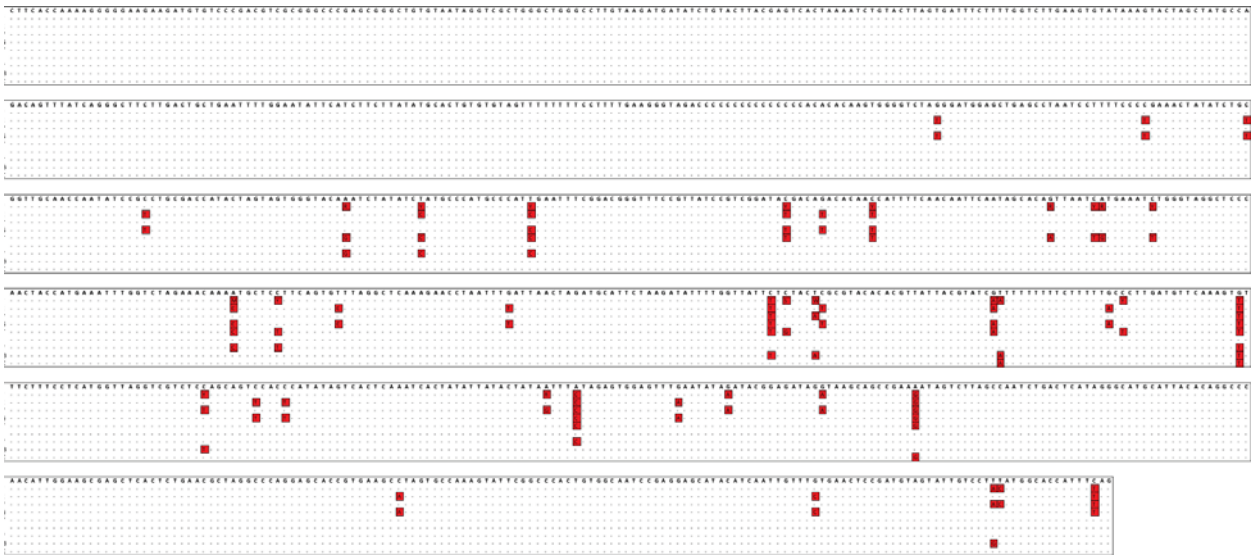
- Created alleles directly in multiple commercial inbreds
- Tested in greenhouse and field under standard testing strategy for late stage hybrids
- Sep 2015 initiated transformation of new elite inbreds "on demand"
- Waxy deletions recovered in all 11 inbred lines



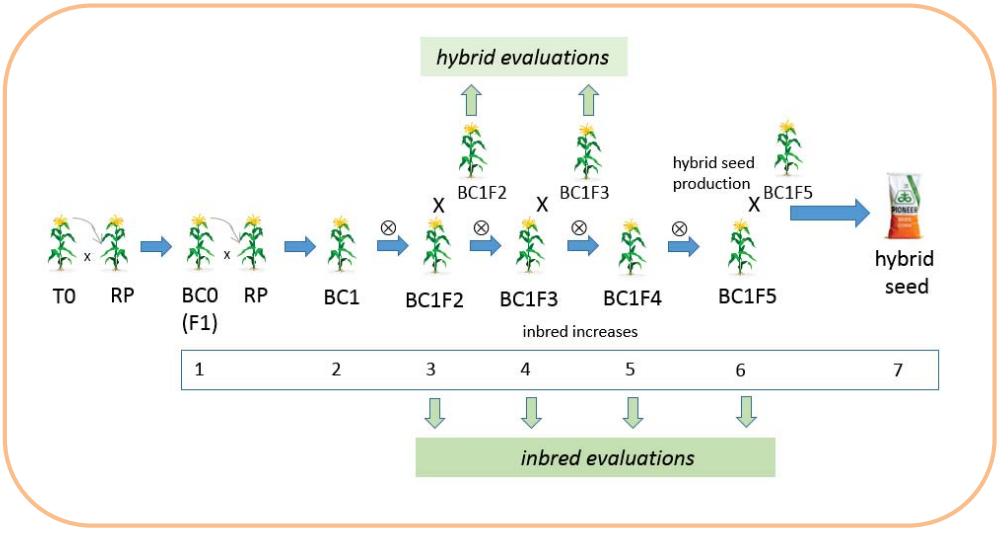
# Identifying edited waxy alleles




# Editing context: native variation at Waxy locus



# Development and evaluation of Next Generation Waxy Corn




**INBREDS**
> 30 characteristics

**HYBRIDS**
> 40 characteristics

- Vegetative characteristics
- Ear photometry
- Yield
- Disease resistance
- Certification traits

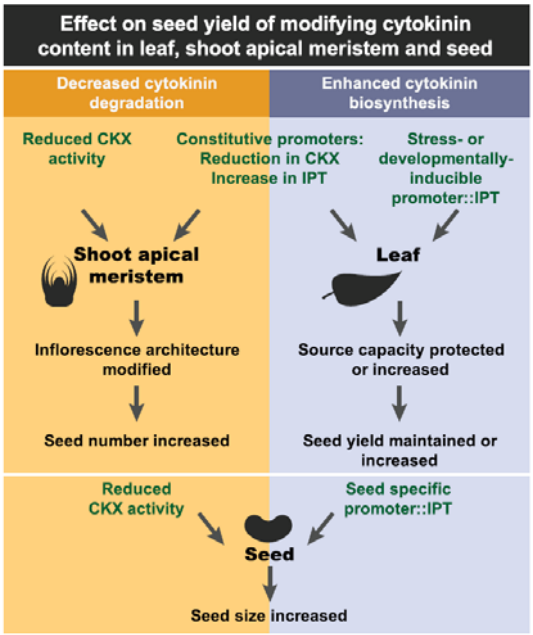
Several years of comprehensive multi-location field evaluations according to common breeding practices



# CRISPR-Cas for improved performance in rice products: a yield example

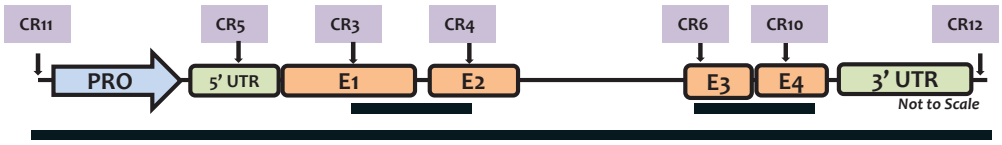
**CKX2 Functionality Impacts Yield and Agronomics**

- Cytokinin oxidase/dehydrogenase2 (CKX2) plays an important role in cytokinin catabolism in plants
- There are 11 CKX genes in rice
- CKX2 plays a critical role in cellular cytokinin level in the inflorescence
- ***Gn1a*** is a major QTL for grain yield in rice and CKX2 is the causal gene
- Natural variation at the CKX2 allele correlates to grain yield per panicle with reduced plant height impacts



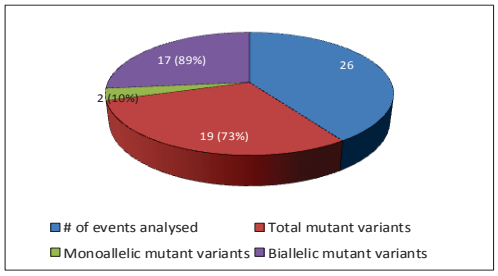

*Jameson and song, 2016*

# CRISPR-Cas allele creation

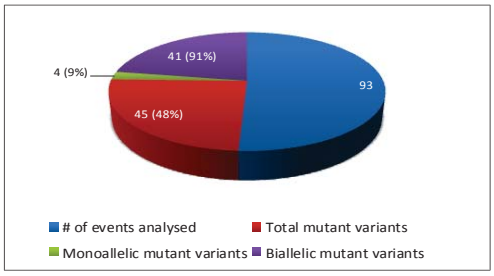



**Schematic: Multiple guides in CKX2 gene**

Variants are screened by PCR first and selected variants are confirmed by NGS



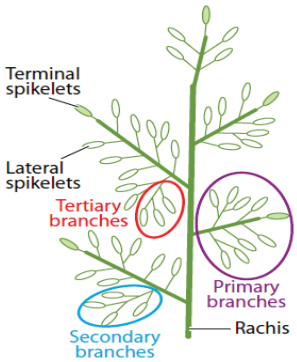

CR4- Mutation frequency (NGS confirmation)

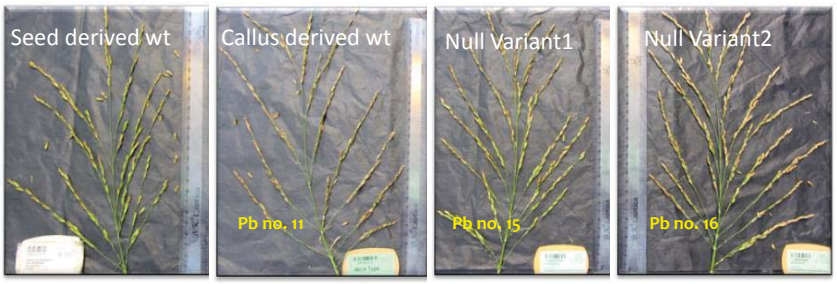



CR3-CR4- Mutation frequency (PCR confirmation)

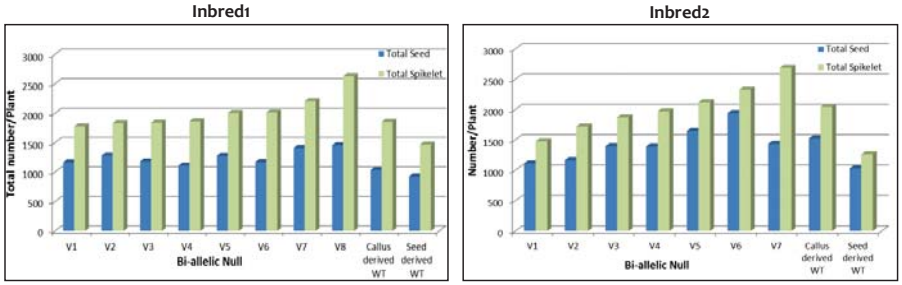
*High frequency of variations obtained across the different edit types*

# CKX2 edits provide expected benefits



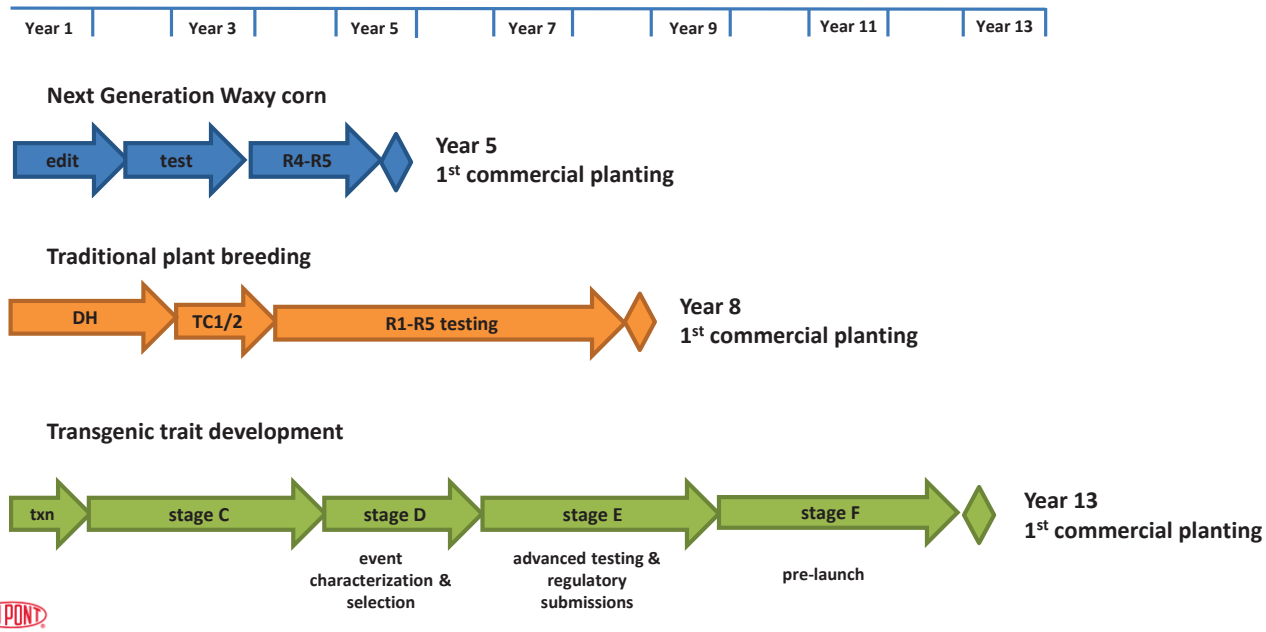




Main panicle showing number of primary branches (Pb)




*CKX2 null variants (T0) show an increase in spikelet and seed per plant*

# Edited variety development can be very efficient

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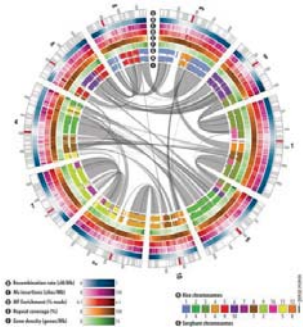
# Editing Context: Native Variation in Crop Genomes

**Tomato fruit morphology**


- SUN genomic transposition
- ↑ gene expression
- Elongated fruit

Xiao et al, Science 2008, 319:15277

**Maize Genome Diversity**



B73 vs Mo17:

- 100's of CNV
- 1,000's PAV
- In/Dels & SNPs ↑

Schnable et al, Science 2009, 326:1112

**SNP rates**

- ~7 SNPs per billion bp per generation
- 1 Ha of soy has >1.8 million novel SNPs

Parrot et al, Nat Biot , 2011, 30: 825

**Naturally occurring genome editing processes are fundamental to crop development and the successful development of high performing elite genetics.**

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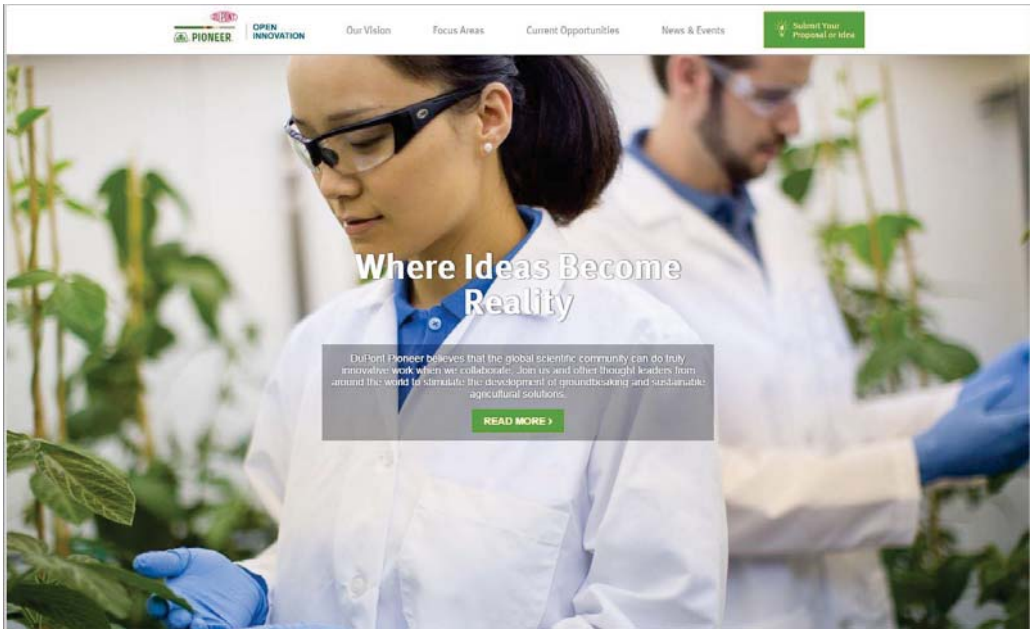
## Enabling a wide array of applications

Developing solutions to the toughest agricultural challenges



## Open Innovation



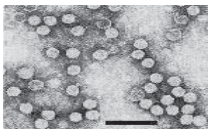

openinnovation.pioneer.com

# CIMMYT & DuPont Pioneer Public-Private Partnership

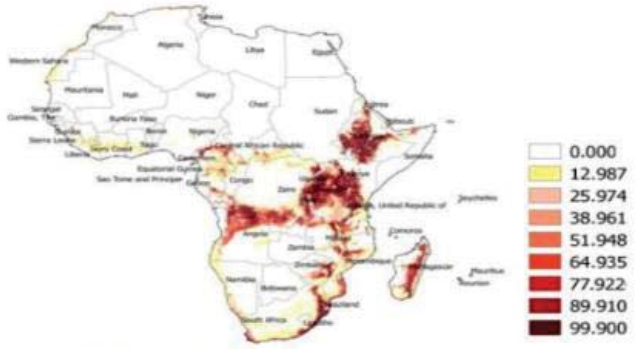
**Sugarcane Mosaic Virus (SCMV)**

**Maize chlorotic mottle viruses (MCMV)**

new to Africa, more severe then SCMV



**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

## 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*

- 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

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## COI Disclosure Information

### Neal Gutterson

**I have the following financial relationships to disclose.**

**Leadership position/advisory role for:** DuPont Pioneer and CIMMYT Board of Trustees

**Patents and royalties from:** Monsanto and Mendel Biotechnology