54th Annual
Illinois Corn Breeders' School
March 5-6, 2018

at the I Hotel
1900 South First Street
Champaign, IL 61820

I ILLINOIS
Crop Sciences
COLLEGE OF AGRICULTURAL, CONSUMER & ENVIRONMENTAL SCIENCES

54th Annual Illinois Corn Breeders' School Program

March 5—6, 2018

At the I Hotel, 1900 South First Street, Champaign, Illinois

Monday, March 5, 2018

7:15-8:15   Registration and Continental Breakfast

Morning Session: Breeding Processes
Chair: Tony Studer

8:15—8:30   **Martin Bohn, University of Illinois**
                           Welcome and Introductory Remarks

10:00-10:30   **BREAK**

12:00-1:15   Lunch

Afternoon Session:
Chair: Steve Moose

2:45-3:15   Break

54th Annual Illinois Corn Breeders' School Program

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At the I Hotel, 1900 South First Street, Champaign, Illinois

Monday Evening:
Chair: Steve Moose

5:30-6:30   **Social Hour**

6:30-7:30   **Dinner in the Chancellor Room**

Tuesday, March 6, 2018

7:30-8:00   **Registration and Continental Breakfast**

Morning Session: Optimizing Corn Performance
Chair: Martin Bohn

9:30-10:00   **Break**

11:30-11:35 **Closing Remarks**

How to make maize seeds that look "not like dad": new insights in double fertilization and prospects for novel breeding tools.

Thomas Widiez, University of Lyon, France

Abstract:

Mixing male and female genetic information during sexual reproduction is considered as key to the evolutionary success of higher eukaryotes and is the basis of plant breeding. Sexual reproduction in flowering plants involves double fertilization, characterized by two separate fusion events between the male and female gametes. A maize line first reported in 1959 deviates from this classic pattern. Crosses using pollen from this so-called haploid inducer line, trigger the development of the egg cell into a haploid embryo with only the maternal genome, a process known as *in vivo* gynogenesis. Derivatives of this maize haploid inducer line have become the preferred tool of numerous maize breeding companies, because it can produce perfectly homozygous plants in only 2 generations instead of 5 to 8 in classical breeding schemes.

Fine mapping restricted a major QTL responsible for gynogenesis in maize to a zone containing a single gene coding for a patatin-like phospholipase A, which was named *NOT LIKE DAD* (*NLD*) because haploid embryos do not have paternal contribution. In all surveyed haploid inducer lines *NLD* carries a 4 pb insertion leading to a predicted truncated protein. This frameshift mutation is responsible for haploid induction as complementation with wildtype *NLD* abolishes the haploid induction capacity. Translational NLD::citrine fusion protein localizes to the sperm cell plasma membrane. In *Arabidopsis* roots, the truncated protein is no longer localized to the plasma membrane, contrary to the wildtype NLD protein. In conclusion, an intact sperm-specific phospholipase is required for successful sexual reproduction and its targeted disruption may allow establishing powerful haploid breeding tools in numerous crops.

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How to Make Maize Seeds that Look "*NOT LIKE DAD*":
the genetics behind maize haploid inducers

Thomas WIDIEZ

thomas.widiez@ens-lyon.fr

www.ens-lyon.fr/RDP

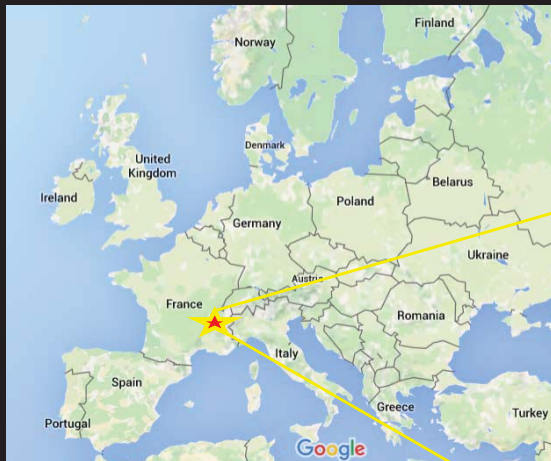The Illinois Corn Breeders' School, 2018 March

INRA SCIENCE & IMPACT · ENS DE LYON · Lyon 1 · cnrs

Where are we?

FRANCE, LYON (gastronomic capital)

Laboratory "**R**eproduction & **D**éveloppement des **P**lantes "

www.ens-lyon.fr/RDP

Who are we?

The "Seed development" team

Gwyneth INGRAM
Audrey CREFF
Sophy CHAMOT

Douglas PYOTT	Post-doc
Jeanne LOU	PhD Student
Angelo GAITI	Master Student

Peter ROGOWSKY
Nathalie DEPEGE-FARGEIX
Thomas WIDIEZ

Anne-Charlotte MARSOLLIER	Post-doc
Laurine GILLES	PhD student
Nicolas DOLL	PhD student

Maize transformation platform:

Ghislaine GENDROT
Christelle RICHARD
Edwige DELAHAYE

Main projects within "Seed dev"

Signaling/communication between seed compartments

Maize

Mechanism involved in haploid embryo induction
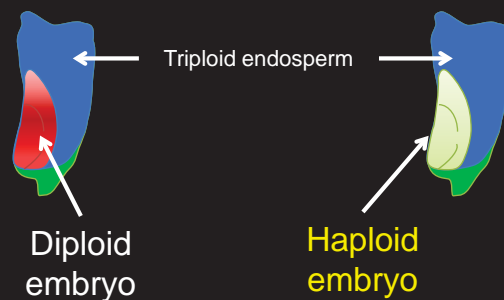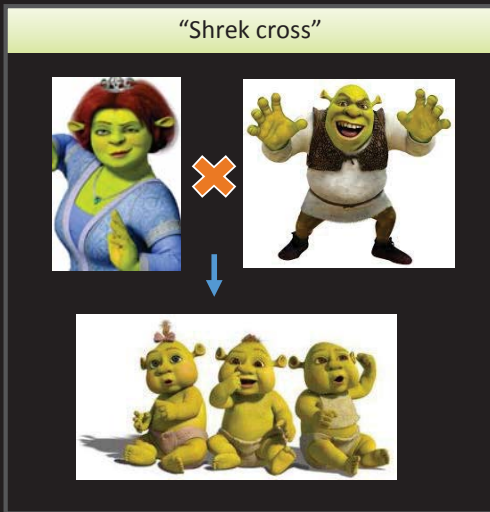
Triploid endosperm

Diploid embryo

Haploid embryo

How to make Kids that Look "*NOT LIKE DAD*"?

✓ **Context:**

How to make Kids that Look "*NOT LIKE DAD*"?

✓ **Context:**

How to make Kids that Look "*NOT LIKE DAD*"?

✓ **Context:**

| "Shrek cross" | Fiona X "special male inducer" |

How to Make Maize Seeds that Look "*NOT LIKE DAD*"?

✓ **Context:**

| "Classical" cross | Cross with inducer as male |

F1

Diploid (2n)

How to Make Maize Seeds that Look "*NOT LIKE DAD*"?

✓ **Context:**

"Classical" cross

F1

Diploid (2n)

Cross with inducer as male

Inducer line

F1

Haploid (n)
(maternal genome)

How to Make Maize Seeds that Look "*NOT LIKE DAD*"?

✓ **Context:**

"Classical" cross

F1

Diploid (2n)

Cross with inducer as male

Inducer line

= PK6

F1
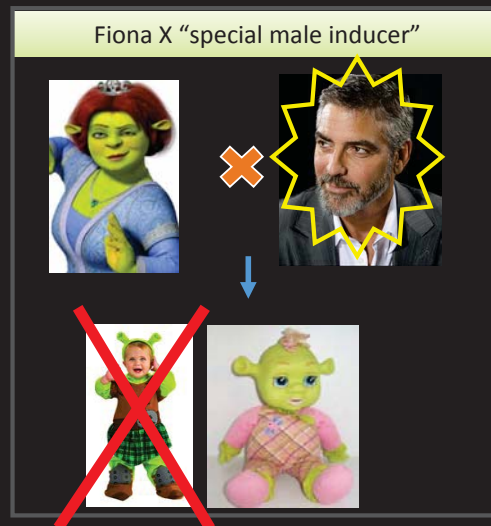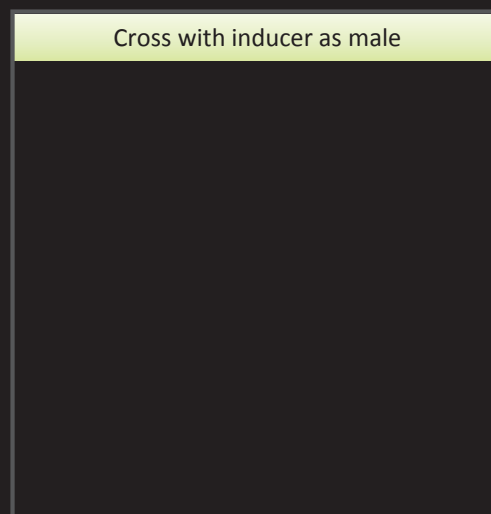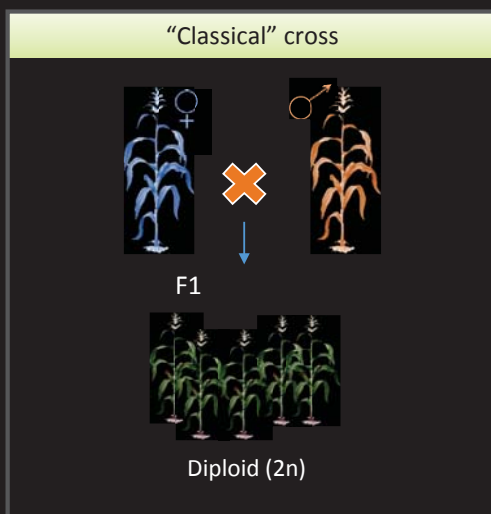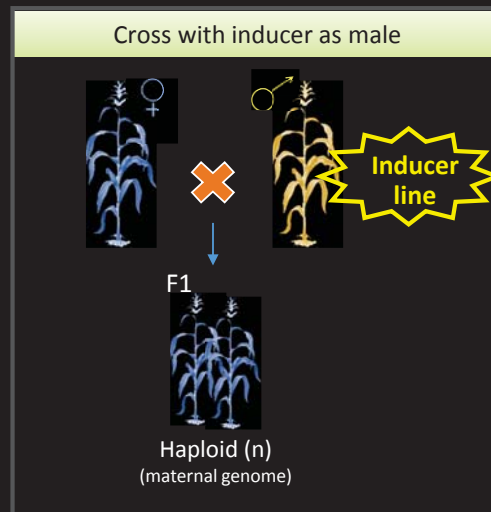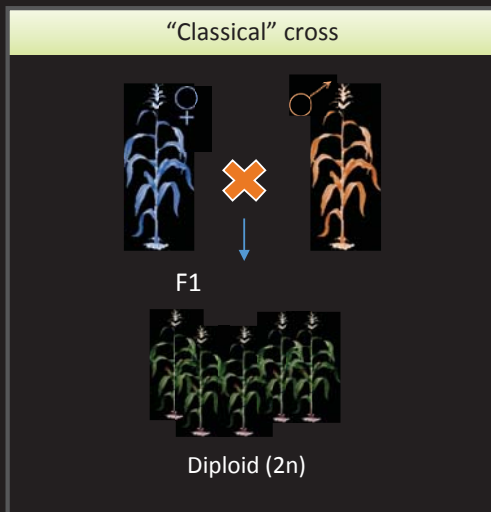
Haploid (n)
(maternal genome)
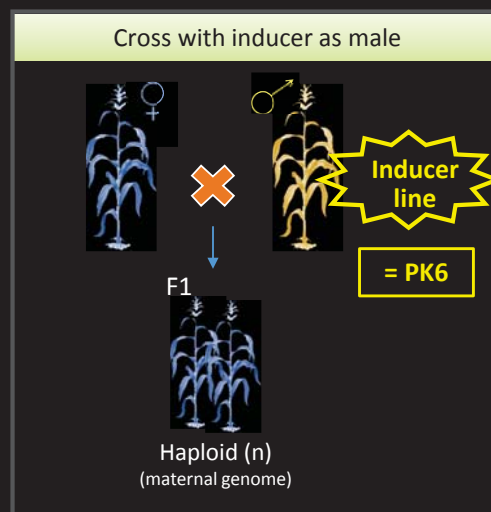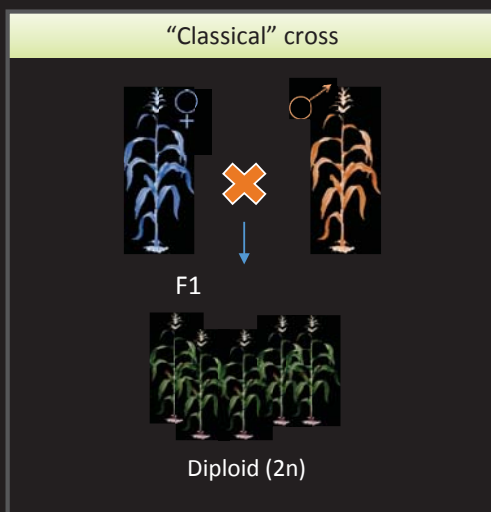
in vivo gynogenesis:
Production of maternal haploids via a male inducer

How to Make Maize Seeds that Look "*NOT LIKE DAD*"?

✓ **Context:**

"Classical" cross	Cross with inducer as male

F1 — Diploid (2n)

F1 — Diploid (2n) ~90% — Haploid (n) (maternal genome) ~10%
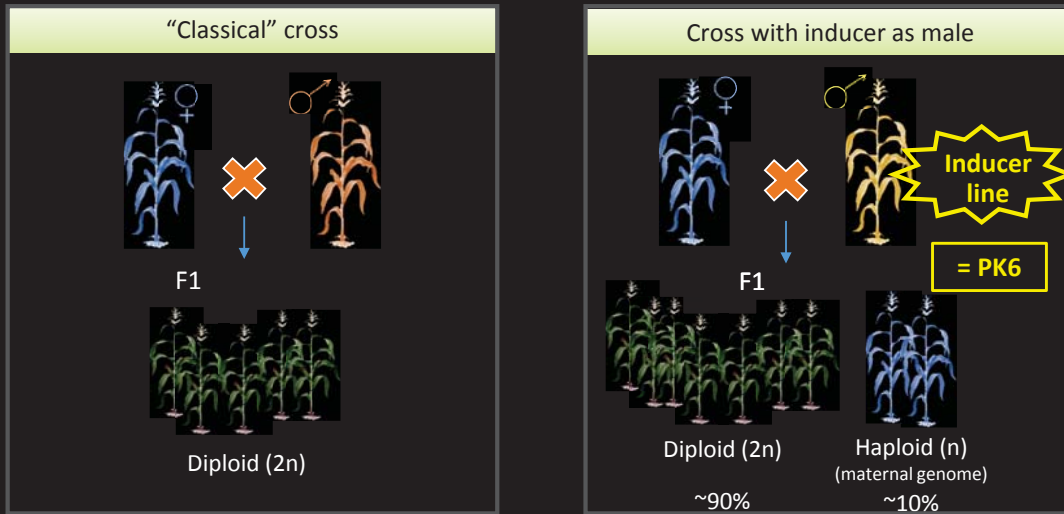
Inducer line = PK6

✓ **History of maize inducer lines :**

Stock6

KEMS — X — WS14 — X — FIGH1 ... MHI — CAU1 — ZMS — UH400

RWS — **PK6**

First haploid inducer line

→ **1-3 %** of haploid induction rate (Coe, 1959)

"modern" inducer lines

✓ **~10%** of haploid induction rate

✓ But reproductive defects:
  - Aborted kernels
  - Segregation distortion

Lashermes and Beckert 1988, Chalyk, 1994, Shatskaya et al. 1994, Eder and Chalyk 2002, Röber et al. 2005, Barret et al. 2008, Kebede et al. 2011…

✓ Haploid plants: what's for?

Haploid
plants
(weak and sterile)

Colchicine
treatment

chromosomes
doubling

DH (2n)
Doubled Haploid plants

➔ Pure Homozygote line

(=inbred line)

Gilles *et al.*, Current Biology 2017

➔ **Greatly accelerates plant breeding efficiency**

Conventional inbred line production

50%   Genetic purity   99,2%

6-7 generations

DH line production

50%   Genetic purity   100 %

2 generations
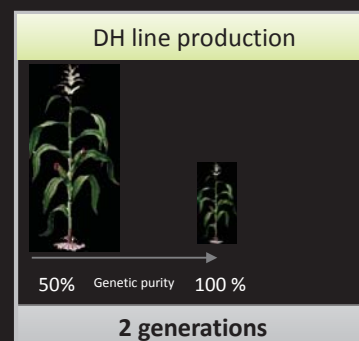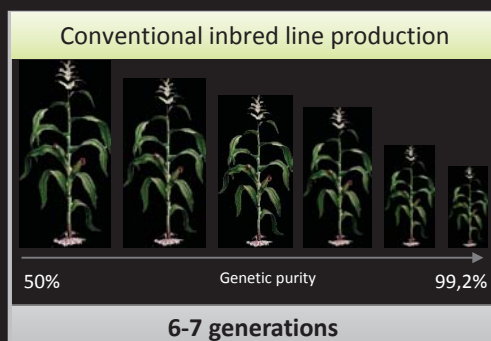
✓ Haploid plants: what's for?
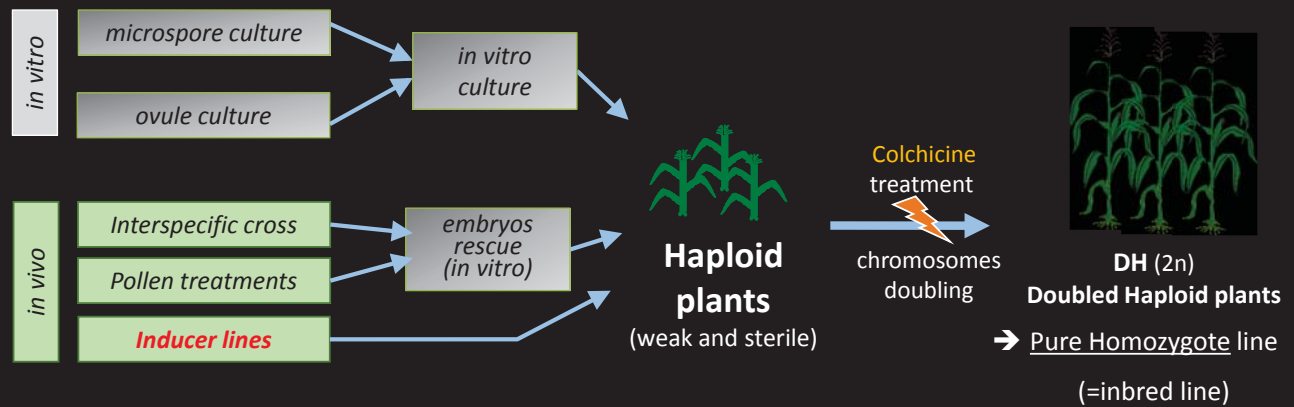
Gilles *et al.*, Current Biology 2017

The reasons to study *in vivo* haploid induction:

➢ Useful tool in maize breeding programs

➢ Tool to understand plant reproduction (Double fertilization process)

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❖ **"Normal" double fertilization:**

The reasons to study *in vivo* haploid induction:

➢ Useful tool in maize breeding programs

➢ Tool to understand plant reproduction (Double fertilization process)

❖ **"Normal" double fertilization:**

❖ *In vivo* **haploid induction (gynogenesis):**

✓ **Objectives:**

Pollen from inducer line
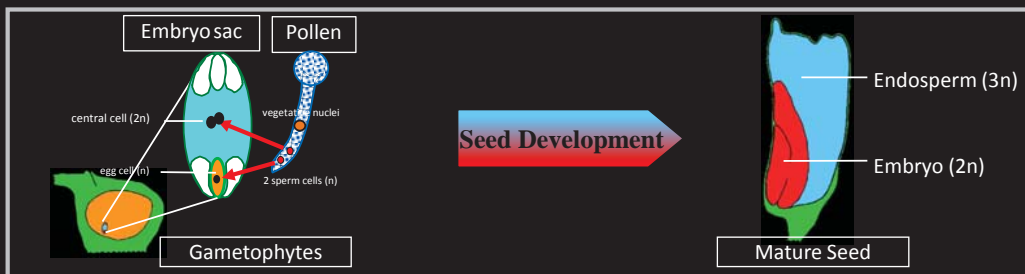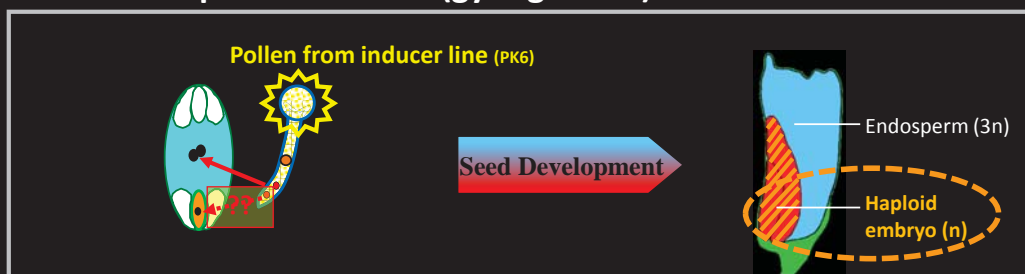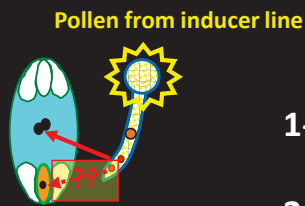
1- Identify the **genetics behind maize haploid inducers**

2- Understand **molecular/cellular mechanisms**

3- Development of **breeding tools**

Fine based mapping of "*ggi1*" QTL

Barret *et al.*, 2008 ➜ *ggi1* (*gynogenesis inducer 1*) the main QTL for haploid induction

Chromosome 1   5,3 Mpb   Barret et al., 2008

Fine based mapping of "*ggi1*" QTL

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Chromosome 1
5,3 Mpb
Barret et al., 2008

Fine mapping
2 Mpb

Creation of new recombinants plants:
-> Genotyping ~**11 000** plants (~15 markers)

-> Identification of **531** with new recombinants

140 kpb

Reference maize sequence = B73 = WT

Phospholipase A

Fine based mapping of "*ggi1*" QTL

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Reference maize sequence = B73 = WT

Phospholipase A

PK6 (inducer line) sequence is different...
? ?

➜ PK6 gDNA region identified thanks to Pac-Bio sequencing of BACs

Gilles *et al.*, EMBO J. 2017

Fine based mapping of *"ggi1"* QTL

B73
=
WT

PK6
=
inducer

TE TE TE TE

4bp insertion

- Deletion (➔ Absence 2 genes)
- Insertion of (lots of) transposable elements (TE)
- Mutation on the unique gene found

TE TE TE TE TE TE TE TE TE TE TE

Fine based mapping of *"ggi1"* QTL

o Reference maize sequence (B73) = WT

o PK6 inducer line

TE TE TE

4bp insertion

TE TE TE TE TE TE TE TE TE TE TE TE

o EM1201

Ears corresponding
to the test cross

WT

PK6

EM1201

0   2   4   6   8   10
% haploid induction

Fine based mapping of "*ggi1*" QTL
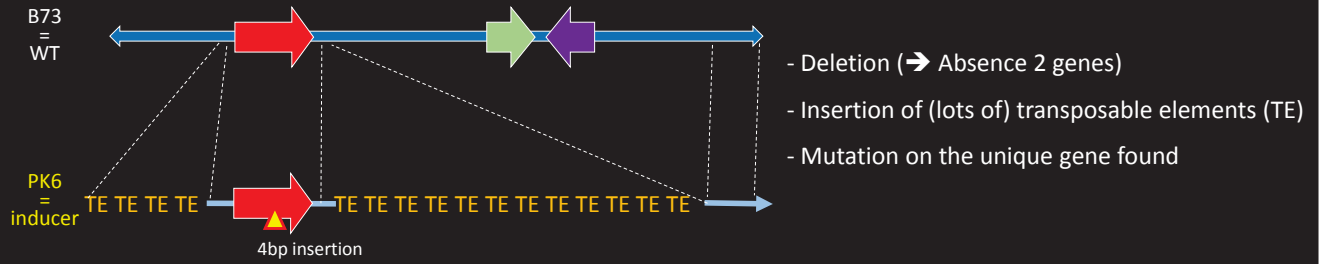
o Reference maize sequence (B73) = WT

o PK6 inducer line

TE TE TE ——■— TE TE TE TE TE TE TE TE TE TE TE ——→

4bp insertion

o EM1201

Ears corresponding to the test cross

WT

PK6
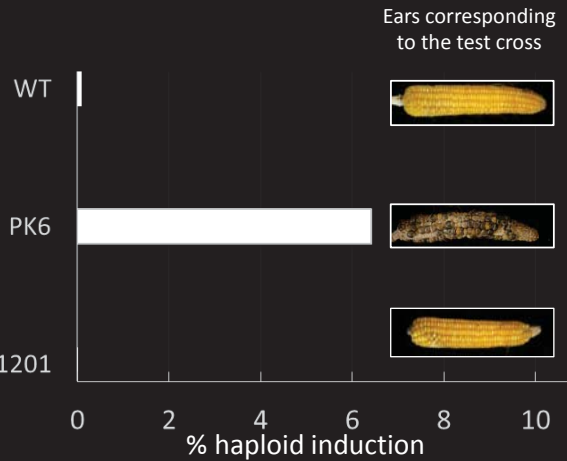
EM1201

0   2   4   6   8   10
% haploid induction

✓ Genotyping of maize diversity Panel

	Nb lines genotyped	4 bp insertion
Inducer lines	12	YES
Non inducer line	92	NO

✓ Functional validation of the candidate gene:

- By complementation

- By genome editing using CRISPR-Cas9

Unpublished results
Gilles *et al.*, EMBO J. 2017

Identification of "*NOT LIKE DAD*" gene = *NLD*

NLD

Identification of "*NOT LIKE DAD*" gene = *NLD*

NLD

Published online: February 22, 2017

Article

THE EMBO JOURNAL

Loss of pollen-specific phospholipase NOT LIKE DAD triggers gynogenesis in maize

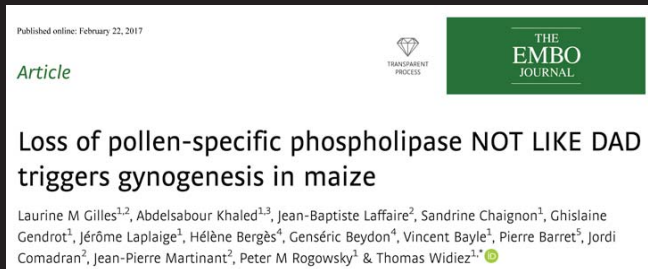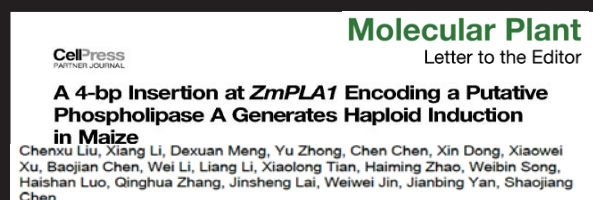Laurine M Gilles[1,2], Abdelsabour Khaled[1,3], Jean-Baptiste Laffaire[2], Sandrine Chaignon[1], Ghislaine Gendrot[1], Jérôme Laplaige[1], Hélène Bergès[4], Genséric Beydon[4], Vincent Bayle[1], Pierre Barret[5], Jordi Comadran[2], Jean-Pierre Martinant[2], Peter M Rogowsky[1] & Thomas Widiez[1,*]

Identification of "*NOT LIKE DAD*" gene = *NLD*

- Two other parallel independent studies identified the same gene:

LETTER **nature**   doi:10.1038/nature20827

MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction

Timothy Kelliher[1], Dakota Starr[1], Lee Richbourg[1], Satya Chintamanani[2], Brent Delzer[3], Michael L. Nuccio[1], Julie Green[1], Zhongying Chen[1], Jamie McCuiston[1], Wenling Wang[1], Tara Liebler[1], Paul Bullock[2] & Barry Martin[1]‡

Molecular Plant
Letter to the Editor

CellPress
PARTNER JOURNAL

A 4-bp Insertion at *ZmPLA1* Encoding a Putative Phospholipase A Generates Haploid Induction in Maize

Chenxu Liu, Xiang Li, Dexuan Meng, Yu Zhong, Chen Chen, Xin Dong, Xiaowei Xu, Baojian Chen, Wei Li, Liang Li, Xiaolong Tian, Haiming Zhao, Weibin Song, Haishan Luo, Qinghua Zhang, Jinsheng Lai, Weiwei Jin, Jianbing Yan, Shaojiang Chen
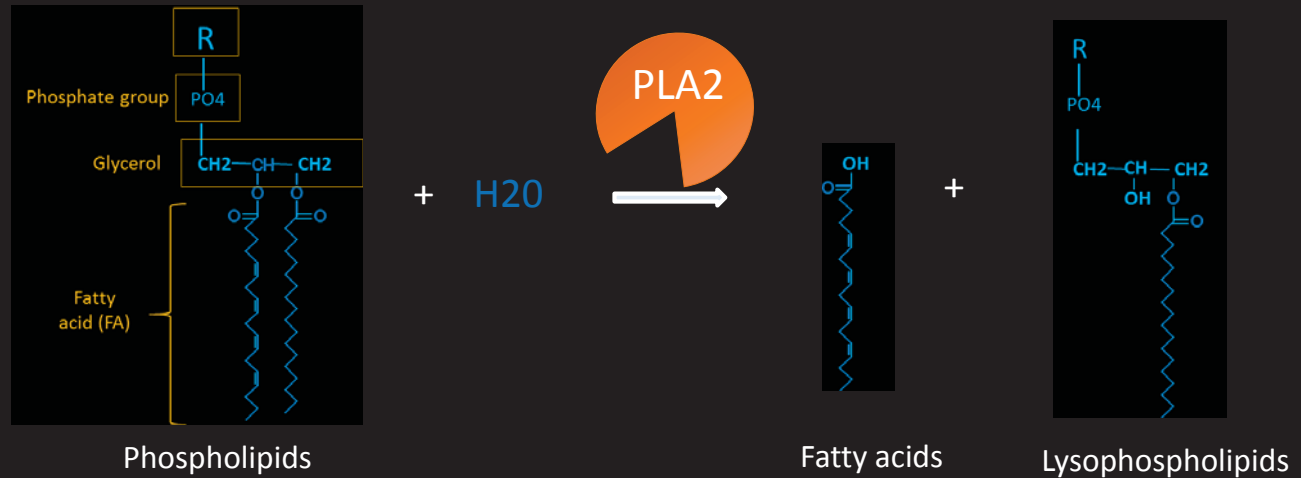
Identification of "*NOT LIKE DAD*" gene = *NLD*

NLD → *NLD* codes for a phospholipase A2: "PLA2"

- Enzyme that hydrolyzes phospholipids
- Broad roles: metabolism, stress, development, signaling…
  (Scherer et al. 2008)

Phospholipids + H20 → PLA2 → Fatty acids + Lysophospholipids

Gene | Protein

NLD-WT

NLD-WT
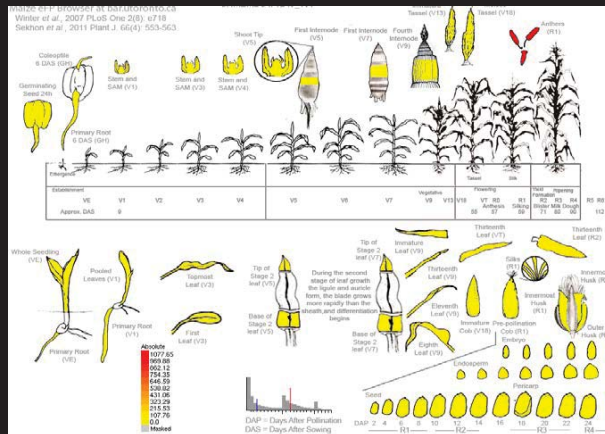1 — 428

NLD-PK6
380 — STOP

→ 4 pb insertion

→ Early STOP codon
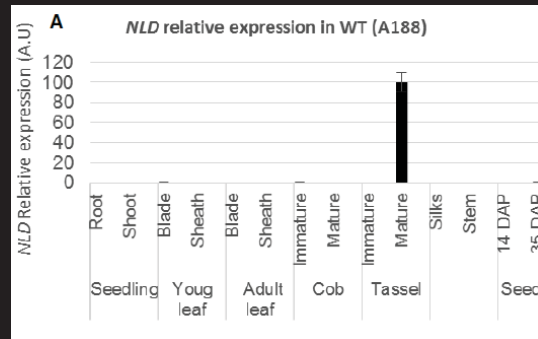(putative truncated protein)

Characterization of *NLD*

✓ ***NLD* is expressed in mature anther**

Q-RT PCR
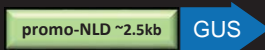

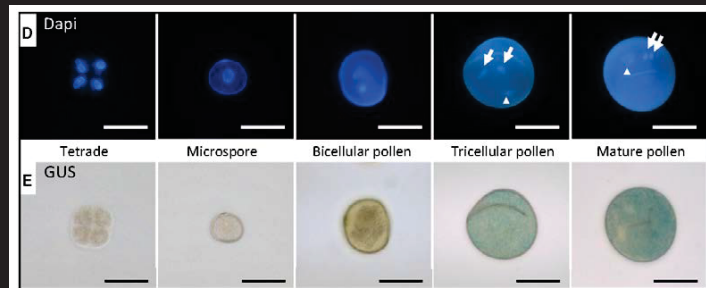Is *NLD* expression pattern sporophytic (Anthers) or gametophytic (pollen)?

Characterization of *NLD*

✓ ***NLD* promoter is ON in mature pollen and OFF 3 days after pollination**

DAPI staining

GUS staining

Characterization of *NLD*

✓ *NLD* promoter is ON in mature pollen and OFF 3 days after pollination

Gilles *et al.*, EMBO J. 2017
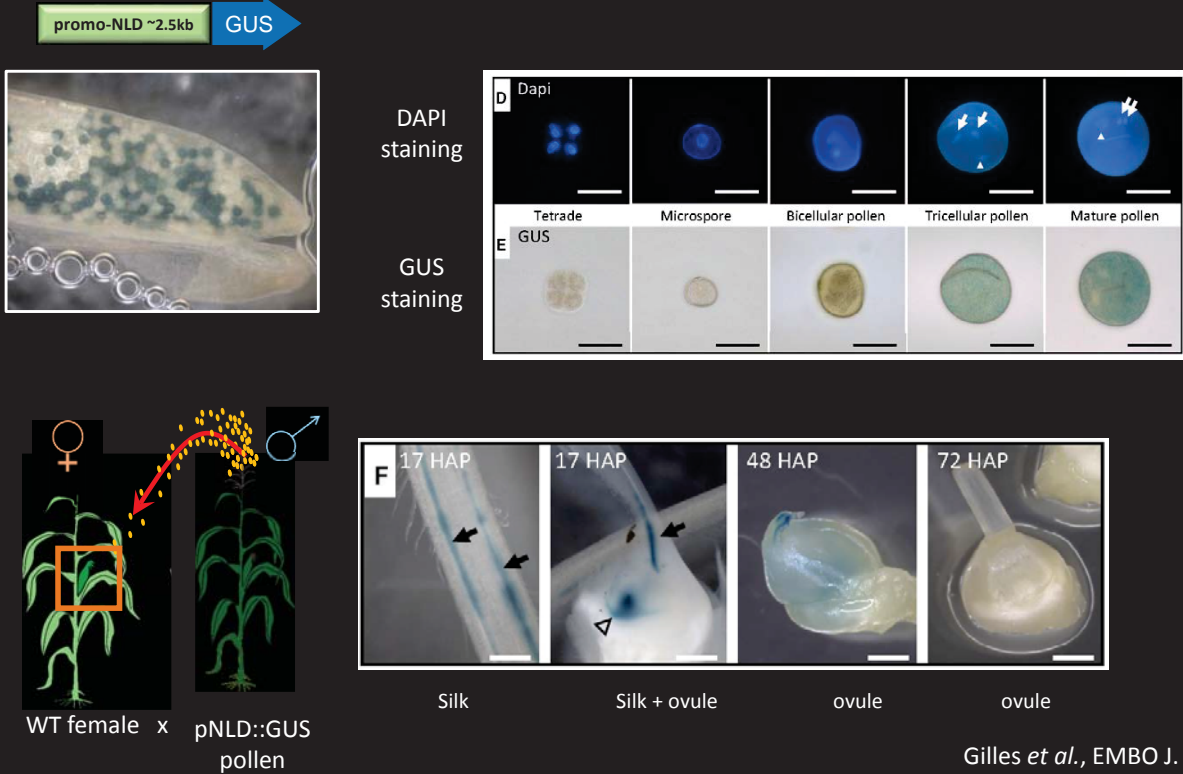
Characterization of *NLD*

✓ Subcellular localization of NLD protein (in Arabidopsis roots)
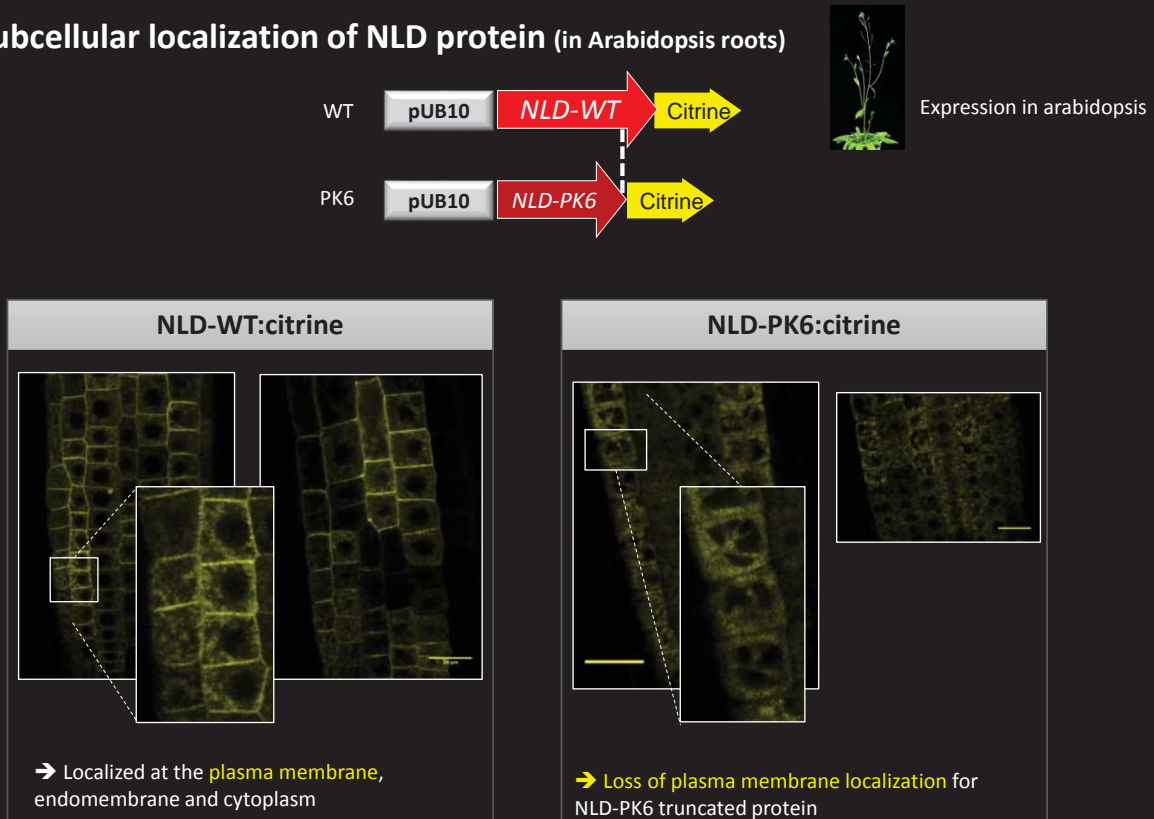
Expression in arabidopsis

NLD-WT:citrine

➜ Localized at the plasma membrane, endomembrane and cytoplasm

NLD-PK6:citrine

➜ Loss of plasma membrane localization for NLD-PK6 truncated protein

Gilles *et al.*, EMBO J. 2017

Characterization of *NLD*

✓ **NLD protein localizes in the sperm cells in maize pollen**

Characterization of *NLD*

✓ **NLD protein localizes in the sperm cells in maize pollen**
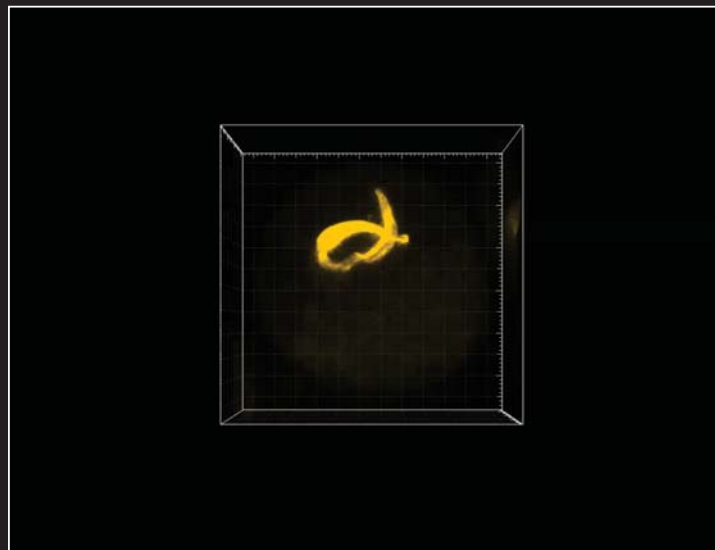
Characterization of *NLD*

✓ **NLD protein localizes in the sperm cells in maize pollen**
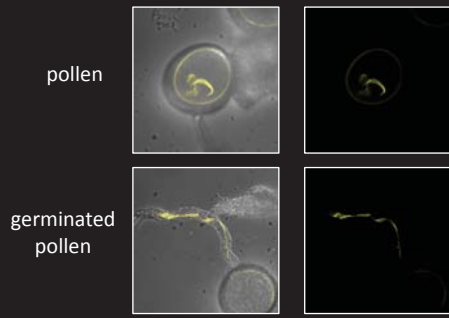
Confocal   Structured illumination microscopy

Gilles *et al.*, EMBO J. 2017

Characterization of *NLD*

✓ **NLD protein localizes in the sperm cells in maize pollen**

Confocal   Structured illumination microscopy

Gilles *et al.*, EMBO J. 2017

Characterization of *NLD*

Denis Falconet

✓ Lipidomics

- "Polar" lipids   2D—TLC
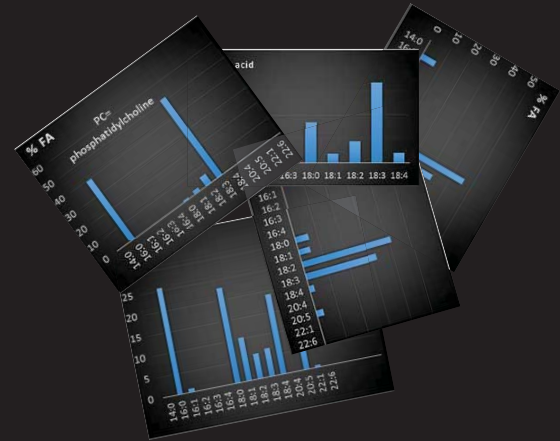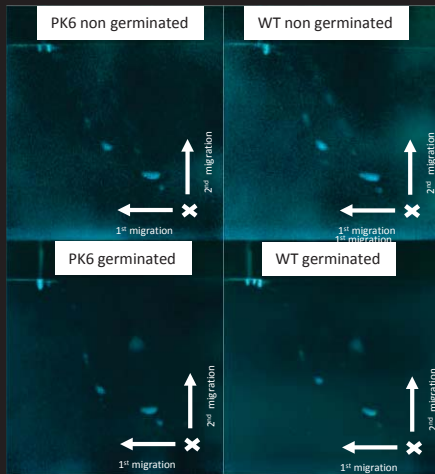
➜ **No major change in lipids composition**

Unpublished results

What's next....

✓ Lots of unanswered questions!!:

NOT LIKE DAD

NLD

Pollen from inducer line

haploid induction (gynogenesis)

??????

Phospholipids of sperm cells
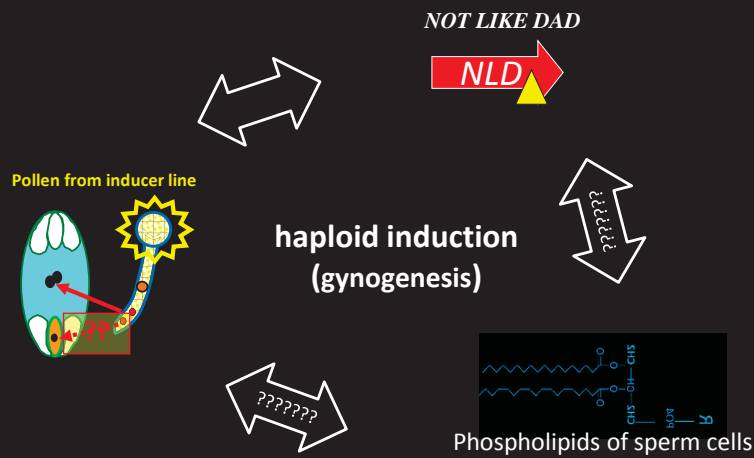
Thank to:

SeedDev Team

Laurine GILLES

- Abdelsabour Khaled
- Sandrine Chaignon
- Ghislaine Gendrot
- Jerome Laplaigne
- Vincent Bayle
- Peter Rogowsky

Limagrain

- Jean-pierre Martinant

- Jean Baptiste Laffaire

- Jordi Comadran

INRA
SCIENCE & IMPACT

GDEC
(Clermont Ferrand)
Pierre Barret

CNRGC (Toulouse)
Hélène Bergès
Genséric Beydon

cea

Denis Falconet

INRA SCIENCE & IMPACT   ENS DE LYON   Lyon 1   Cnrs   Cifre

Use of Landrace Maize in Plant Breeding at CIMMYT

Dr. Martha Willcox, Maize Landrace Improvement Coordinator
International Maize and Wheat Improvement Center (CIMMYT)

Mexico, as the center of origin of maize, has contributed important genetic resources to germplasm banks, but native maize landraces, still sown as the majority of hectares of maize grown in Mexico also play a role as a reserve of genetic diversity still evolving through local farmer selection. CIMMYT has sought to use genetic resources, both *ex situ* and *in situ,* in improving lives of farmers and in providing information to breeders worldwide. As part of this effort the Seeds of Discovery Project, conducted both genotypic and phenotypic characterization on a large collection (over 4000) of maize landraces from the Breeders Core Collection of the Maize Germplasm Bank. This work has contributed information on allelic diversity but has also identified specific germplasm bank accessions that have used directly in breeding efforts for small farmers. Further work to improve landraces of marginalized communities in Oaxaca has focused on participatory breeding and agronomic improvements in areas where landrace maize is still the best option for farmers. This work has included farmer training, local diversity and selection studies, and cost benefit analysis of agronomic treatments. . During the course of this project, we have connected excess production of landrace maize by smallholder subsistence farmers of the project with culinary markets, opening a market that did not before exist. These market connections have increased prices of landrace maize to farmers and the rapid expansion of this market has created questions as to how to maintain the benefit of this market to smallholder subsistence farmers.

ILLINOIS

Crop Sciences

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& ENVIRONMENTAL SCIENCES

Use of Landrace Maize in Plant Breeding at CIMMMYT

Martha Willcox, CIMMYT

Fernando Castillo Gonzalez, Colegio de Posgraduados

Flavio Aragon Cuevas, INIFAP

Humberto Castro Garcia, Universidad de Chapingo

Leodegario Osorio Alcala, INIFAP

CIMMYT

Why Landraces?

- 60%+ of the maize hectares planted in Mexico are sown to Native Maize (traditional landraces).

- Mexico is the center of origin of maize and conservation of native maize *in situ* is an international public benefit.

- **There are agroecological niches not served by hybrid programs.**

- Native Mexican Maizes are integral to the culture and culinary traditions of Mexico.

- **Selling price of grain of Natives Maize is often higher than hybrid grain price**.

Percentage of Area Sown to Non-Improved Maize

%
- 100 - 85
- 84 - 73
- 74 - 45
- 45 - 25
- 25 - 10
- 10 - 0

N W E S
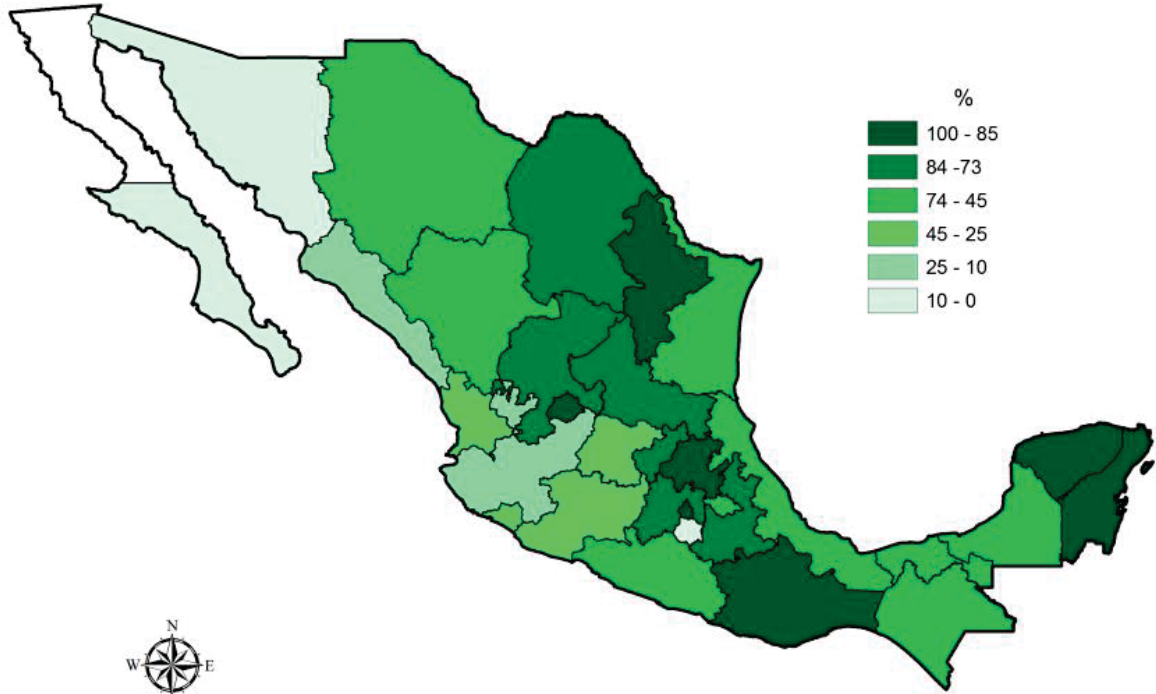
0   250   500   1,000 km

Fuente: SIAP, 2012. Estadística de uso tecnológico y de servicios en la superficie agrícola.
Disponible en: http://www.siap.gob.mx/tecnificacion/

Diversity of Culinary Uses

Seeds of Discovery

Genome Wide Association Study (GWAS) in Maize

- Breeder's Core Collection from the CIMMYT Maize Germplasm Bank (4471 accessions).
- One plant per accession crossed with a CIMMYT hybrid to make a series of modified topcrosses.
  - The same plant was sampled for DNA extraction/GBS
- Accessions were crossed to hybrids of their same environmental adaptation (where possible).

Tropical Accessions X Tropical Hybrid

      Subtropical Accessions X Subtropical Hybrids

           Highland Accessions X Highland Hybrid

CIMMYT

Trial Sites in Mexico: GWAS

Drought; Heat
Heat & Drought — Obregon, Sonora / Torreon, Coahila

Stalk Rot; Quality

Low Nitrogen, Ear Rot, Quality, Turcicum

Cercospora

San Pedro Lagunillas, Nay
Celaya, GTO
Cortazar, GTO
Numaran, Mi
Tarimbaro Michoacan
moloya, Mex
El Batán, Mex
Agua Fría, Pueb
Tlaltizapán, Mor
guala, GRO
Ocotito, Guerrero

Stalk Rot

Low Nitrogen

Drought

Guadalupe-Victoria, Chiapas

Tar Spot Complex

CIMMYT

Yield and Agronomic Data Taken : All Locations

- Yield (field weight, grain and cob weight, moisture, number of ears)
- Plant Height and Ear Height
- Male and Female Flowering (50% of row)
- Stalk and Root Lodging

CIMMYT.

Phenotypic Data   Genotypic Data

Lines
Top 10%

Participatory Breeding

Improved Native Maizes for Subsistence Farmers

Selected S1 or S2 Lines for Public Breeders

Searchable Genetic Database for Worldwide Use of Maize Germplasm on Allelic Level
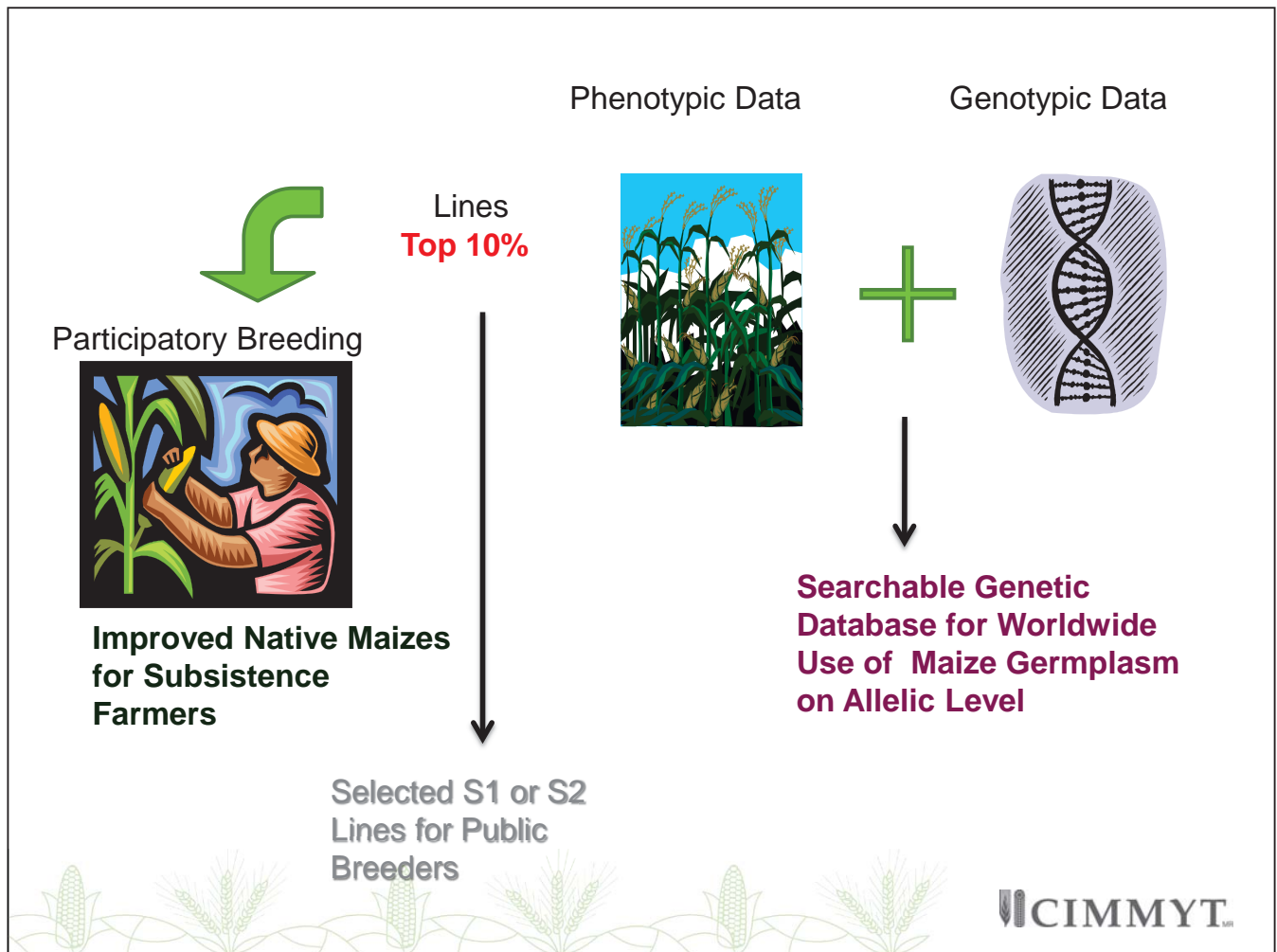
CIMMYT.

Tar Spot Complex

The symptom caused by *Monographella maydis*

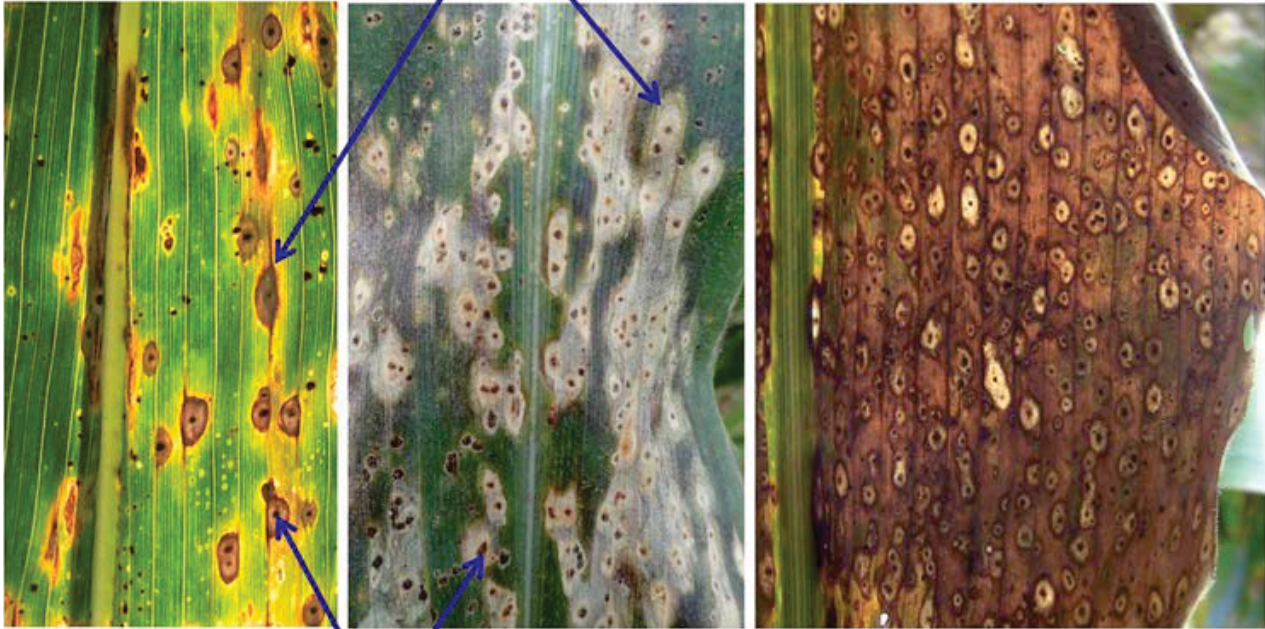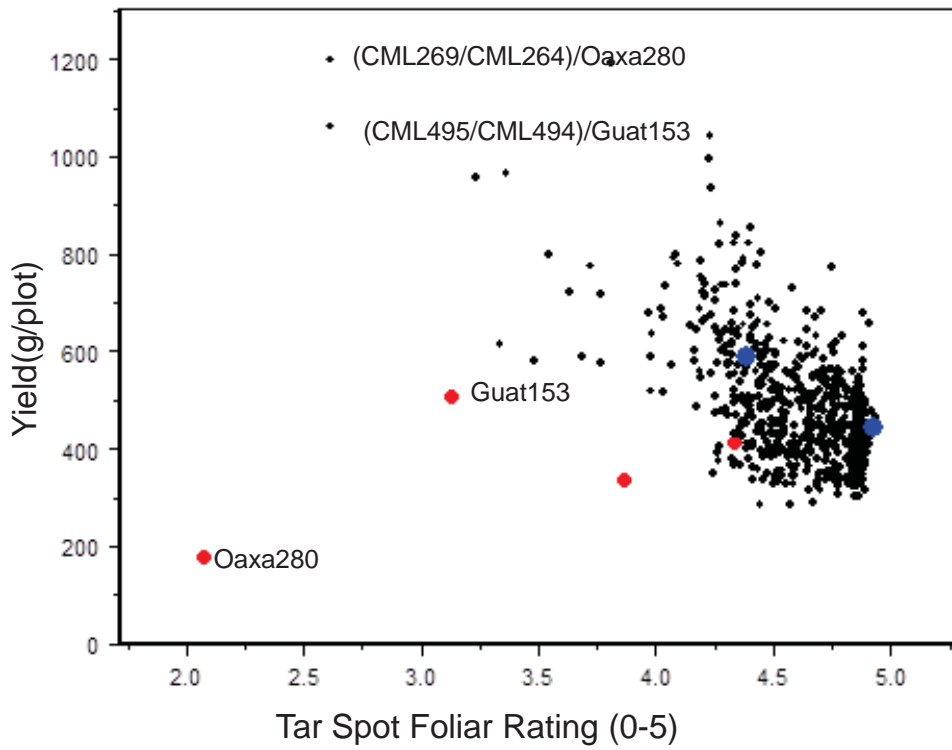The symptom caused by *Phyllachora maydis*

CIMMYT

Tar Spot Complex

Photo: Rosemary Shrestha
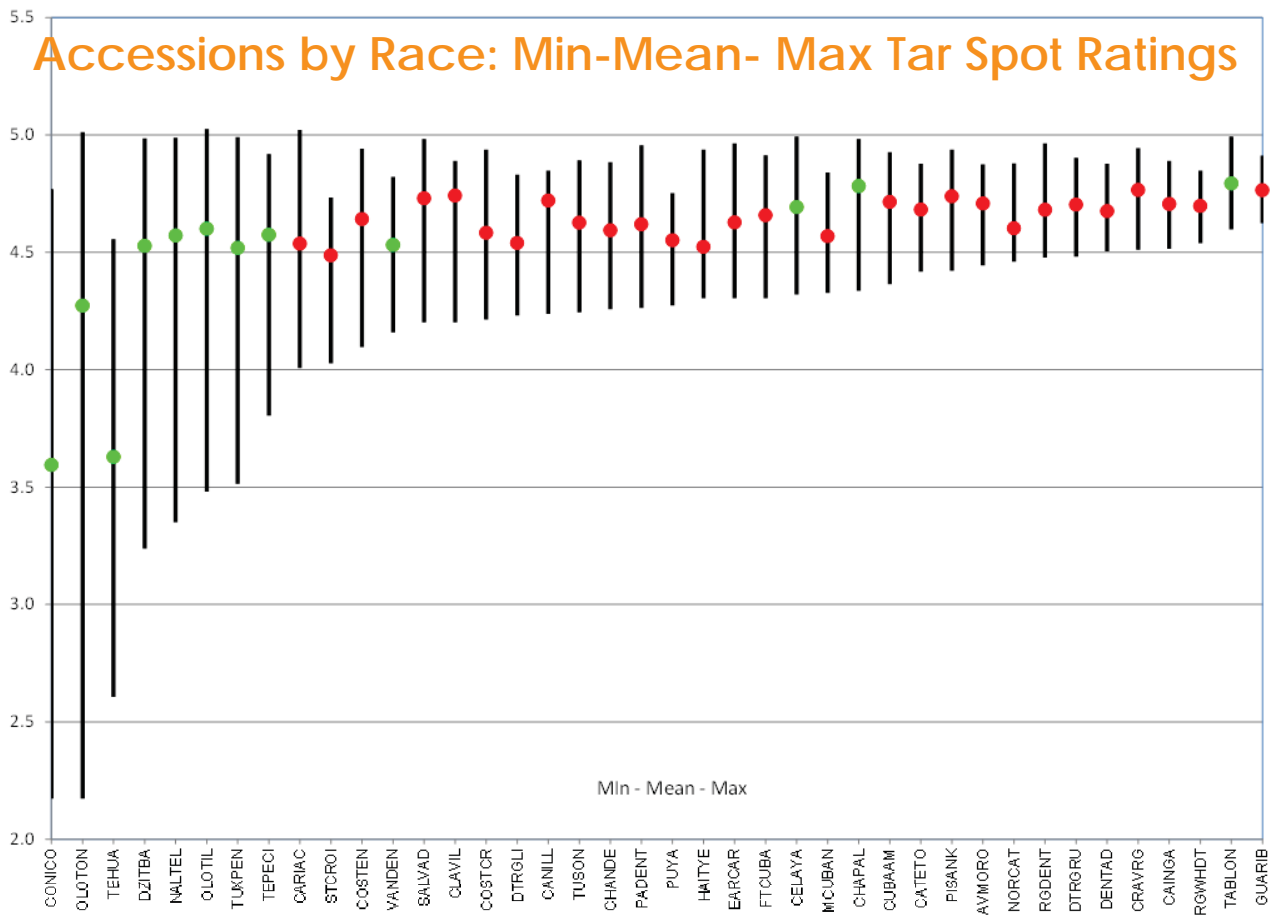
Relationship between Tar Spot rating and Yield (2nd foliar rating: scale 0-5; average of 6 plantas)
🔴=Accessions; ⚫️ =Topcrosses; 🔵 = Commercial Checks

Accessions by Race: Min-Mean- Max Tar Spot Ratings

Min - Mean - Max

Selection Under Tar Spot Complex

- 2011B and 2012B Evaluation of TC under Seeds of Discovery
- 2013B Crosses between best Accessions and 4 CMLs.
- 2014A Backcross to Accessions Oaxa280 and Guat153
- 2014B Produce BC1S1s
- 2015B BC1S1 evaluated in Chiapas and Oaxaca (bulks 3 locations)
- 2016A Increase farmer voted BC1
- **2016B**
  - ➢ **BC1 Oaxa280/CML324//Oaxa280 in farmer fields in Santiago Yaitepec, Oaxaca**

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Tar Spot Disease Complex

Oaxa280//Oaxa280/CML 324

- **Accession identified in field trials.**
- **Selected by farmers along with local farmer samples**

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Evaluation under Low nitrogen

CIMMYT

2014 B: S1 Trial

2016B: Highland Tester Topcross Trial

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S1 line from Germplasm Bank Accession Dura159 in Conditions of Low Nitrogen Fertility

CIMMYT

Participatory Plant Breeding of Native Maizes in Marginalized areas of Oaxaca

Collaborators

M.C. Flavio Aragón
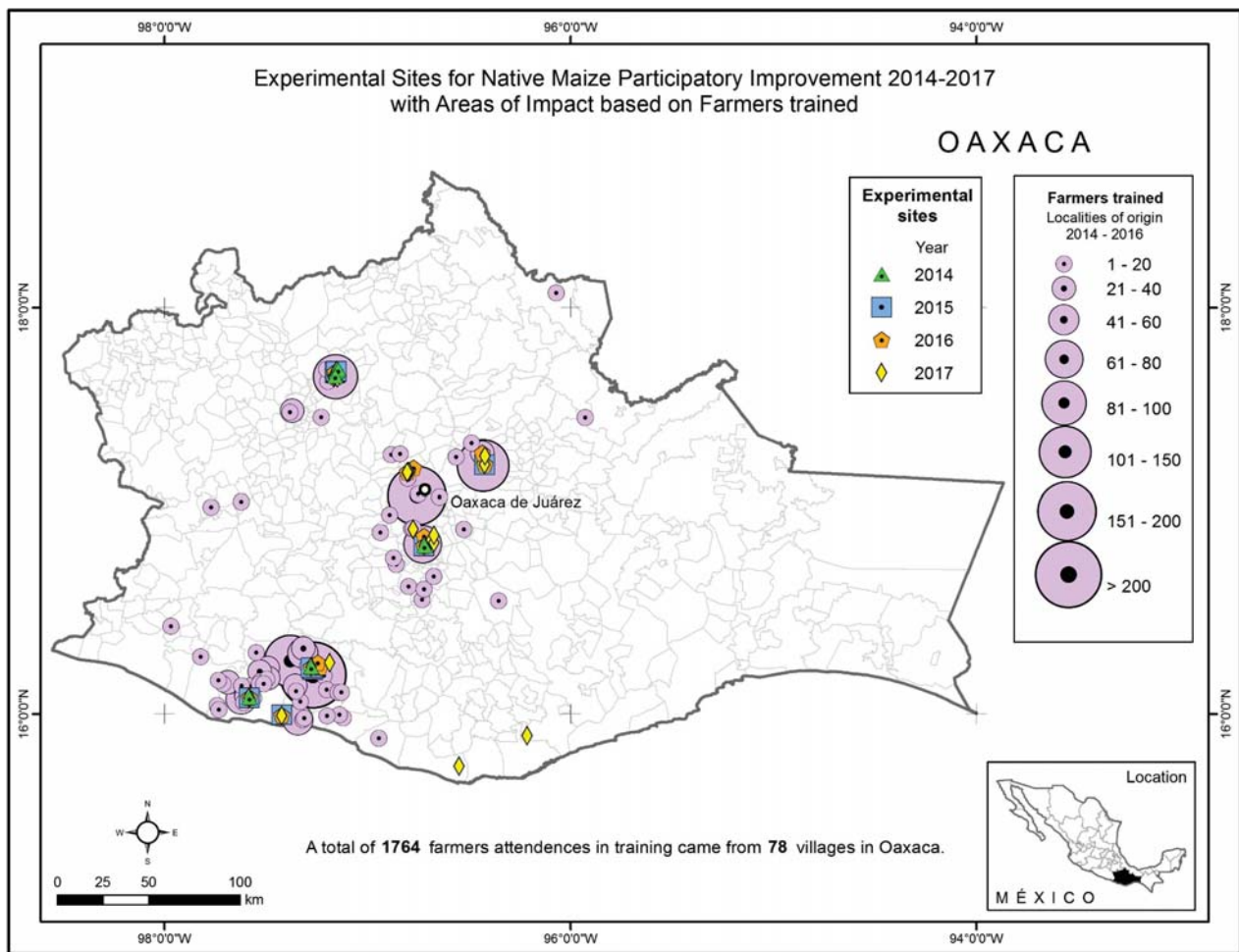INIFAP

Ing. Humberto Castro
UACH

M.C. Leodegario Osorio
INIFAP
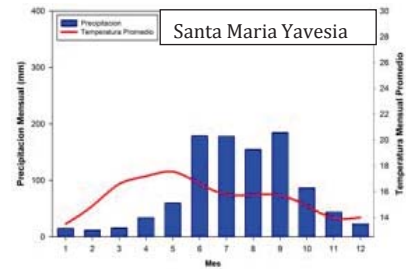
CIMMYT

Community Based
Participatory
Plant Improvement

CIMMYT

Experimental Sites for Native Maize Participatory Improvement 2014-2017
with Areas of Impact based on Farmers trained

OAXACA

Experimental sites
Year
▲ 2014
■ 2015
⬠ 2016
◆ 2017

Farmers trained
Localities of origin
2014 - 2016
1 - 20
21 - 40
41 - 60
61 - 80
81 - 100
101 - 150
151 - 200
> 200

Oaxaca de Juárez

A total of 1764 farmers attendances in training came from 78 villages in Oaxaca.

0 25 50 100 km

Location

MÉXICO

Biodiversity, Participatory Plant Breeding and Agronomic Trials:

Community and Municipality	Altitude (masl)	Incline	Environment	Maize Races	Indigenous Groups	Percentage of the population in extreme poverty
El Sanjon & Rio Grande, Villa Tututepec	60	Flat	Arid Tropics	Conejo, Tuxpeño, & Olotillo	Mixtecos & Mestizos	20.94
Santiago Yaitepec, Juquila	1900	Hillslopes	Transition Zone	Comiteco	Chatinos	46.04
Santa Ana Zegache, Ocotlan	1600	Flat	Subtropics	Bolita	Zapotecos de Valle	29.16
Nduayaco & Jazmín Morelos, Santiago Apoala	2200- 2100	5 – 25%	Semi-arid Highlands	Chalqueño & Cónico	Mixtecos	49.3
Santa María Yavesia, Santa Maria Yavesia	2000 - 2100	5 – 35%	Humid Highlands	Cónico x Bolita, Elotes Occidentales, Serrano	Zapotecos	13.13

Environments

San Antonio Nduayaco

Santiago Yaitepec

El Zanjón

Santa Ana Zegache

Santa Maria Yavesia
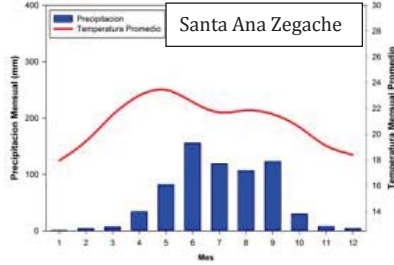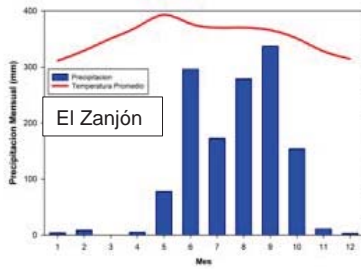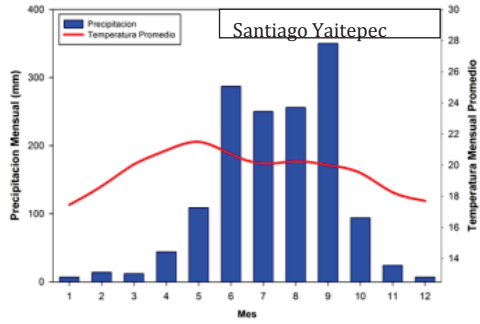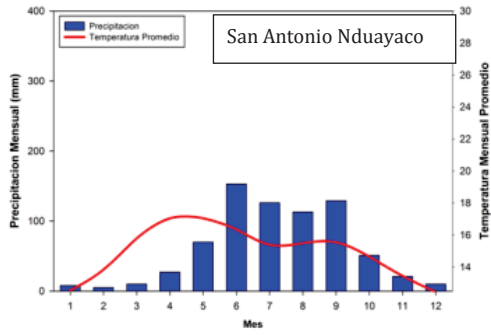
Genetic Improvement

Agronomic Improvement

Participatory Improvement of Native Maize in Marginalized Areas of Oaxaca: San Antonio Nduayaco
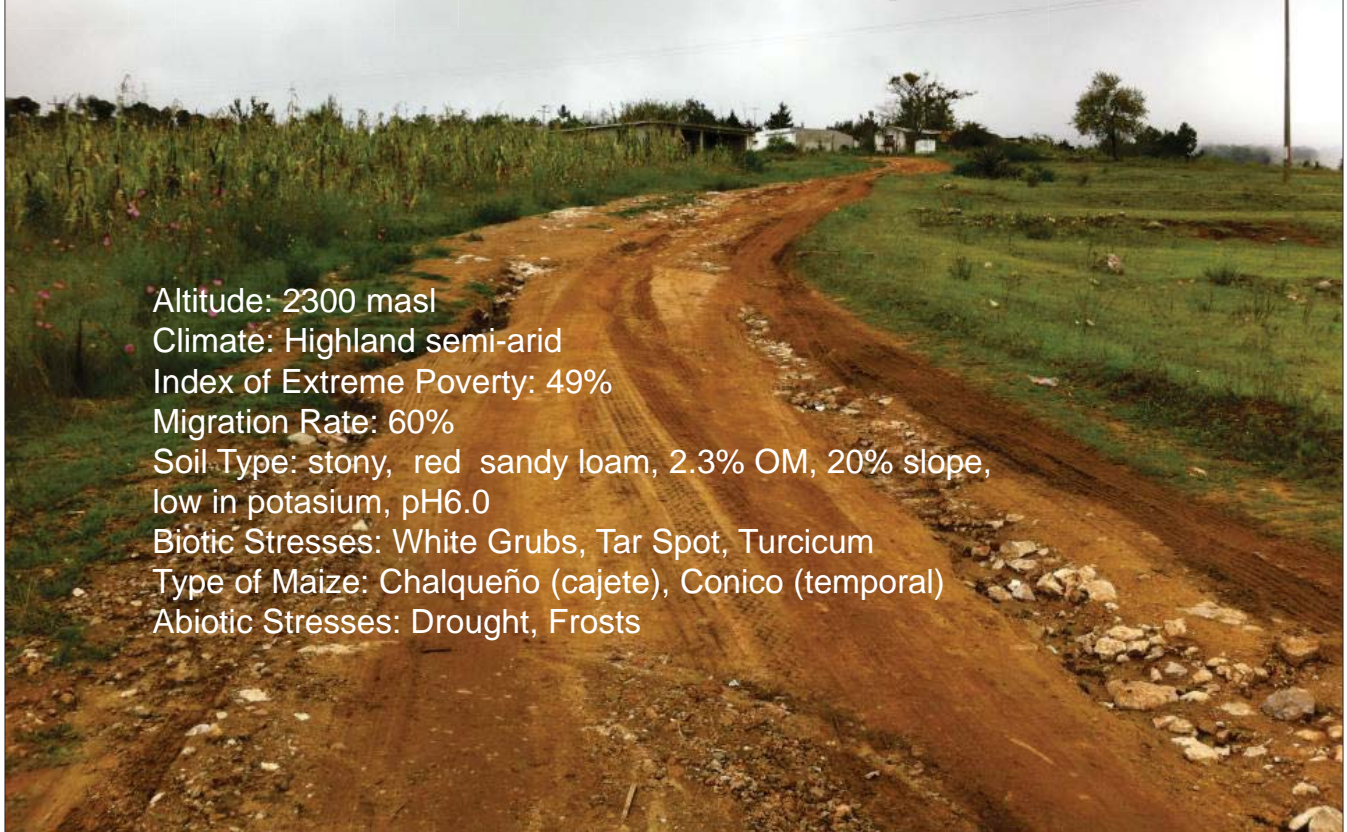
Altitude: 2300 masl
Climate: Highland semi-arid
Index of Extreme Poverty: 49%
Migration Rate: 60%
Soil Type: stony, red sandy loam, 2.3% OM, 20% slope, low in potassium, pH6.0
Biotic Stresses: White Grubs, Tar Spot, Turcicum
Type of Maize: Chalqueño (cajete), Conico (temporal)
Abiotic Stresses: Drought, Frosts

Planting Systems
San Antonio Nduayaco

Cajete- Deep planting 1-3 months before initiation of rains	Temporal – at initiation of rains

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San Antonio Nduayaco, Oaxaca 2300 masl Mean Yields Affected by Treatments		
Density Plants/ha	**Plant Spacing**	**Grain Yield (Kg/ha 14% Hum**
40,797	D1= 2 pl a 0.7 m	1494.875
40,797	D2= 4 pl a 1.4 m	802.125
	Farmer Variety	**Grain Yield (Kg/ha 14% Hum**
	V4= Salvador	1503.00
	V3= Juan	1461.25
	V2= Isauro	840.25
	V1= Andrés	789.50
	Fertility	**Grain Yield (Kg/ha 14% Hum**
	F1= Balanced (soil test)	1862.375
	F3= 50% of Balanced	1681.25
	F2= Micorriza	672.125
	F4= No Fertilizer	378.25

Genotype Management Interaction
Maiz de Cajete San Antonio Nduayaco
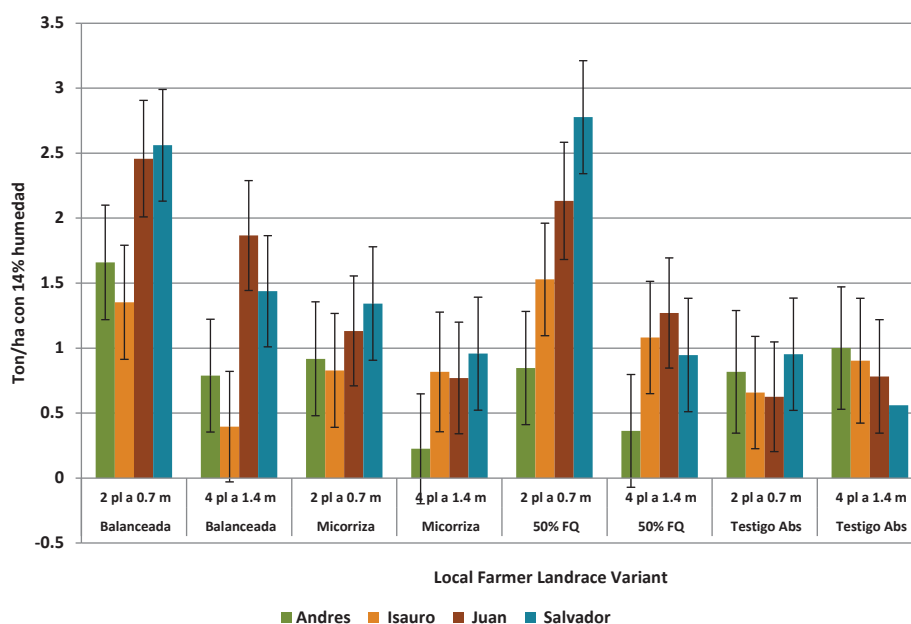
Production Costs of Treatments

		No fertilizer	Fert. 50% (3.6 - 20-25 NPK)	Fert. 100% (7.2-40-50 NPK)	Mycrorrhiza
Land Preparation					
Plowing $350/team	4 teams/ha	1400	1400	1400	1400
Planting					
Landrace Seed $7/kg	22 kg/ha	154	154	154	154
Labor $150/day	12	1800	1800	1800	1800
Fertilization					
18-46-0		0	418.47	836.94	0
KCL			352.8	705.6	0
Micorriza		0	0	0	150
Application Labor	$150/ person	0	150	300	0
Transport fertilizer	$ 50/sack	0	85.5	171	0
Pest Control					
Seed Treatment		372.6	372.6	372.6	372.6
Cultivation $300/team	2 teams/ha	600	600	600	600
Labor $150/day	3	450	450	450	450
Manual weeding	4 laborers	600	600	600	600
Karate (2 aplic)	0.25 lt/ha		300	300	
Aplication Labor	$ 150/person		300	300	
Harvest					
Labor	$ 150/person	300	600	750	600
Shelling		300	600	750	600
Transport from field			300	600	
Total ($/ha)		**5976.6**	**8483.37**	**10090.14**	**6726.6**

Cost Benefit Analysis

Farmer Seed Sample	Fertility Treatment	Yield (T/ha)	Cost Prod. ($/ha)	Value Prod, ($/ha)	Utility ($/ha)	C/B Ratio
Sra. Antonia Alvarado	Per Soil Analysis (95-50-60 NPK)	5.09	11521.4	35,630	24109	3.09
Sra. Petra Pérez	Per Soil Analysis (95-50-60 NPK)	4.69	11521.4	32,830	21309	2.85
Sra. Antonia Alvarado	50% Recommended (47.5-25-30 NPK)	3.61	9399.4	25,270	15871	2.69
Sra. Petra Pérez	50% Recommended (47.5-25-30 NPK)	4.23	9399.4	29,610	20211	3.15
Sra. Antonia Alvarado	Mycorrhiza + 50% recommended (47.5-25-30 NPK)	3.82	9549.4	26,740	17191	2.80
Sra. Petra Pérez	Mycorrhiza + 50% recommended (47.5-25-30 NPK)	3.92	9549.4	27,440	17891	2.87
Sra. Antonia Alvarado	Farmer Fert. (50-0-0 NPK)	3.67	6761.8	25,690	18928	**3.80**
Sra. Petra Pérez	Farmer Fert. (50-0-0 NPK)	4.71	6761.8	32,970	26208	**4.88**
Sra. Antonia Alvarado	No Fertilizer	1.47	5928.0	10,290	4362	1.74
Sra. Petra Pérez	No Fertilizer	1.73	5928.0	12,110	6182	2.04

Local Price of Native Maize $ 7.0/kg

Participatory Plant Breeding

Photo: Flavio Aragon

Planting of Farmer Seed Samples within the Community
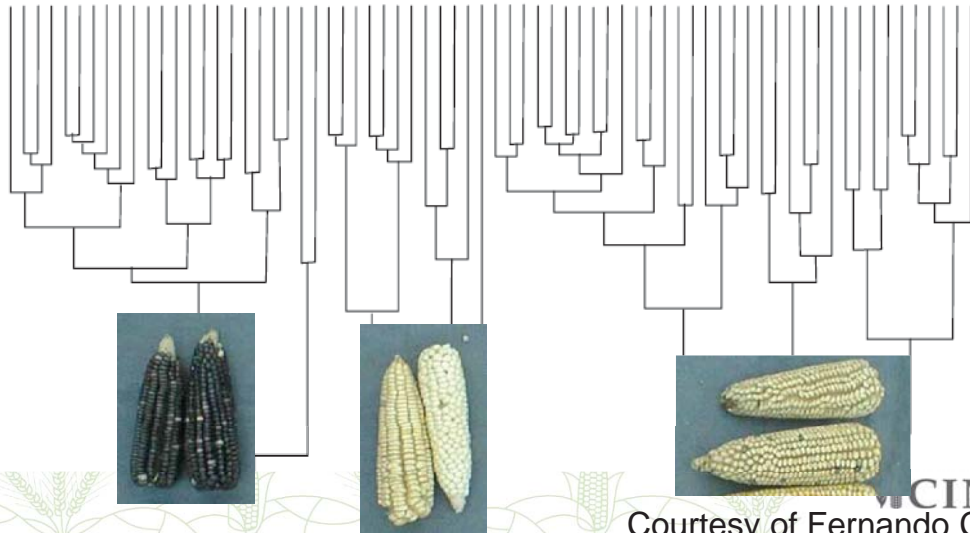
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Harvest
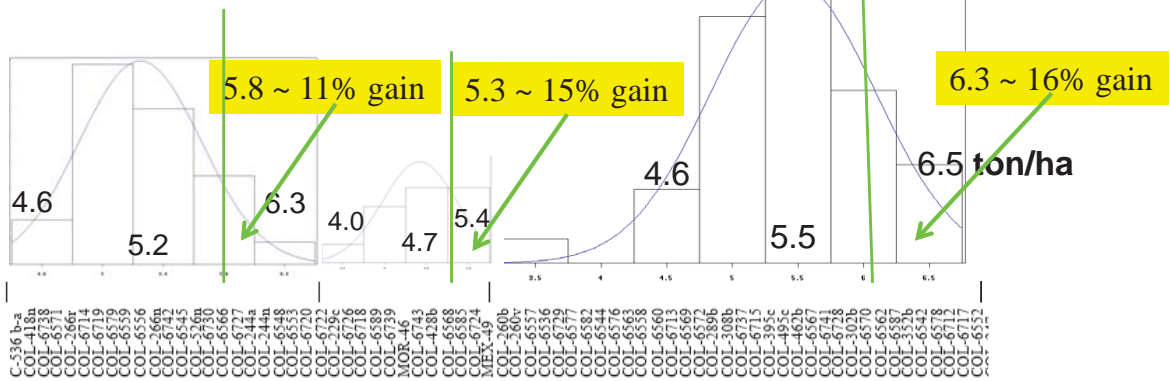
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Field Day Demonstrations

Community Evaluation

Evaluación participativa en 4 sitios
Herrera *et al.* 2004, 2013

Gains through detection of 20% best yielding, within each morphological type

5.8 ~ 11% gain

5.3 ~ 15% gain

6.3 ~ 16% gain
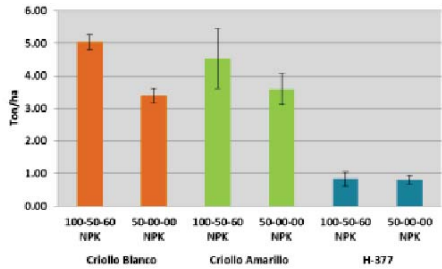
4.6

6.5 **ton/ha**

4.6

6.3

4.0

5.4

5.2

4.7

5.5

Courtesy of Fernando Castillo

Participatory Selection

Agronomic Improvement

Market Access

Genetic and Agronomic improvement of native maize in Oaxaca, México

FALL PREVIEW 2014

Enrique Olvera to Serve 'Single-Origin' Tortilla at Cosme, Opening Next Week
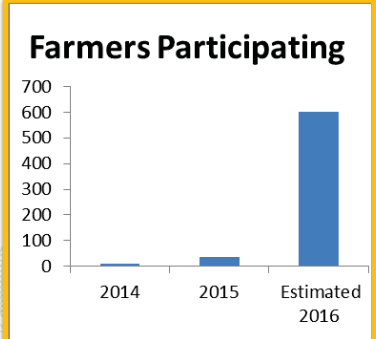
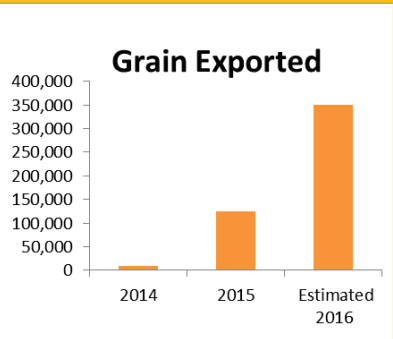By Robin Raisfeld and Rob Patronite

end user

Native Maize Landrace Improvement

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MASIENDA

Small Farmers to Chefs

Benefits of Culinary Markets for Landrace Maize:
- **Premium price for quality and diversity**
- **Small farmers directly targeted**
- **Promotes *in situ* conservation of maize landraces**

Grain Exported

Farmers Participating

Connecting Farmers to Culinary Markets

Training Farmer Groups to Connect to Export Markets

Grain Cleaning

Record Keeping

Aflatoxin Testing

Hermetic Bagging

Storage

Effects of Expanding Culinary Markets on Native Maize Prices and Exports 2014-2017
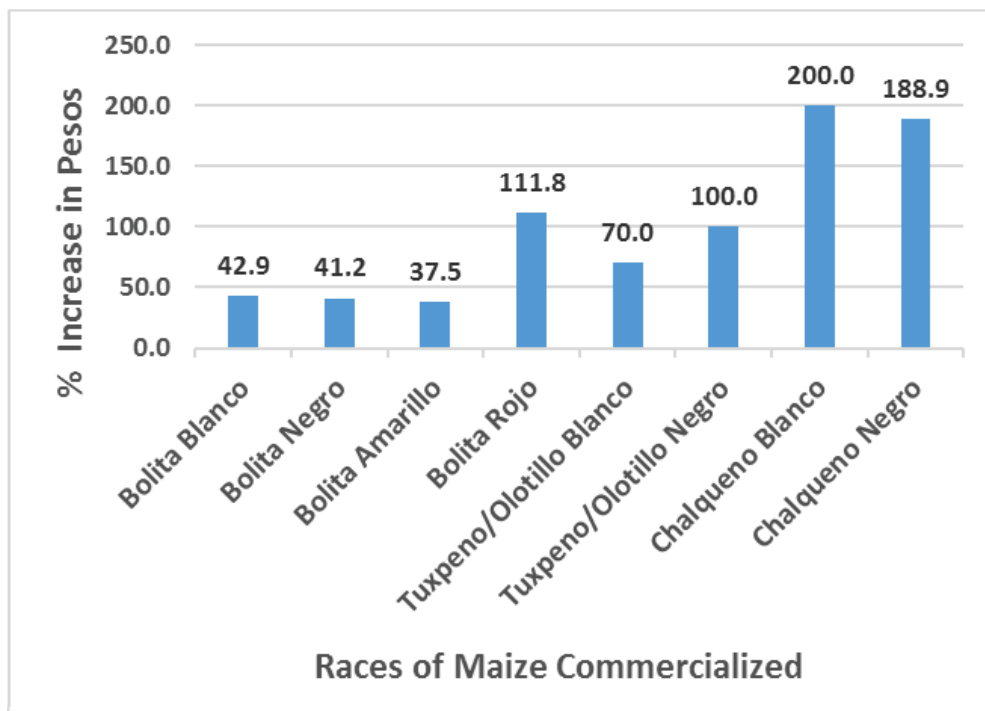
	Kgs Grain Exported
2014	10,000
2015	125,000
2016	350,000
2017	925,000

Commercialization Guidelines for Native Maize Landraces

Absent an authentication process for Native landraces, rapid expansion in the culinary market could cause inundation by non-landrace maize and large commercial producers.

Experts in Native Mexican Maize were convened to:
- Define standards for distinguishing native maize
- Form a panel of experts to identify native maize
- Define types of farmers and communities that can be targeted.

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Centro Internacional de Mejoramiento de Maíz y Trigo

CONABIO
COMISIÓN NACIONAL PARA EL CONOCIMIENTO Y USO DE LA BIODIVERSIDAD

Landrace Farmer Profitability and Wellbeing

- Markets
- Genetic Improvement
- Agronomic Optimization
- Grain Storage
- Coordination of Farmers

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Thank you !

CIMMYT
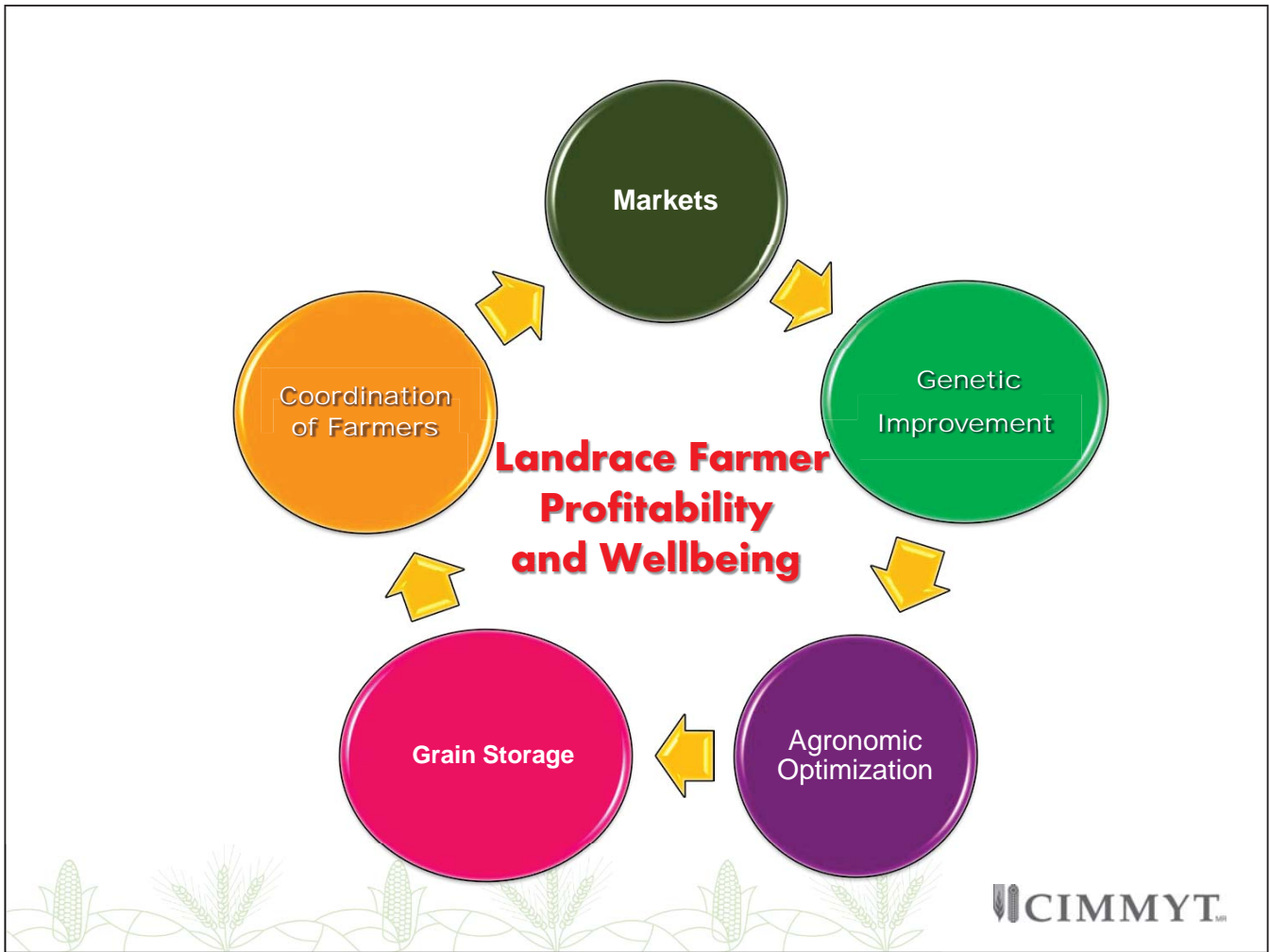
Progenitor lineages within proprietary dent corn germplasm

Mark A. Mikel, Associate Director Biotech Center
Institute for Genomic Biology, University of Illinois at Urbana-Champaign

During the past 40 years, the number of corn breeding programs has plummeted due to merger and acquisition, resulting in Pioneer Hi-Bred (PHI), Monsanto, and Syngenta owning ~95% of the U.S. PVP/utility patent registered lines. The first breeding cycles of proprietary germplasm began with publicly available germplasm and quickly transitioned to breeding within each company's own material. However, there are common progenitors across programs from using public/foundation germplasm and extensive breeding of PHI commercial hybrids during the early breeding cycles. For example, pedigree examination of PVP/patented lines registered since 2010 identify the two largest progenitors as B73 within the Stiff Stalk and PH207 within the Iodent heterotic family. Interestingly, B73 and PH207 contribute across proprietary germplasm pools. Among 461 non-Stiff Stalk inbreds registered since 2010, all (228 of 228) of PHI, 99% (167 of 169) of Monsanto, and 84% (54 of 64) of Syngenta lines are descendants of PH207. Among 391 Stiff Stalk inbreds registered since 2010, 95% (187 of 196) of PHI, 98% (143 of 146) of Monsanto, and 98% (48 of 49) of Syngenta lines are descendants of B73. As crosses between Stiff Stalk and Iodent heterotic families are the predominate commercial hybrid formulation, it is most likely that a hybrid grown in a farmers' field today in the heart of the U.S. corn belt is a cross between a B73 descendant and a PH207 descendant. Understanding the major lineages of commercial germplasm enables optimizing ex-PVP germplasm in forward breeding in predictable and sometimes novel ways.

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& ENVIRONMENTAL SCIENCES

Progenitor lineages within proprietary dent corn germplasm

Mark A. Mikel
University of Illinois

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& Environmental Sciences

Examples of Genetic Diversity in Vegetable, Fruit, and Field Crops

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The 'nuts and bolts' of contemporary N.A. Dent Corn Germplasm

Transition from many to few companies

U.S. hybrid corn industry thirty years ago:

- **Era of "snow white" and the seven dwarfs and 100s of small companies**
- **~400-500 U.S. located seed companies of all sizes retailing hybrid corn, soybean, and or small grains seed**
  - **~40 had breeding program**
  - **~100 <u>multi-location</u> hybrid evaluation in small plot or strip trials**
  - **nearly all had some form of hybrid evaluation (strip test or small plot)**
- **All but Pioneer Hi-Bred used foundation seed from Holden's, Illinois Foundation, or Brayton Seeds as sole source or supplementary germplasm**

U.S. corn % market share 1973 - 1983

Table 15—-U.S. market shares of corn seed by company[1]

Company	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983
						Percent					
Pioneer	23.8	25.5	24.6	27.3	30.9	26.2	32.9	36.9	34.8	38.8	38.1
DeKalb[2]	21.0	18.8	18.8	19.5	15.8	17.9	13.3	13.0	15.9	12.2	10.3
Asgrow	0	0	0	0	0	0	0	0	0	0	0
Funk[3]	8.8	9.4	8.9	9.2	6.4	8.1	6.7	5.7	5.4	5.2	3.9
Trojan[4]	5.9	5.1	6.8	5.6	4.2	5.4	3.8	2.0	0	0	0
Northrup-King[5]	6.1	4.5	4.7	3.4	3.8	3.3	3.8	4.9	3.4	2.6	2.5
Zeneca/ICI	0	0	0	0	0	0	0	0	0	0	0
Cargill/PAG[6]	4.8	6.8	3.9	3.5	4.1	4.6	3.3	4.7	5.6	5.4	4.2
Golden Harvest	0	0	1.8	2.4	2.5	3.1	2.9	1.3	3.2	2.3	2.6
Dow/Mycogen	0	0	0	0	0	0	0	0	0	0	0
Jacques/Agrigenetics[7]	0	1.3	1.7	2	1.9	2.1	2.7	2.2	0	0	0
Other	29.6	28.6	29.8	27.1	30.4	29.3	30.6	29.3	31.7	33.6	38.4
Largest 8 firms	72.5	70.7	69.8	71.2	68.1	67.0	69.7	69.4	70.0	68.3	64.0
Largest 4 firms	59.7	58.8	59.1	61.6	57.3	55.6	56.7	60.5	59.5	59.1	54.9
Herfindahl index	0.1171	0.1159	0.112	0.1269	0.1049	0.1138	0.1354	0.1609	0.1501	0.1723	0.1604

from USDA Economic Research "The seed industry in U.S. agriculture" / AIB-786

Seed Industry Structure 1996 - 2013

Phil Howard, Associate Professor, Michigan State University
http://www.msu.edu/~howardp

- Seed Companies
- Chemical Companies
- Other Companies
- Full Ownership
- Partial Ownership
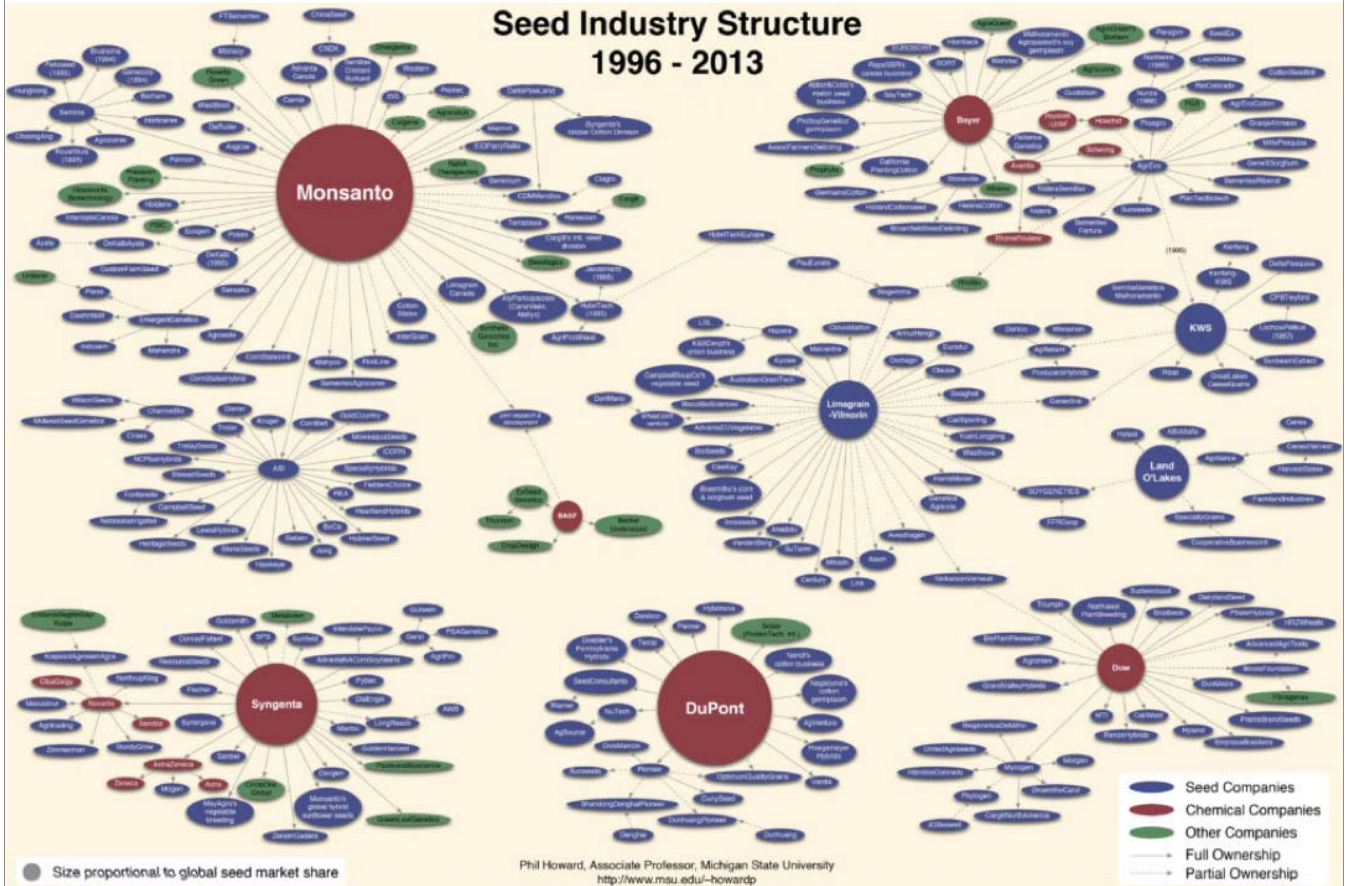
Size proportional to global seed market share

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Five Years of U.S. Seed Market Share

CORN

	2015	2014	2013	2012	2011
Monsanto	36.7	35.5	34.9	34.4	33.4
DuPont	34.6	34.7	36.0	36.1	35.8
Local & Regional Companies	10.5	11.1	11.0	11.8	13.0
AgReliant	7.1	7.0	6.7	6.5	6.6
Dow AgroSciences	6.1	6.0	5.3	5.2	4.7
Syngenta	5.0	5.7	6.1	6.0	6.5

https://www.agweb.com/article/seed-competition-heats-up-naa-sonja-gjerde/

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Bottom line of mergers and acquisitions is …

Hybrid seed cost per acre

1975	1995	2011	2017
$9	$24	$86	$125

What is the foundation of proprietary germplasm?

Input of public inbreds in first breeding cycles of registered proprietary germplasm (1980-1999)

Line	% Genetic contribution
B73	12%
B37	4%
B14	4%
A632	2%
Mo17	6%
Lancaster	6%
Oh43	3%
W117	1%

Footprint of PHI commercial hybrids in Monsanto and Syngenta inbreds registered 2010-present

PHI hybrid	# lines parent of	%GC in lines registered 2010-present	
		Monsanto	Syngenta
PHI3901	15	1%	6%
PHI3737	12	23%	10%
PHI3394	7	1%	2%
PHI3732	5	0%	0%
PHI3751	4	0%	8%
PHI3713	4	0%	0%
PHI3378	4	2%	0%
PHI3475	4	0%	0%
PHI3540	3	0%	5%
PHI3377	3	0%	0%
PHI3358	3	0%	0%
PHI3527	3	1%	0%
PHI3535	2	1%	5%
PHI3180	2	4%	0%
PHI3293	2	0%	0%
		34%	36%

Also 2 lines developed from PHI hybrids: 3163, 3615, 3790, 3902, 3953, 3165, 3704, 3780,; and 1 line from 3147, 3160, 3369, 3720, 3978, 3162, 3195, 3199, 3245, 3603, 3769, 3558, 3861, 3921, and 3921, and LIZA.

Building block of first few 'PVP' breeding cycles

1980-1990: Most used germplasm (last breeding cross) of development of 271 registered Monsanto lines

Germplasm	Pedigree	Parent of
FBLL	5B2C-A / PB80	18
LH82	610 / LH7	17
B73	BSSS_C5	16
LH132	B73 *2 / H93	14
LH123	PHI3535	13
LH51	Mo17 isoline	13
3IIH6	PHI3737 (PHG29 / PHG47)	10
2FACC	4676A / PB80	8
LH74	A632 / B73	8
LH38	?A619 / L120?	7
Mo17	C103 / CI 187-2	7

1980-1990: Most used germplasm (last breeding cross) of development of 252 registered PHI lines

Germplasm	Pedigree	Parent of
PH207	PHB3BD2 / PHG3RZ1	17
PHR03	PHT19 / PHG84	16
PHJ40	PHB09 / B36	14
PHW52	B73 / PHG39	13
PHG39	PHA33GB4 / PHA34CB4	12
PHP02	PHG44 / PHG29	12
PHK29	PHB47 / PHAC54	10
PHP38	PHG39 / PHK29	10
PHG47	PH041 / MKSDTE	9
PHR25	PHB83 / PH207	8
PH595	50%FC, MYD, Oh07	7
PHHB9	PHG86 / PHW52	7

1980-1990: Most used germplasm (last breeding cross) of development of 78 registered Syngenta lines

Germplasm	Pedigree	Parent of
B73	BSSS_C5	10
PHI3737	=PHG29 / PHG47	9
LH123	PHI3535	5
Mo17	C103 / CI 187-2	4
NP235	uk / A635	4
W117	643 / MINN13	4

What is contemporary (2008-present) proprietary germplasm composted of?

Most used non-Stiff Stalk parent in Monsanto/PHI hybrids 2008 - present

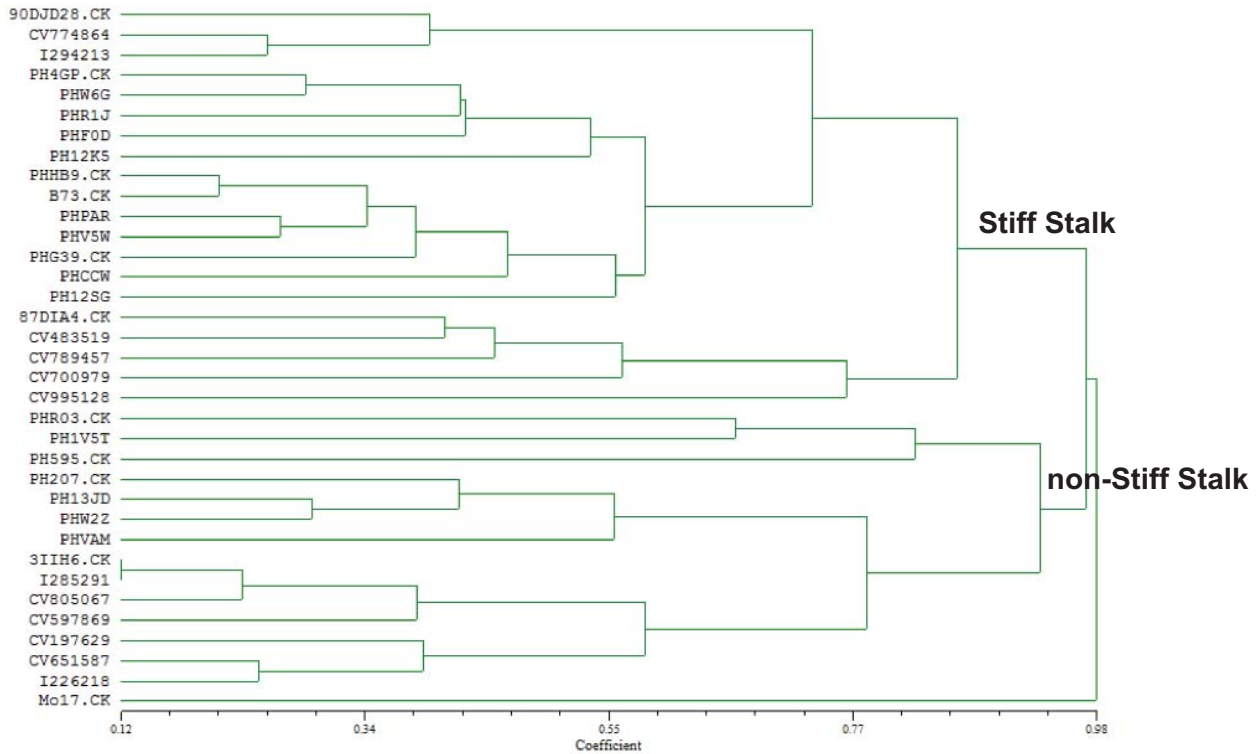| Pedigee	Company/inbred	# hybrids	% Genetic Contribution			
			3IIH6	PH207	PHR03	Mo17
Monsanto Non-Stiff Stalk						
01INL1 / 17INI20	I285291	27	63%	33%		
I283669 / I226218	CV197629	23	47%	18%		
I211988 / 2* I226218	CV651587	23	28%	11%		
01INL1 *2 / ASG5750	CV597869	21	56%	21%		
I119149 / I900429	CV805067	21	69%	30%		2%
01INL1 / LH283	I226218	17	38%	14%		
PHI Non-Stiff Stalk						
PHEDR / PH8JR	PH13JD	21		48%		
PHAVD / PH8CW	PHVAM	16		34%	19%	
PHVNV / PHNTV	PH1V5T	15		17%	31%	3%
PH7DD / PH8JR	PHW2Z	15		51%		
PHACE / PHACV	PH128Z	10		37%	25%	
PHHAT / PH8JR	PH17RM	10		44%	6%	

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Most used Stiff Stalk parent in Monsanto/PHI hybrids 2008 - present

Pedigree	Company/Inbred	# hybrids	% Genetic Contribution				
			PHG39	90DJD28	2FACC	3AZA1	B73
Monsanto Stiff Stalks							
I900420 / I180421	CV995128	54			13%		28%
PA2121 / 2* I294213	CV774864	32		38%	19%		35%
01DHD10 / 90DJD28	I294213	20		50%	25%		47%
I119135 / I054029	CV483519	19			25%	38%	9%
I325350 / I119135	CV700979	19			13%	63%	5%
I054029 / I090372	CV789457	17		25%	25%	13%	19%
PHI Stiff Stalks							
PHE71 / PH7CH	PH12K5	20	27%				21%
PH5WA / PH890	PHPAR	20	38%				31%
PHAPT / PH890	PHV5W	18	38%				31%
PH4GP / PH91V	PHW6G	17	38%				16%
PHE0T / PH7CR	PH12SG	16	30%				15%
PH4GP / PH714	PHF0D	15	31%				14%
PH4GP / PH6WA	PHR1J	15	31%				14%
PH09B / PH07D	PHCCW	13	31%				19%

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Pedigree distance of inbreds used most in hybrids 2008-present

Stiff Stalk

non-Stiff Stalk

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Contribution among non-Stiff Stalk lines developed 2010-present

Line	Monsanto		PHI		Syngenta	
	% GC	Progeny	% GC	Progeny	% GC	Progeny
Non-Stiff Stalk						
Mo17	1%	30% (52 of 169)	1%	14% (32 of 228)	6%	31% (20 of 64)
PH207	18%	99% (167 of 169)	29%	100% (228 of 228)	16%	84% (54 of 64)
PHR03			18%	72% (165 of 228)		
3IIH6 *(carries 3/8 PH207)*	38%	98% (165 of 169)				

Contribution among Stiff Stalk lines developed 2010-present

Line	Monsanto		PHI		Syngenta	
	% GC	Progeny	% GC	Progeny	% GC	Progeny
Stiff Stalk						
B73	25%	98% (143 of 146)	17%	95% (187 of 196)	33%	98% (48 of 49)
90DJD28	19%	67% (98 of 146)				
PHG39			26%	100% (196 of 196)		

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In the U.S. Corn Belt the predominant hybrid 'formula' grown in a farmers' field is a Stiff Stalk female (B73 descendant) x Iodent male (PH207 descendant)

Should we stick to the Stiff Stalk x non-Stiff Stalk paradigm?

Grain yield (bu/acre) half diallel 12 progenitors from 2 years (6 locations, ~12 reps)

	B73	PHG39	LH1	PHJ40	PH207	LH82	PHG47	Mo17	PHG35	PHG84	LH123
B73											
PHG39	166										
LH1	165	117									
PHJ40	159	146	130								
PH207	113	159	127	157							
LH82	179	146	139	152	147						
PHG47	150	156	149	158	139	162					
Mo17	175	146	156	139	153	153	135				
PHG35	183	161	149	138	170	150	169	137			
PHG84	183	188	161	142	168	183	179	144	146		
LH123	180	170	152	167	152	178	164	141	171	181	
PHZ51	175	172	173	160	152	167	165	151	158	126	166

BSS (rows B73 through PH207), NONBSS (rows LH82 through PHZ51)

←BSS   NON-BSS →

Hauck, Johnson, Mikel, Mahone, Morales, Rocheford, and Bohn. 2014. Crop Sci.

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AMERICAN SOCIETY OF PLANT BIOLOGISTS

The Iodent inbred PH207 genome

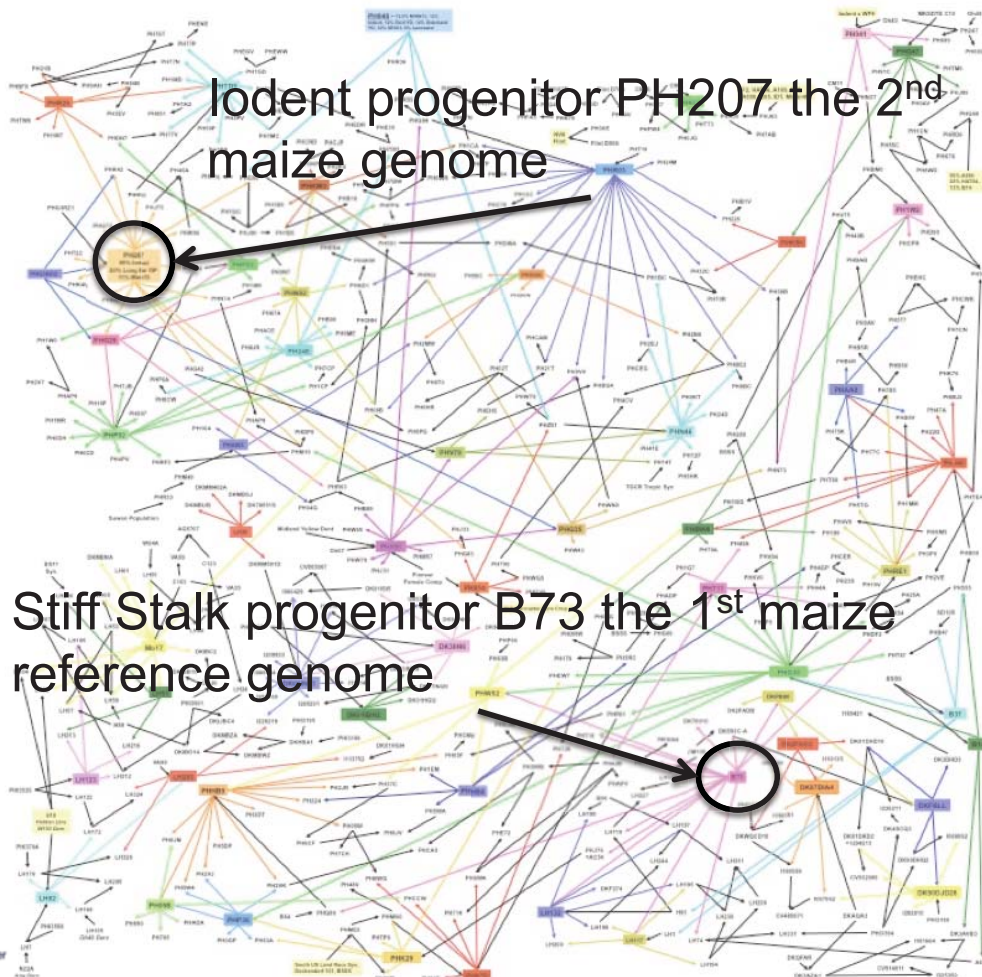Draft Assembly of Elite Inbred Line PH207 Provides Insights into Genomic and Transcriptome Diversity in Maize[OPEN]

Candice N. Hirsch,[a,1] Cory D. Hirsch,[b] Alex B. Brohammer,[a] Megan J. Bowman,[c] Ilya Soifer,[d] Omer Barad,[e] Doron Shem-Tov,[e] Kobi Baruch,[e] Fei Lu,[f] Alvaro G. Hernandez,[g] Christopher J. Fields,[g] Chris L. Wright,[g] Klaus Koehler,[h] Nathan M. Springer,[i] Edward Buckler,[f,j] C. Robin Buell,[c,k] Natalia de Leon,[l,m] Shawn M. Kaeppler,[l,m] Kevin L. Childs,[c,n] and Mark A. Mikel[g,o]

Author information ► Article notes ► Copyright and License information ►

ABSTRACT

Go to: ⊠

Intense artificial selection over the last 100 years has produced elite maize (*Zea mays*) inbred lines that combine to produce high-yielding hybrids. To further our understanding of how genome and transcriptome variation contribute to the production of high-yielding hybrids, we generated a draft genome assembly of the inbred line PH207 to complement and compare with the existing B73 reference sequence. B73 is a founder of the Stiff Stalk germplasm pool, while PH207 is a founder of Iodent germplasm, both of which have contributed substantially to the production of temperate commercial maize and are combined to make heterotic hybrids. Comparison of these two assemblies revealed over 2500 genes present in only one of the two genotypes and 136 gene families that have undergone extensive expansion or contraction.

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Iodent progenitor PH207 the 2nd maize genome

Stiff Stalk progenitor B73 the 1st maize reference genome

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The PH207 annotated assembly

- **Over 500Gb of sequence data from multiple pair-end, mate-pair jumps, and TSLR libraries**

- **Assembly consists of 132,022 scaffolds with a final N50 of ~630Kb**

- **Estimated genome size 2.45Gb slightly larger than B73 ~2.3Gb**

- **PH207 genome containg 40,557 gene models vs 39,656 in B73**

- **Available at** https://www.maizegdb.org/genome/assemblies_overview#Zm-PH207-REFERENCE_NS-UIUC_UMN-1.0 **:**
  - **PH207 genome assembly** "Zm-PH207-Reference_NS-UIUC_UMN-1.0"
  - **PH207 gene set** ZM008a0000001-ZM0000008a040557

Identification of dispensable genes that are present in one genome but absent in the other through comparison of the two de novo assemblies and their specific annotation gene set

- **1,545 B73 specific genes that were present in B73 but absent in PH207 genome**

- **2,042 PH207 specific genes present in PH207 but absent in B73 genome**

Thank You!

Reimagining our Fields

Jenna Lynn Hoffman, Breeding Digital Phenomics and Statistics Lead, Monsanto

Valuable data layers can be derived from images sourced from satellites, manned aircraft, drones and smartphones. These data layers, when fused with other agronomic and product information, promise to deliver on insights specific to the acre. This will become the foundation for the combination of targeted breeding and personalized product placement. In this talk, I will highlight the new scientific insights that have resulted from this approach progress at Monsanto and Climate toward using this approach.

Reimagining our Fields

Illinois Corn Breeders' School
Jenna Hoffman

PEOPLE ARE THE KEY TO DIGITAL PHENOMICS

| Plan. Coordinate. Execute. | Analyze. Enrich. Decide. | Teach. Learn. Inspire. | Design. Create. Build. | Dream. Question. Innovate. |

Always work hard on something uncomfortably exciting

~Larry Page one sentence on how to change the world

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Slide 1

# Uniquely Positioned to Address Grower Requirements with Digitally Integrated Solutions

Industry's leading technology platforms and digital integration are addressing evolving grower needs

BREEDING
Leading germplasm positions in corn, soybeans, cotton and vegetables enhanced by cutting edge breeding technology to widen gap

BIOTECH
Industry leader with broadly licensed biotech traits reaching >350M acres globally in corn, soybeans, cotton, canola. Multi-generation of traits in pipeline to provide new modes of action.

Biologicals

Plant Breeding

Leading Technology Platforms

Data Science

Biotechnology   Chemistry
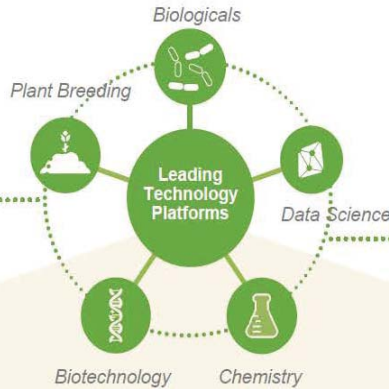
BIOLOGICALS
BioAg Alliance with Novozymes; industry leader in fermentation technology

DATA SCIENCE
Leading platform with >92M acres of penetration and leading connectivity, retail & equipment partnerships

CHEMISTRY
Herbicide leader with glyphosate; partner of choice for new molecule development

YIELD — INSECT CONTROL — WEED CONTROL — DISEASE & OTHER — FERTILITY

DIGITALLY INTEGRATED SOLUTIONS

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Slide 2

Shifting the Paradigm: Moving from measuring yield to understanding what is driving yield

Current: Example Yield Trial Data Collection

Data Points
% Total Data

Planting   In Season   Harvest

Current Field Season
- Targeted data collection
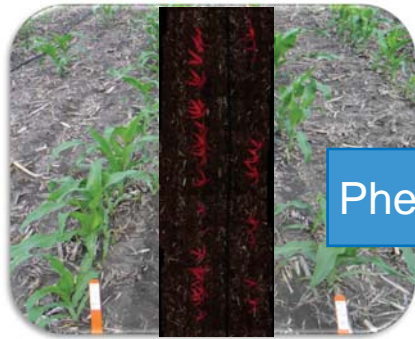- Trial assessment
- Contextual understanding
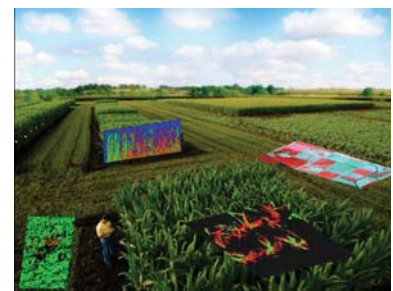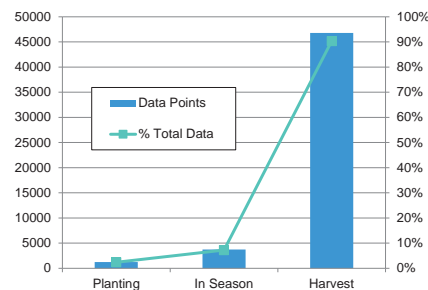
Future Field Season
- Full season data collection
- Embrace complexity
- Expand perspective
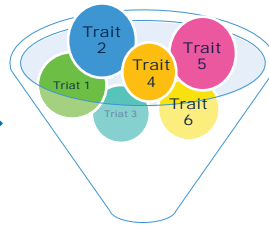
MONSANTO

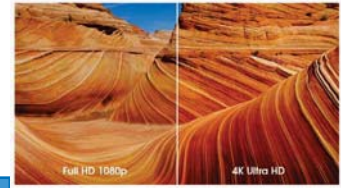# Shifting from subjective to quantitative measurements

Traditional vigor assessment

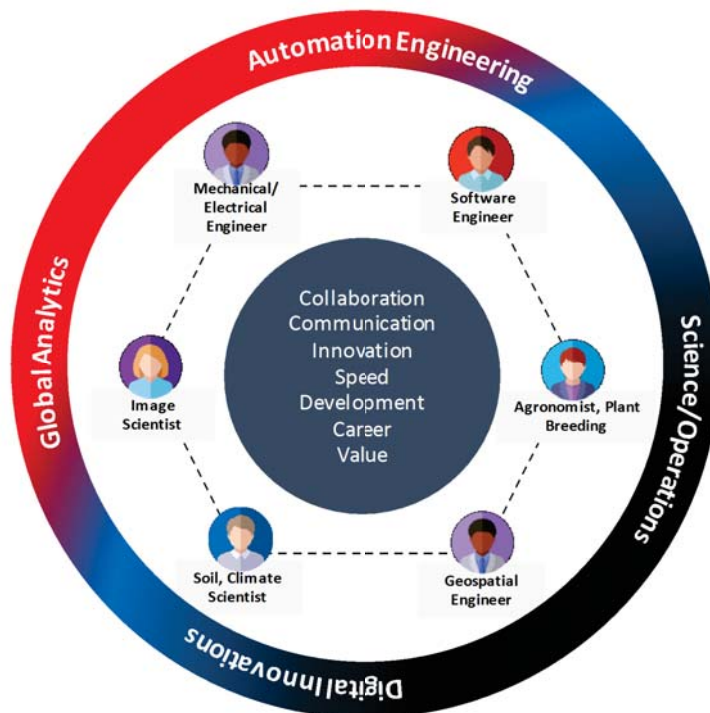Rating 1-9

Phenomics

Quantitative vigor surrogate

Trait 1, Trait 2, Trait 3, Trait 4, Trait 5, Trait 6

Enhanced Resolution

Full HD 1080p — 4K Ultra HD

Increased Efficiency

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PHENOME 2018
TUCSON, AZ FEBRUARY 14-17

Phenomics Community at Monsanto
Who we are, skills we need, structure that invites collaboration

Automation Engineering

Global Analytics

Science/Operations

Digital Innovations

Mechanical/Electrical Engineer

Software Engineer

Image Scientist

Agronomist, Plant Breeding

Soil, Climate Scientist

Geospatial Engineer

Collaboration
Communication
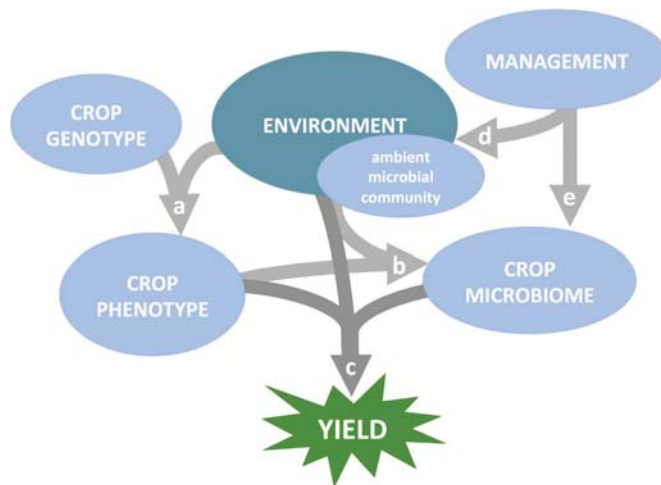Innovation
Speed
Development
Career
Value

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Data Science as a Center of Excellence

Phenomics vision: Sense first, Sense global, Sense Remote, Sense smart

Genotype by phenotype specific sensing

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Thank You!

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Cereal transformation at DuPont Pioneer – meeting future demands for genome modification.

William Gordon-Kamm
Pioneer H-Bred International

Abstract:

Keith Lowe, Emily Wu, Ning Wang, George Hoerster, Ajith Anand, Mauricio La Rota, Craig Hastings, Brian Lenderts, Mark Chamberlin, Maren Arling, Visu Annaluru, Candy Sweeney, Todd Jones & Bill Gordon-Kamm.

While transformation methods for monocot crops continue to improve, the process has remained constrained to a few genotypes per crop, and the methods have been slow and labor intensive, placing these methods beyond the reach of most academic labs.   Recent progress in our labs is rapidly changing this situation for monocots. By focusing on the overexpression of the maize Babyboom (BBM) and Wuschel2 (WUS2) genes, we can routinely produce high transformation frequencies in numerous previously non-transformable maize inbreds. This was accomplished by altering the expression of our BBM and WUS cassettes in such a way that we can eliminate all callus steps and obtain transgenic T0 plants via direct germination of somatic embryos, making maize inbreds such as B73 and Mo17 easily transformable. Of even greater import to genome editing, this process is largely genotype independent and transgenic plants can be sent to the greenhouse in less than half the time of conventional methods. Another limitation for many monocots is the intensive labor and greenhouse space required to supply immature embryos for transformation. As a new alternative to immature embryos, we use BBM and WUS2 to recover transgenic events directly from either embryo slices from mature seed or leaf segments from seedlings in a variety of Pioneer inbreds, routinely recovering healthy, fertile T0 plants. Finally, we demonstrate that the maize BBM and WUS2 genes stimulate transformation in cereals.

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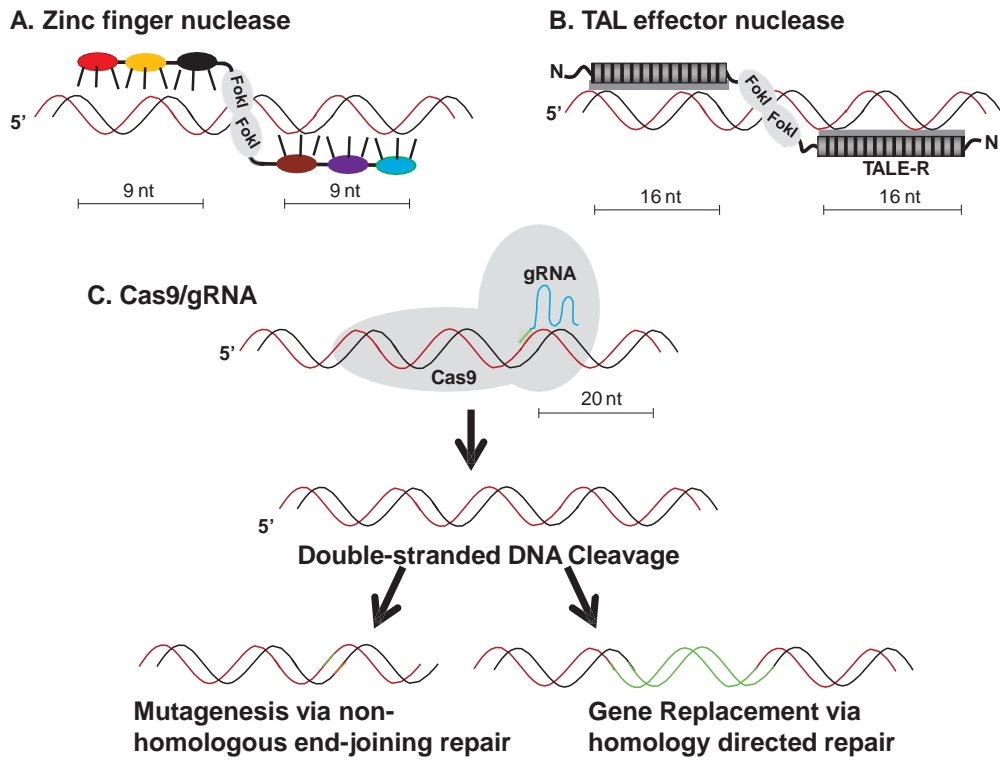
Genome editing in maize

Bing Yang, Associate Professor
Iowa State University, Ames, Iowa

Programmable nucleases (zinc finger nucleases, transcription activator-like nucleases, and CRISPR RNA guided Cas nucleases) have been successfully engineered to induce site-specific mutations at genomic loci in maize. The genome editing tools have significantly advance our basic understanding of gene function and engineering beneficial traits in maize. In my presentation, I will provide our experience in developing and utilizing TALENs (transcription activator-like nucleases) and CRISPR/Cas9 technologies for targeted mutagenesis in maize.

Genome editing in maize
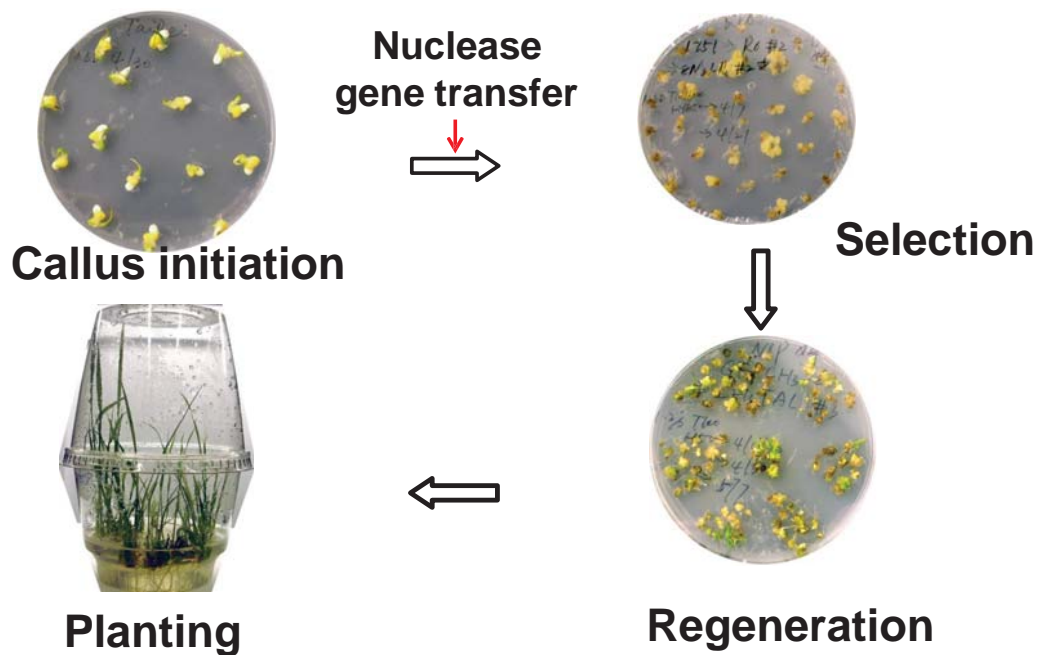
Bing Yang

byang@iastate.edu

Iowa State University

Ames, Iowa 50011

Outline:

- Introduction of genome editing in plants
- An example of maize gene mutagenized by using TALEN technology
- Development and application of CRISPR/Cas9 in maize
- Conclusion

A. Zinc finger nuclease

5'

9 nt   9 nt

FokI FokI

B. TAL effector nuclease

N

5'

FokI FokI

TALE-R

16 nt   16 nt

N

C. Cas9/gRNA

gRNA

5'

Cas9

20 nt

Double-stranded DNA Cleavage

Mutagenesis via non-homologous end-joining repair

Gene Replacement via homology directed repair

Plant genome editing requires transgenics

Callus initiation

Nuclease gene transfer

Selection

Planting

Regeneration

Delivery systems for genome editing

DNA constructs:

Ubi:Cas9:T   U6:gRNA:T

Homo. L   Homo. R

Template DNA

mRNA molecules:

Cas9 mRNA   sgRNAs

Ribonucleoproteins (RNP):

Cas9/gRNA (pre-assembled mixture)

Transgene-free, gene-edited plants can be obtained from the segregating population

T0   T1

T-DNA

Edit

Edit only

segregation
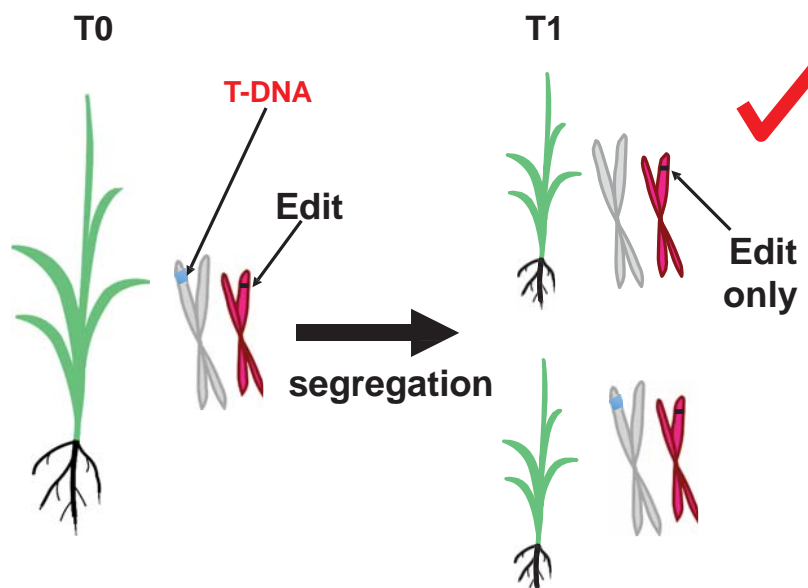
Major plant species targeted for gene editing with engineered nucleases

Plant	Mega-nuclease	ZFNs	TALENs	Cas9/gRNA
Arabidopsis	√	√	√	√
Canola		√		
Cotton	√			
Potato			√	√
Soy bean		√	√	√
Tobacco		√	√	√
Tomato			√	√
Barley			√	√
Maize	√	√	√	√
Rice			√	√
Sorghum				√
Wheat			√	√

The Role of Genome Editing in Plant Biology and Agriculture

- Basic biology - e.g., Functional genomics

- Gene/trait discovery - e.g., SNP variations in gene expression and function

- Applied biology - e.g., create novel germplasm with precise edits

Types of DNA Modifications with Genome Editing in Plant

- SNPs (single nucleotide polymorphisms

- Indels (Insertions/deletions)

- Large chromosomal deletions

- Insertion of gene or regulatory DNA

- Gene replacement

Benefits of Genome Editing in Agriculture

- Increase crop productivity and food production

- Increase resistance to plant pathogen and pest

- Increase tolerance to abiotic stress

- Better manage weeds

- Make healthier and more nutritious food

LETTERS

Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases

Vipula K. Shukla[1], Yannick Doyon[2], Jeffrey C. Miller[2], Russell C. DeKelver[2], Erica A. Moehle[2], Sarah E. Worden[1], Jon C. Mitchell[1], Nicole L. Arnold[1], Sunita Gopalan[2], Xiangdong Meng[2], Vivian M. Choi[2], Jeremy M. Rock[2], Ying-Ying Wu[2], George E. Katibah[2], Gao Zhifang[1], David McCaskill[1], Matthew A. Simpson[1], Beth Blakeslee[1], Scott A. Greenwalt[1], Holly J. Butler[1], Sarah J. Hinkley[2], Lei Zhang[2], Edward J. Rebar[2], Philip D. Gregory[2] & Fyodor D. Urnov[2]

IPK1: *Inositol-1,3,4,5,6-pentakisphosphate 2-kinase*, a gene encoding an enzyme that catalyses the final step in phytate biosynthesis in maize seeds.
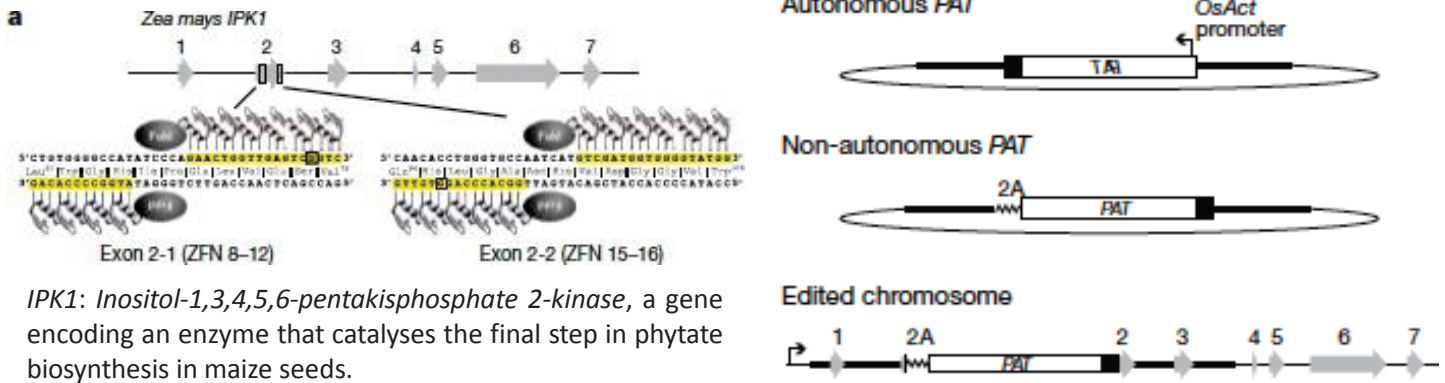
Structure of TAL effectors


```
5 LTPDQVVAIAS NI GGKQALETVQRLLPVLCQDHG
6 LTPDQVVAIAS NG GGKQALETVQRLLPVLCQDHG
7 LTPDQVVAIANN NN GGKQALETVQRLLPVLCQDHG
8 LTPDQVVAIAS HD GGKQALETVQRLLPVLCQDHG
  1    5    10    15    20    25    30  34
```

- Repeat region determines the gene specificity
- Both NLS and AD are required for TALE activity

Yang *et al.* 2004 *MPMI* 17:1192-1200   Yang *et al.* 2000 *PNAS* 97:9807-9812   Zhu *et al.* 1999 *Plant Cell* 11:1665-1674

Zhu *et al.* 1998 *MPMI* 11:824-832

TALE recognition code

Science
AAAS

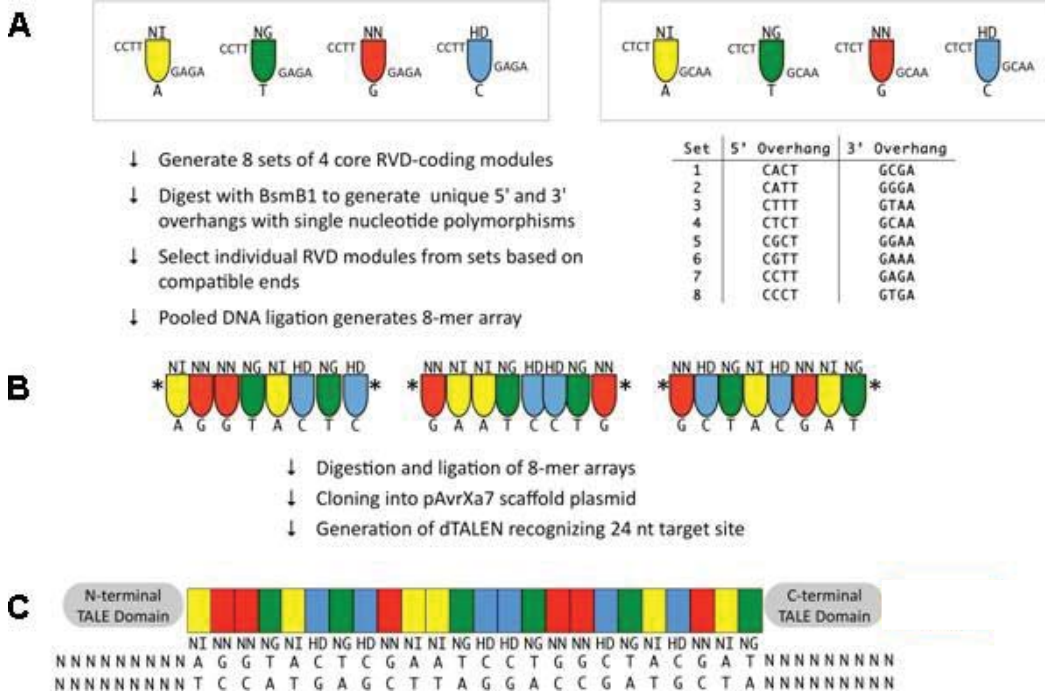**Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors**
J. Boch, et al. *Science* **326**, 1509 (2009).

	HD	NG	NI	NS	NN	N*	HG	H*	IG
A	19	7	**55**	20	7	1	0	0	0
C	**69**	12	5	10	2	7	2	0	0
G	0	0	0	4	7	0	0	0	0
T	5	**50**	0	3	1	1	2	1	1

	HD	NI	NG	NN	NS	N*	HG	HA	ND	NK	HI	HN	NA	IG
N	129	84	82	69	35	27	27	6	4	2	1	1	1	1
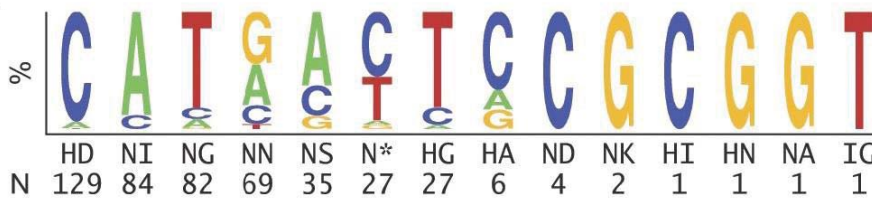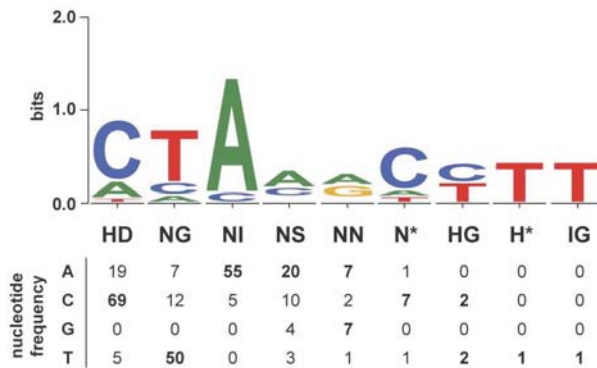
A Simple Cipher Governs DNA Recognition by TAL Effectors
M. J. Moscou and A. J. Bogdanove. *Science* **326**, 1501 (2009).

A

↓ Generate 8 sets of 4 core RVD-coding modules
↓ Digest with BsmB1 to generate unique 5' and 3' overhangs with single nucleotide polymorphisms
↓ Select individual RVD modules from sets based on compatible ends
↓ Pooled DNA ligation generates 8-mer array

Set	5' Overhang	3' Overhang
1	CACT	GCGA
2	CATT	GGGA
3	CTTT	GTAA
4	CTCT	GCAA
5	CGCT	GGAA
6	CGTT	GAAA
7	CCTT	GAGA
8	CCCT	GTGA

B

↓ Digestion and ligation of 8-mer arrays
↓ Cloning into pAvrXa7 scaffold plasmid
↓ Generation of dTALEN recognizing 24 nt target site

C

N-terminal TALE Domain ... C-terminal TALE Domain

NI NN NN NG NI HD NG HD NN NI NI NG HD HD NG NN NN HD NG NI HD NN NI NG
NNNNNNNNNA G G T A C T C G A A T C C T G G C T A C G A T NNNNNNNNN
NNNNNNNNNT C C A T G A G C T T A G G A C C G A T G C T A NNNNNNNNN

Heritable site-specific mutagenesis using TALENs in maize

Si Nian Char[1,†], Erica Unger-Wallace[1,†], Bronwyn Frame[2], Sarah A. Briggs[1], Marcy Main[2], Martin H. Spalding[1], Erik Vollbrecht[1], Kan Wang[2] and Bing Yang[1,*]

[1]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA, USA
[2]Department of Agronomy, Iowa State University, Ames, IA, USA

Summary

Transcription activator-like effector nuclease (TALEN) technology has been utilized widely for targeted gene mutagenesis, especially for gene inactivation, in many organisms, including agriculturally important plants such as rice, wheat, tomato and barley. This report describes application of this technology to generate heritable genome modifications in maize. TALENs were employed to generate stable, heritable mutations at the maize *glossy2* (*gl2*) locus. Transgenic lines containing mono- or di-allelic mutations were obtained from the maize genotype Hi-II at a frequency of about 10% (nine mutated events in 91 transgenic events). In addition, three of the novel alleles were tested for function in progeny seedlings, where they were able to confer the glossy phenotype. In a majority of the events, the integrated TALEN T-DNA segregated independently from the new loss of function alleles, producing mutated null-segregant progeny in T1 generation. Our results demonstrate that TALENs are an effective tool for genome mutagenesis in maize, empowering the discovery of gene function and the development of trait improvement.

TALEN construct and the target maize *Glossy2* sequences

a

Glossy2 locus (GRMZM2G098239)

Exon 1 — Exon 2

+110   +176

5′–TGGCGGACGCGGACCTGGCCTTCAAGCTGCACTACCTGCGCGGGGTGTACTACTACCGGTCCGGGGA
   ACCGCCTGCGCCTGGACCGGAAGTTCGACGTGATGGACGCGCCCCACATGATGATGGCCAGGCCCCT–5′

b
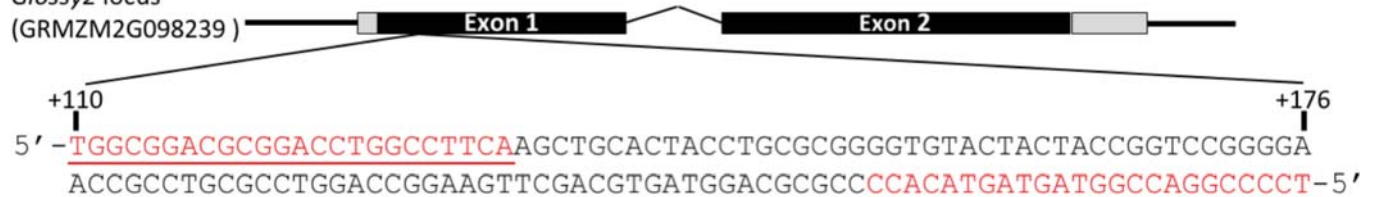
p467   LB — Tvsp-bar-P35S — PZmUbi — TALE-R FN Tnos — P35S — TALE-L FN Tnos — RB

TALENs induce mutations in *glossy2*

a

```
WT          TGGCGGACGCGGACCTGGCCTTCAAGCTGCACTACCTGCGCGGGGTGTACTACTACCGGTCCGGGGA
HT  467.3   TGGCGGACGCGGACCTGGCCTTCAAGCTGCACT-------CGGGGTGTACTACTACCGGTCCGGGGA -7a
HT  467.14 ┌TGGCGGACGCGGACCTGGCCTTCAAGCTGCACTA---GCGCGGGGTGTACTACTACCGGTCCGGGGA -3a
           └TGGCGGACGCGGACCTGGCCTTCAAGCTGC---------GCGGGGTGTACTACTACCGGTCCGGGGA -9
HT  467.18  TGGCGGACGCGGACCTGGCCTTCAAGCTGCACT----GCGGGGTGTACTACTACCGGTCCGGGGA -4
HT  467.22  TGGCGGACGCGGACCTGGCCTTCAAGCTGCACT-----CGCGGGGTGTACTACTACCGGTCCGGGGA -5
HT  467.25  TGGCGGACGCGGACCTGGCCTTCAAGCTGCACT-----CGCGGGGTGTACTACTACCGGTCCGGGGA -5
HT  467.37  TGGCGGACGCGGACCTGGCCTTCAAGCTGCA------GCGCGGGGTGTACTACTACCGGTCCGGGGA -6
HT  467.44  TGGCGGACGCGGACCTGGCCTTCAAGCTGCACT-----CGCGGGGTGTACTACTACCGGTCCGGGGA -5
HT  467.46 ┌TGGCGGACGCGGACCTGGCCTTCAAGCTGCACTA-------GGGGTGTACTACTACCGGTCCGGGGA -7b
           └TGGCGGACGCGGACCTGGCCTTCAAGCTGCACTAC---CGCGGGGTGTACTACTACCGGTCCGGGGA -3b
B104 555.12 TGGCGGACGCGGACCTGGCCTTCAAGCTGCACTA-------GGGGTGTACTACTACCGGTCCGGGGA -7c
```

b

WT

HT #3 -7 bp/WT

Genetic segregation produces null segregants of edited *gl2* in the T1 generation

a

b
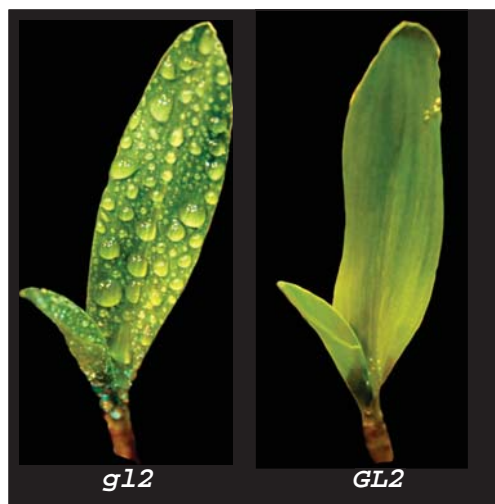
Glossy phenotype of TALEN-mutagenized *glossy2* maize

TAL effector nucleases targeting the Glossy2 gene induce site-specific mutations that confer classic glossy phenotype. Water drops adhered to the surface of the mutant leaf (left) due to reduced epicuticular wax caused by loss of function of the *gl2* gene but not the wild type (right).

An *Agrobacterium*-delivered CRISPR/Cas9 system for high-frequency targeted mutagenesis in maize

Si Nian Char[1,†], Anjanasree K. Neelakandan[1,†], Hartinio Nahampun[2], Bronwyn Frame[2], Marcy Main[2], Martin H. Spalding[1], Philip W. Becraft[1], Blake C. Meyers[3], Virginia Walbot[4], Kan Wang[2,*] and Bing Yang[1,*]

[1]*Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA, USA*
[2]*Department of Agronomy, Iowa State University, Ames, IA, USA*
[3]*Donald Danforth Plant Science Center, St. Louis, MO, USA*
[4]*Department of Biology, Stanford University, Stanford, CA, USA*

Summary

CRISPR/Cas9 is a powerful genome editing tool in many organisms, including a number of monocots and dicots. Although the design and application of CRISPR/Cas9 is simpler compared to other nuclease-based genome editing tools, optimization requires the consideration of the DNA delivery and tissue regeneration methods for a particular species to achieve accuracy and efficiency. Here, we describe a public sector system, ISU Maize CRISPR, utilizing *Agrobacterium*-delivered CRISPR/Cas9 for high-frequency targeted mutagenesis in maize. This system consists of an *Escherichia coli* cloning vector and an *Agrobacterium* binary vector. It can be used to clone up to four guide RNAs for single or multiplex gene targeting. We evaluated this system for its

CRISPR/Cas9 system for targeted mutagenesis in maize
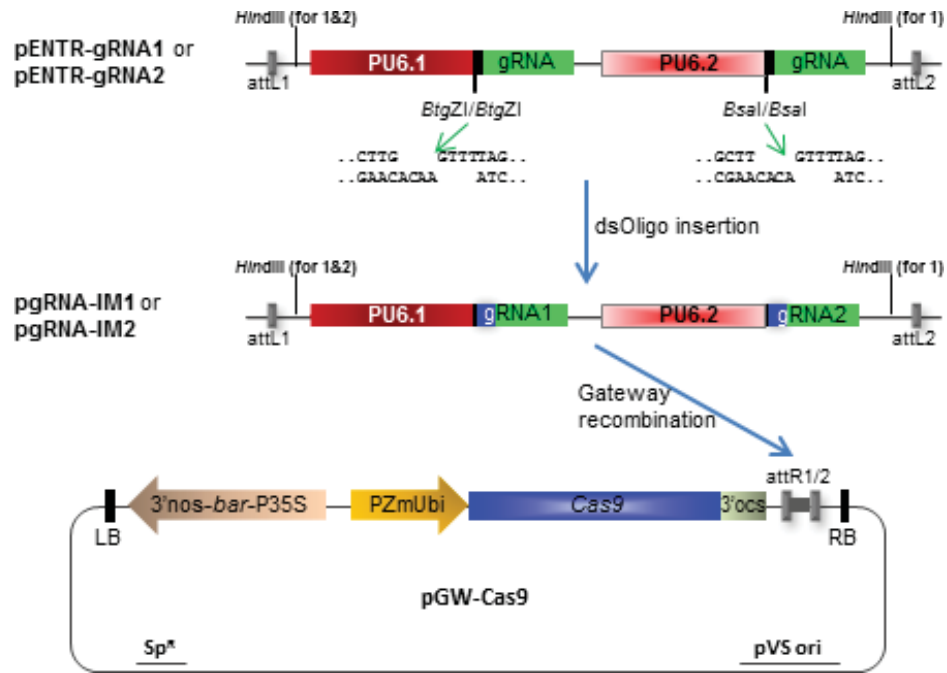
Represented mutations in *ago18a*, *ago18b*, *a1*, *a4*
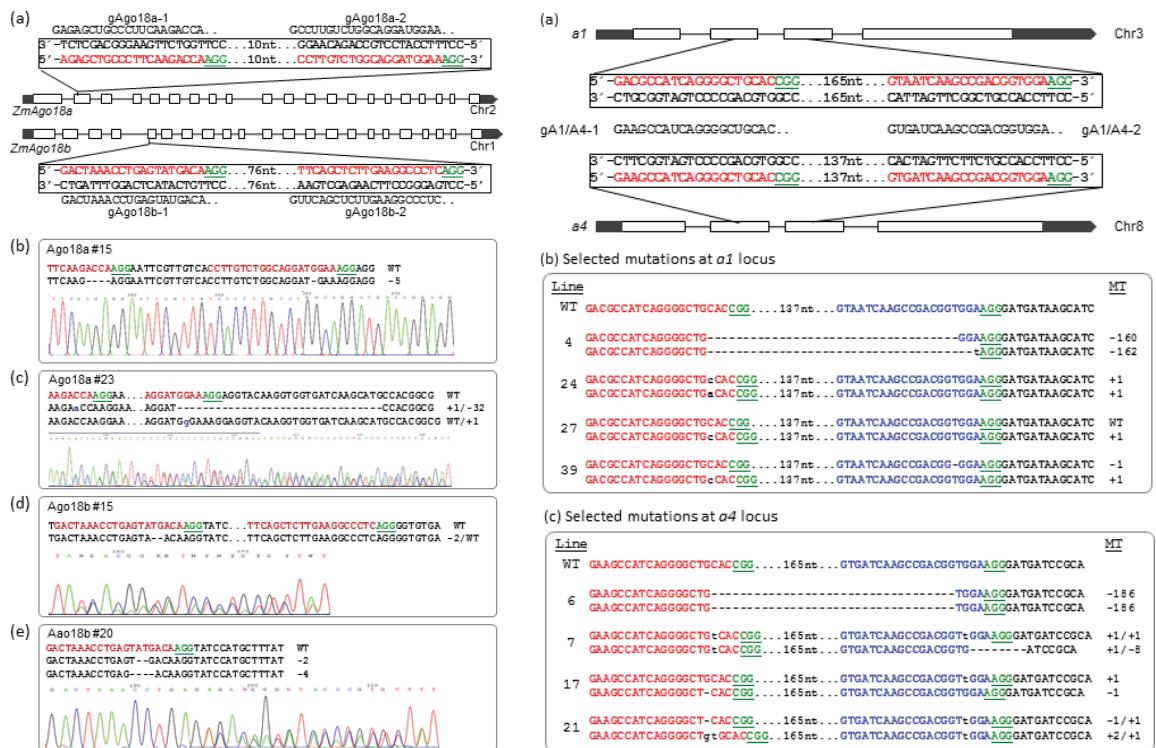
Cas9/gRNA continuously induces mutations in progeny plants
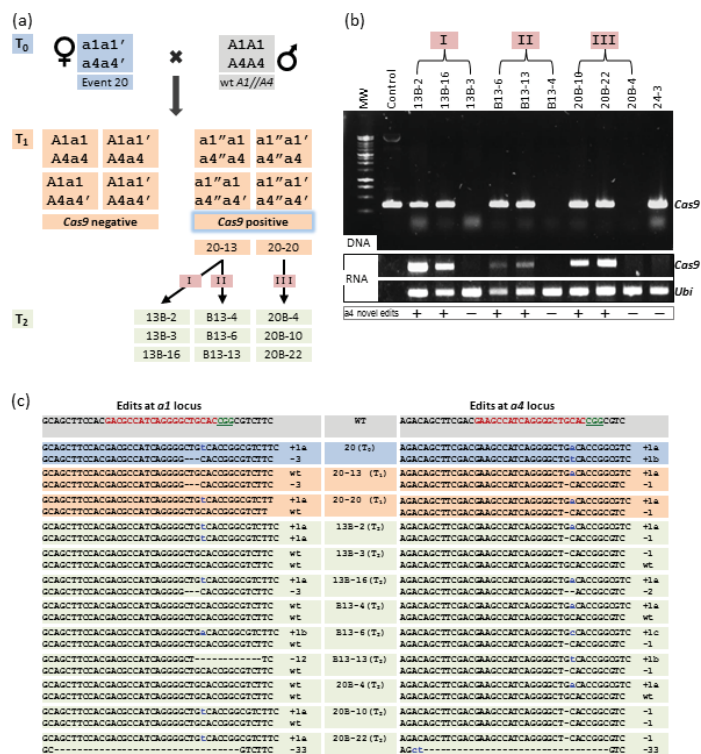
Table 1 Summary of CRISPR mutagenesis frequencies on four genes in maize Hi-II genotype

gRNA	Target gene	# bar+ callus line analysed	# Mutation+ callus line	% Mutation frequency	# Monoallelic mutant	# Diallelic mutant	# Mutation+ line regenerated
gAGO18a	ZmAgo18a	23	17	74	12	5	17
gAGO18b	ZmAgo18b	23	16	70	9	7	16
gAGO18a/b	ZmAgo18a	26	3	12	1	2	22
	ZmAgo18b		4	15	3	1	
	ZmAgo18a&18b		15	58	11 (18a), 10 (18b)	4 (18a),5 (18b)	
gA1/A4	a1	47	7	15	1	6	35
	a4		23	49	1	20	
	a1 & a4		7	15	0 (a1), 0 (a4)	7 (a1), 7 (a4)	

CRISPR/Cas can be used for multiplex targeting, e.g., producing up to 8 guide RNAs

---
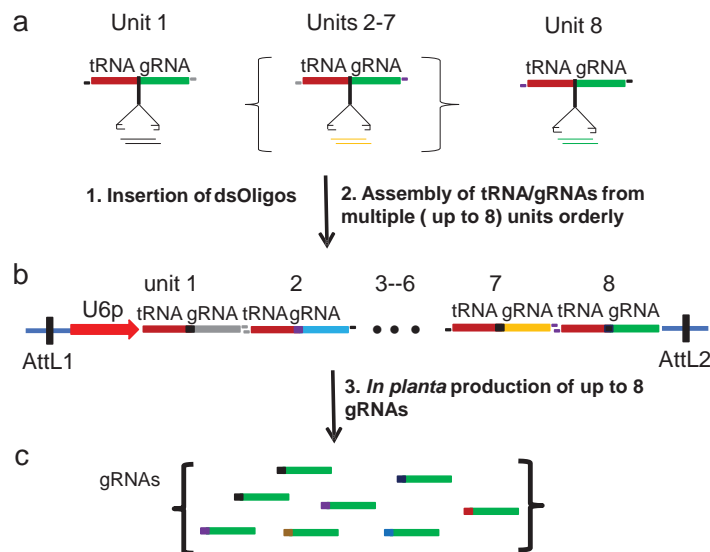
Conclusion

- Engineered nucleases (ZFNs, TALENs, and guide RNA-directed Cas9) are promising genetic tools for genome editing in plants;

- Engineered TALENs are feasible for targeted mutagenesis in maize

- CRISPR/Cas9 is highly efficient to induce site-specific gene mutations in maize

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Virginia Walbot
Stanford University

Donald Weeks
University of Nebraska

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Response to Selection in the ILTSE and a population of epigenetic NILs

Nicholas Heller, Graduate Research Fellow, Department of Crop Sciences and Steve Moose, Professor, Department of Crop Sciences, University of Illinois at Urbana-Champaign

Advances in sequence technology have allowed incredible discoveries about the genomes of many organisms and elucidated relationships between gene and phenotype. These advances have allowed a deeper look into how variation in phenotype is created, selection for this variation is realized, and how phenotypes are inherited. However, many studies found that the heritability of many phenotypes is not fully explained by genomic DNA sequence, especially for quantitative, complex traits.

Here, I present some insights into the contributions to phenotypic response to selection in plants using a genetic system (utilizing the Illinois Long Term Selection Experiment, ILTSE), transgenic system (utilizing the red fluorescent protein driven by the *Floury2* promoter in the maize kernel), and an epigenetic system (utilizing variation created by the *mop1* mutation). Briefly, the ILTSE materials provide a unique resource because they have undergone continuous selection for the same trait for over 100 years and the last 50 years of seed is preserved. Reverse selection experiments are still underway to determine the plasticity of the populations' genomes after 50, 90, and even 100 years of forward selection. Finally, we utilize an inbred system to look more closely at the possibility that some of the response to selection is due to heritable, non-genetic factors.

Response to Selection in the Illinois Long-Term Selection Experiment and a Population of epigenetic NILs

Presented by Nicholas Heller

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The Moose Laboratory

Past and Present

Illinois Long-Term Selection Experiment

ILTSE through 2017

Red Fluorescent Protein

IHP: RFP/0

ILP: RFP/RFP

Relative Frequency Histogram 2015 ---- IRHP2
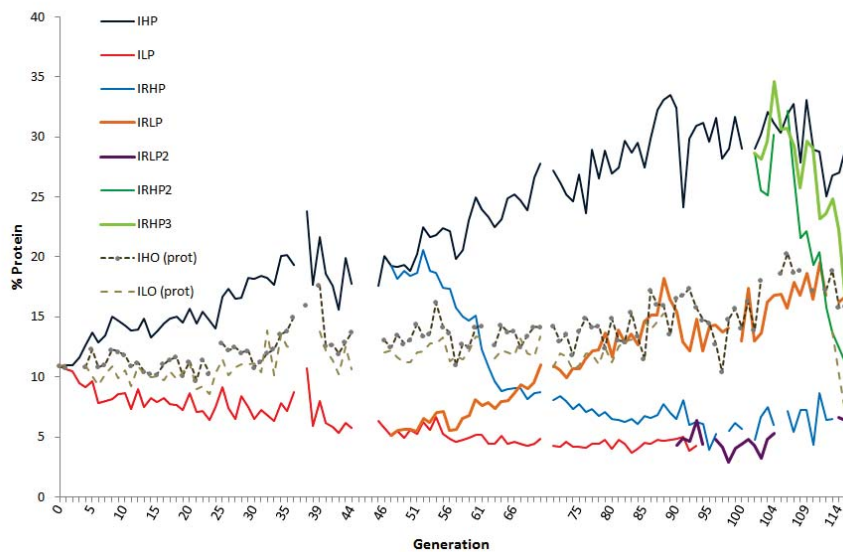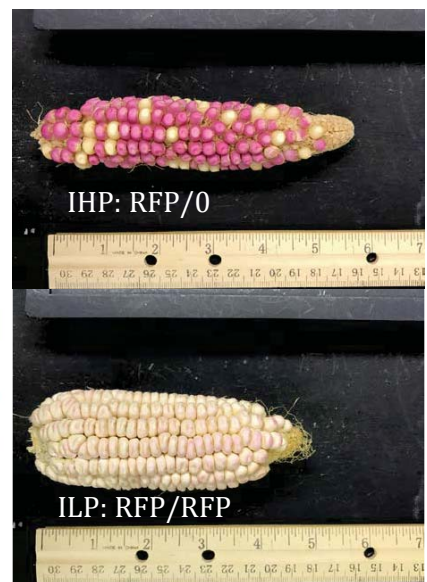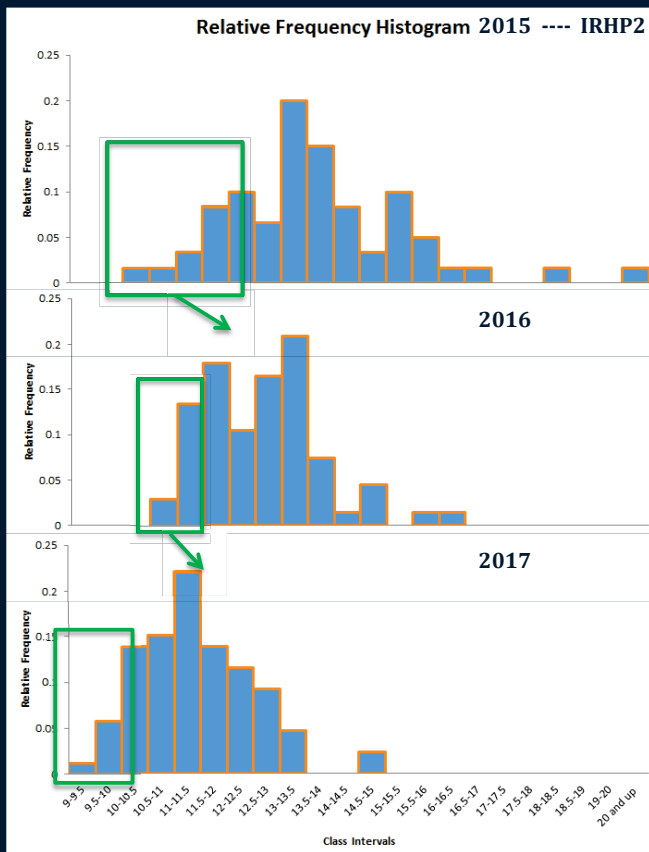
	$\bar{P}_S$	$\bar{P}$
2015	11.6	13.7
2016	11.2	12.6
2017	10.0	11.4

$\bar{P}$ population mean
☐   selected individuals
$\bar{P}_S$ mean of selected individuals

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ILTSE through 2017

The *mop1* mutation

The *mop1* mutation results in a dysfunctional RDR2

and thus, a global reduction in 24-nt small RNA molecules

Barber et al., 2012

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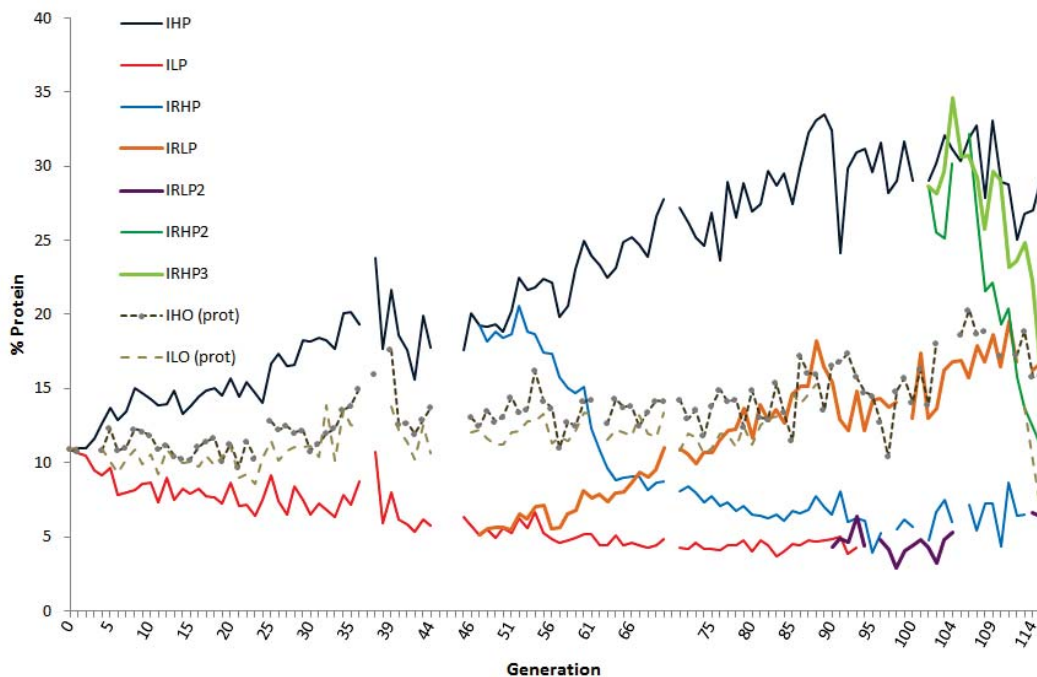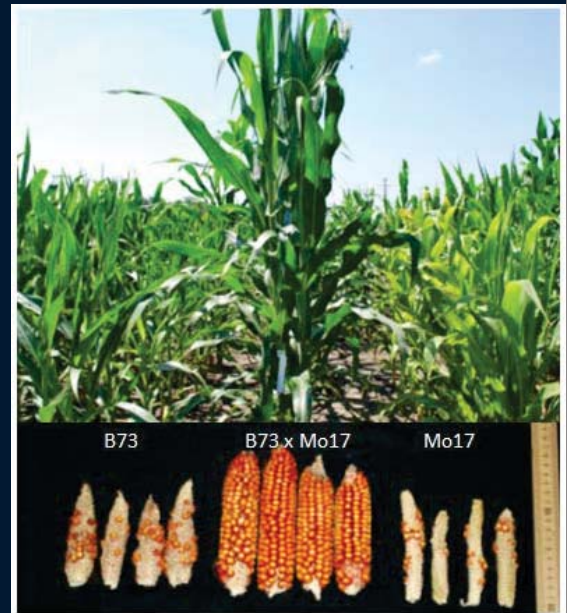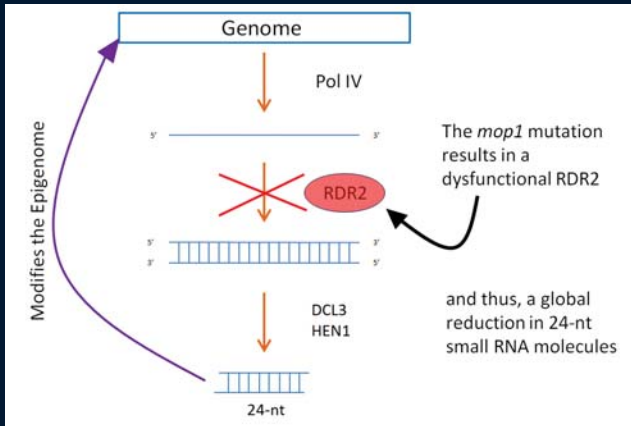
Selection on a Reporter Phenotype – the Red Fluorescent Protein

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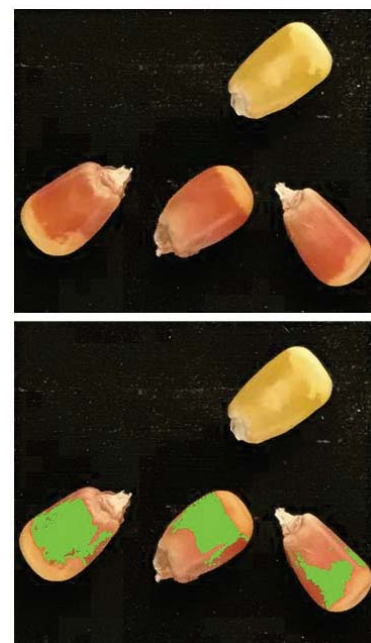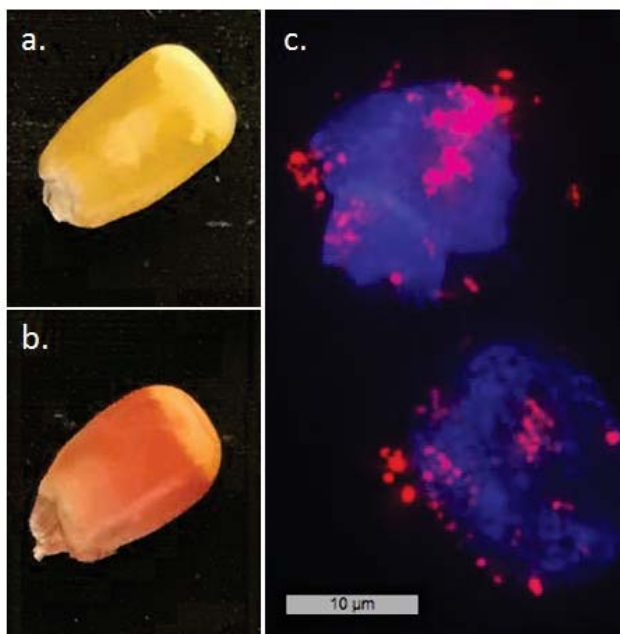
The Zein-RFP system is a worthy reporter for three reasons:

1) **the phenotype (red seed) can be quantitatively measured through imaging techniques;**

2) **the alpha-zeins are responsive to nitrogen supply; and**

3) **alpha-zein genes are known to be sensitive to epigenetic regulation (Miclaus, *et al.*, 2011)**

mop1-induced Phenotypic Variation

Normal   Different 'Light Shows'

mop1–induced Phenotypic Variation in the Reporter RFP
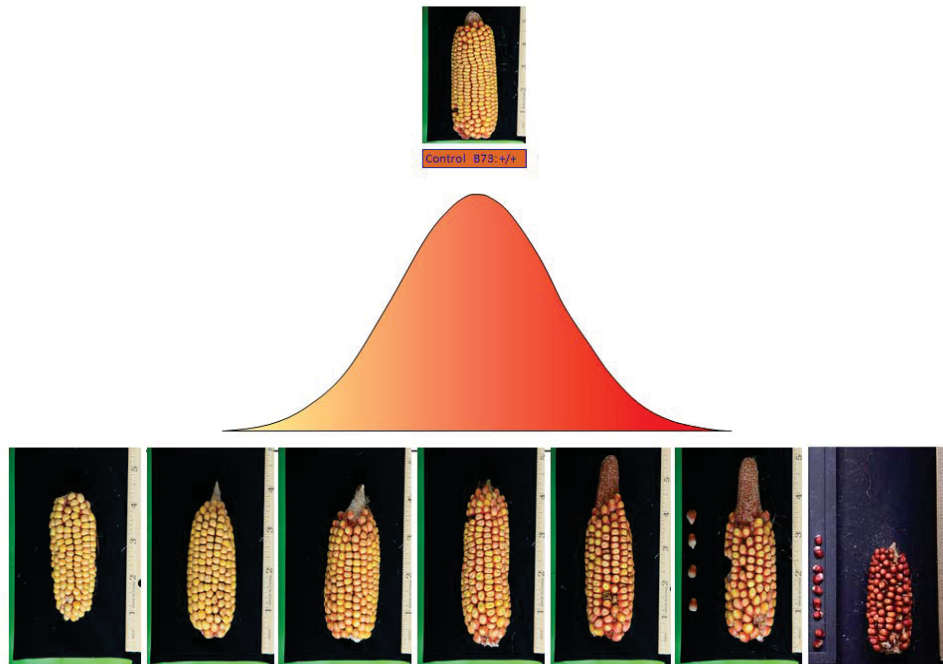
- **Creation of epiNIL population**

- **Genetic similarity of epiNILs (to each other and to B73 control)**

- **Selected on RFP phenotype to create 34 'versions' of B73 (15 High RFP, 15 Low RFP, 4 Medium RFP) plus 4 lineages of the control B73:RFP**

Is the created variation heritable to hybrids?

Cross 14 inbred parents
to the epiNILs:

B73	Mo17	PH207
A632	PHZ51	LH82
PHG39	PHG84	PHJ31
LH1	PHJ33	IHP1
	NC350	ILP1

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- **Subsets of the ~500 hybrids grown over two years**

- **Included select other control crosses between non-epiNIL inbreds**

- **Measured Height, Lodging, Grain Yield, Grain composition (Protein, Starch, Oil, etc.), and the RFP phenotype**

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CS squared: Crop Science x Computer Science

Matthew Hudson, Professor of Bioinformatics, Department of Crop Sciences University of Illinois at Urbana-Champaign

Abstract

The influx of data into Crop Sciences research and development, especially corn breeding, is becoming a flood. At the same time, the increase in the speed of computers (Moore's Law) is slowing down. In order to use the huge amounts of data that are being generated in Agriculture, new types of students and degree programs are needed. The University of Illinois is now offering a joint degree program in Crop Sciences and Computer Science, with the first students starting in Fall 2018. The need for this program and the curriculum will be described in the presentation.

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(CS)²

Crop Science x Computer Science

Matt Hudson

University of Illinois

Science vs Malthus (1766-1834)

- Population, when unchecked, increases in a geometrical ratio, Subsistence, increases only in an arithmetical ratio.

- The power of population is so superior to the power in the earth to produce subsistence for man, that premature death must in some shape or other visit the human race.

- But

- The main peculiarity which distinguishes man from other animals, is the means of his support, is the power which he possesses of very greatly increasing these means.

History of corn yields

U.S. Corn Grain Yield Trends Since 1866
Data Source: USDA-NASS (as of Jan 2017)

Back to 9,000 BC or so
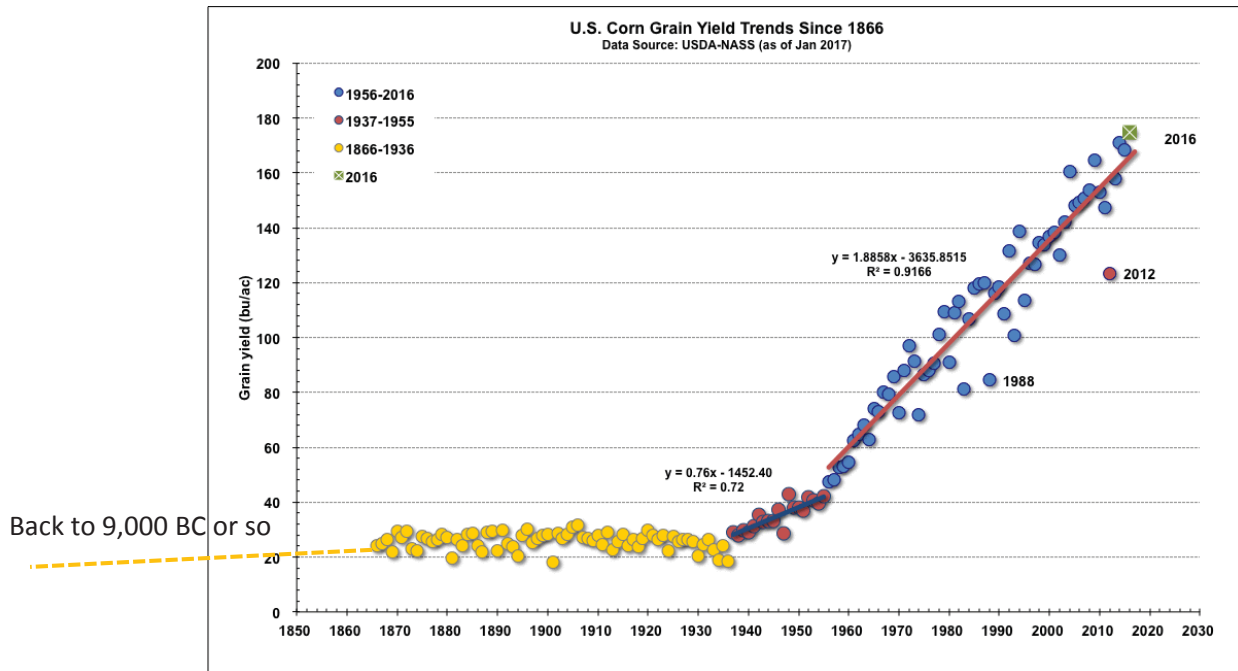
Bob Nielsen

For most of that time, analyzing, moving, and storing data wasn't the biggest problem

Crop Genome sequencing – the first "big data" problem in breeding

human
genome
3.2 Gb
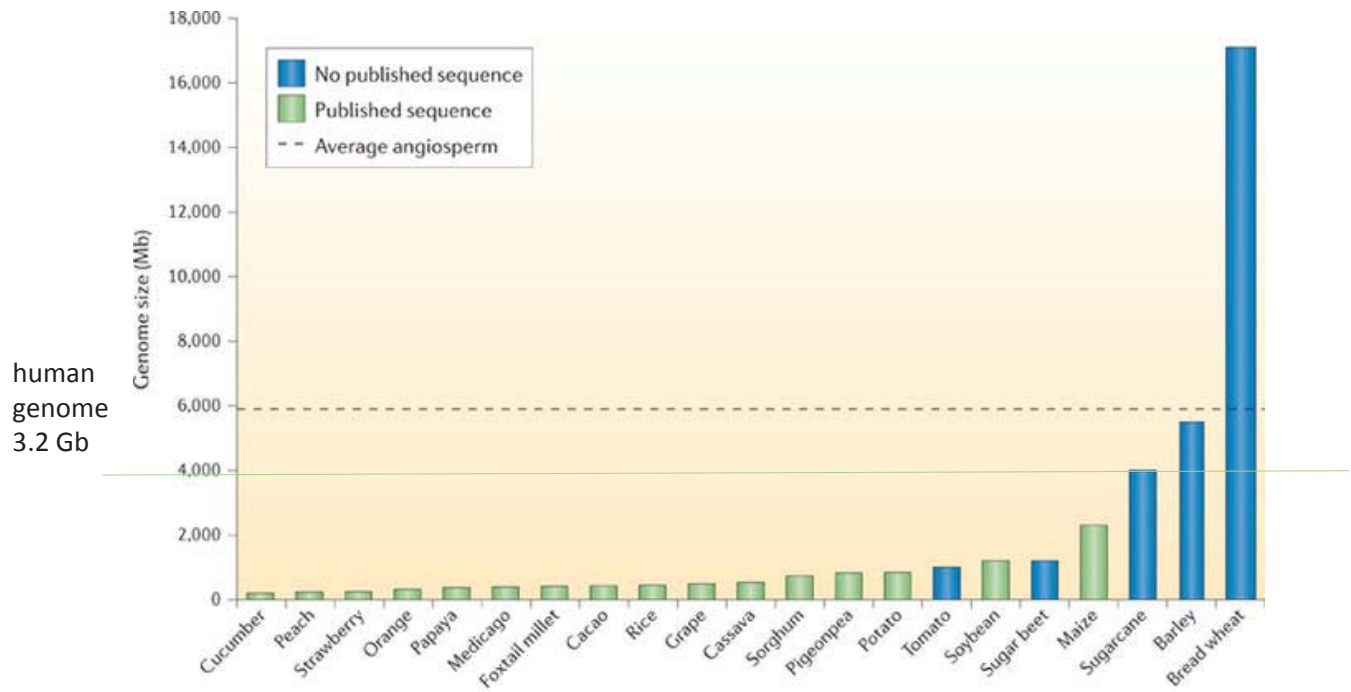
Nature Reviews | Genetics
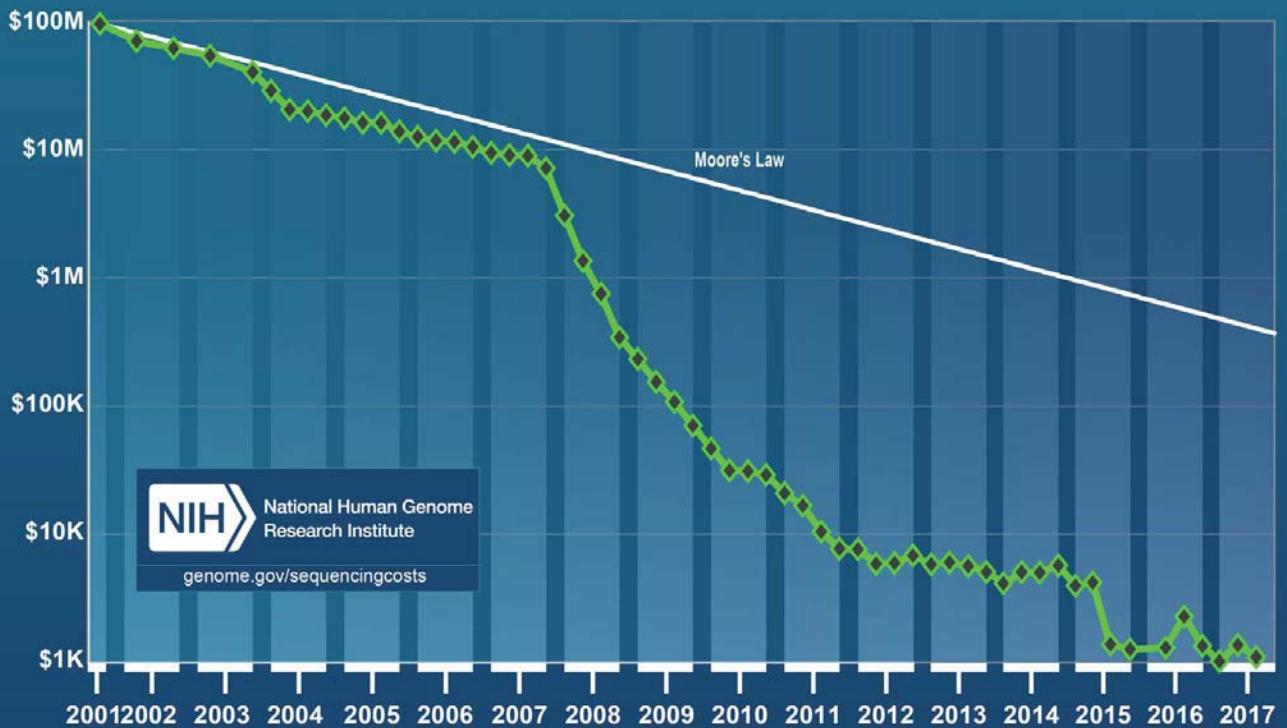
Growth of DNA Sequencing

Stephens et al., PLoS Biol 2015
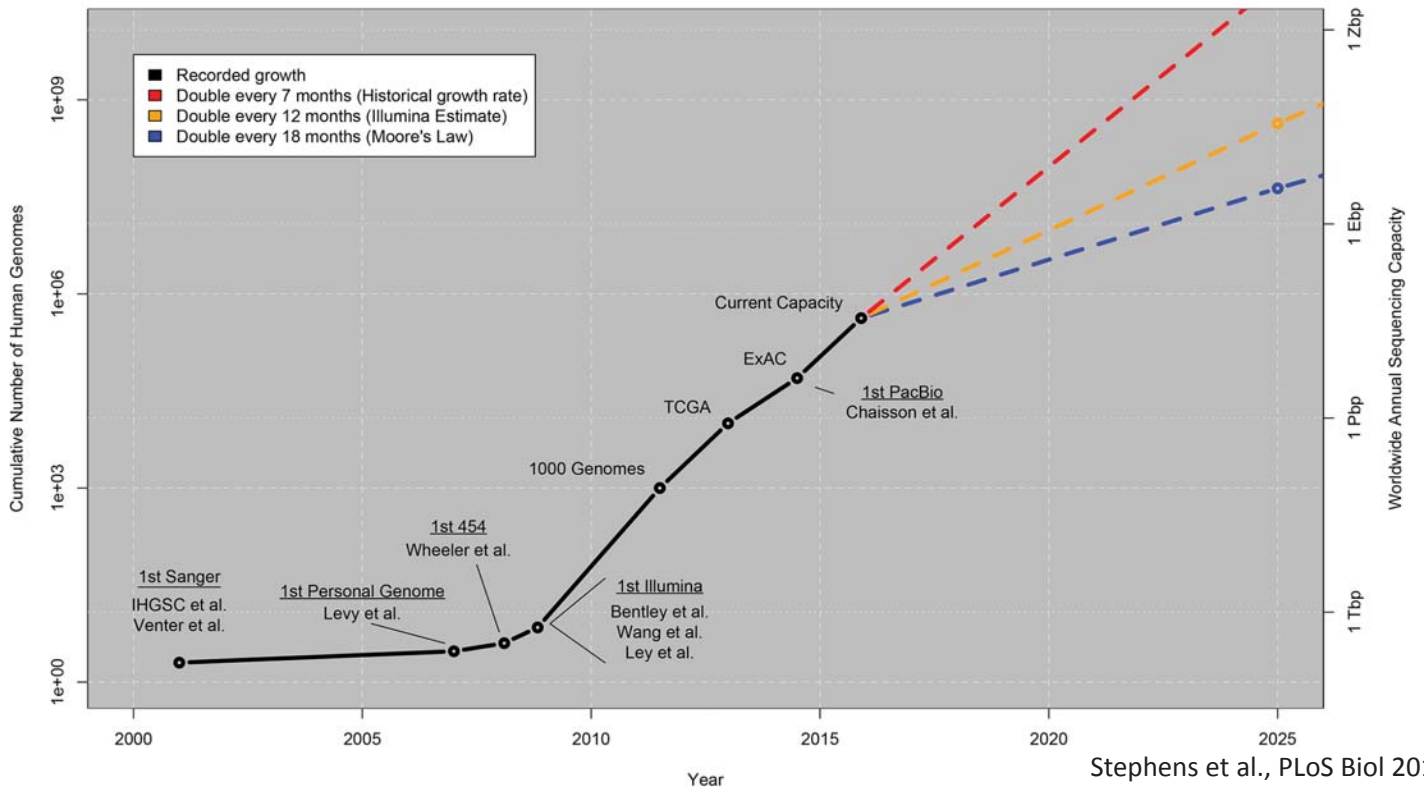
World population growth, 1750-2100
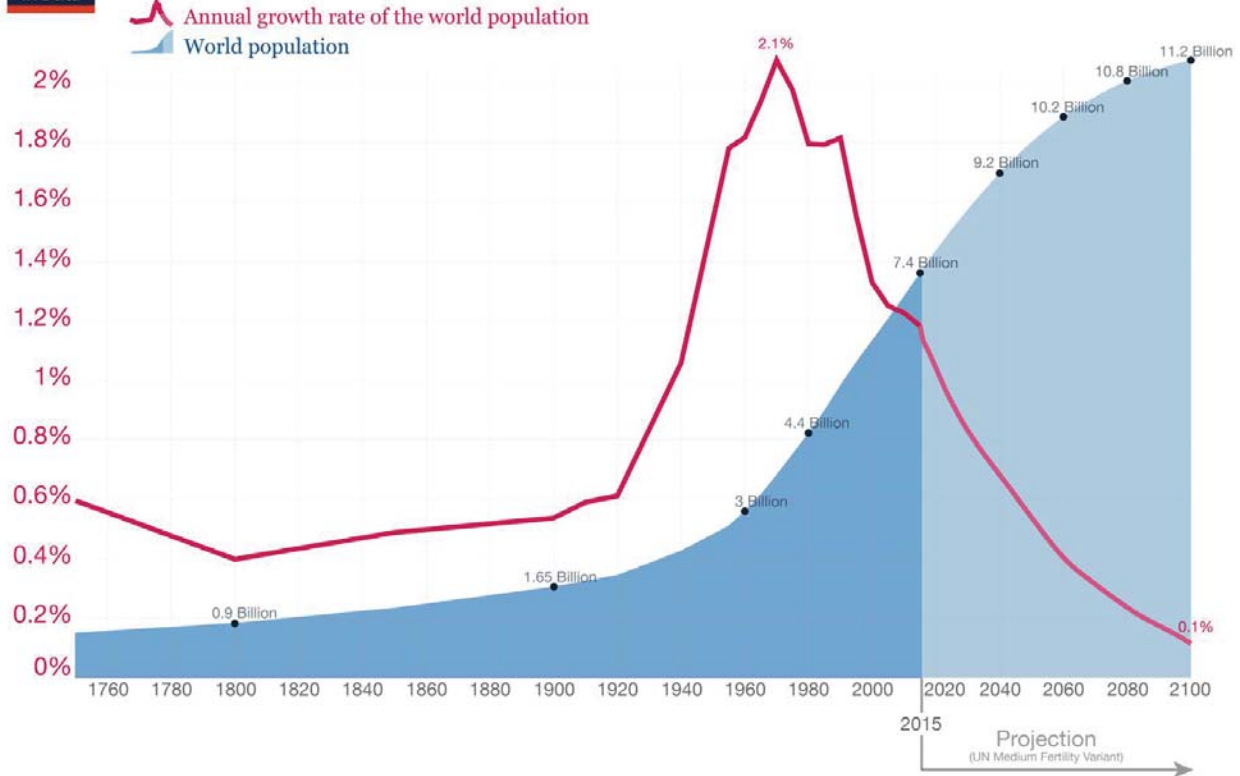
Data sources: Up to 2015 OurWorldInData series based on UN and HYDE. Projections for 2015 to 2100: UN Population Division (2015) – Medium Variant.
The data visualization is taken from OurWorldInData.org. There you find the raw data and more visualizations on this topic.

Licensed under CC-BY-SA by the author Max Roser.

How many of those people have wireless internet?

Mobile subscriptions by technology (billion)
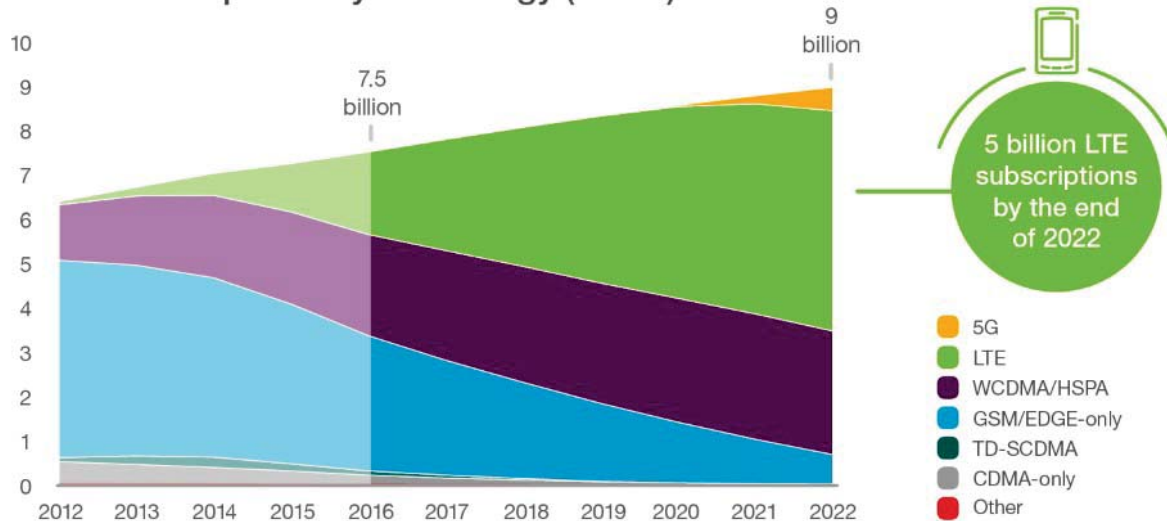
Ericsson 2017

Computers also have a long history

Antikythera mechansim, ~100 BCE
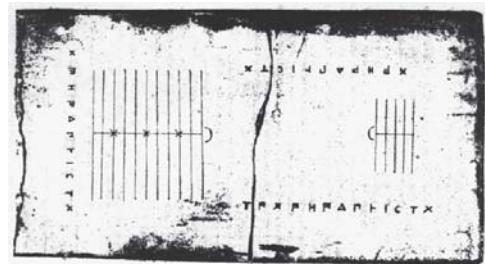
Salamis Tablet, 300 BCE

History of processor performance

Back to 300 BC or so
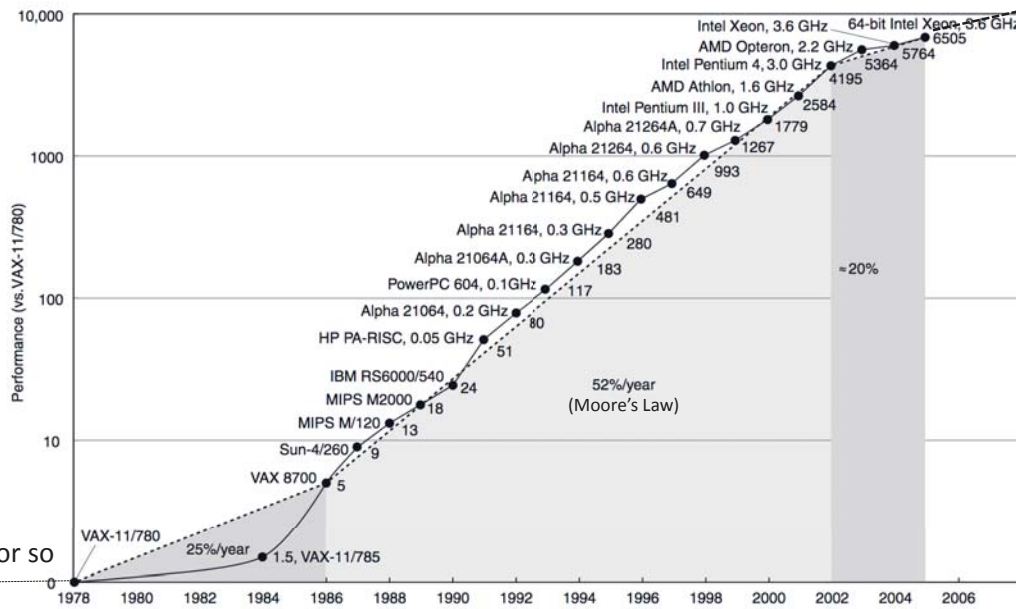
Consistently ~20% per year since 2002

Copyright Elsevier 2008

So many more sources of big data than DNA now

• Soils, physiology sensor data	GBs / field /yr	Pickup bed full of paper
• **Genomics**, phenotypes, models	GBs - TBs / plant	University library
• GPS and planter / sprayer / combine data	TBs - PBs / region	All US academic libraries
• Weather and climate data and models	TBs - PBs/ region	
• Image data from machinery, remote sensing	PBs - EBs / region	Every word ever spoken by every human being

Geo-Eye1 – about 8 terabytes / day

Moore's law isn't going to fix the problem

- We need CS experts qualified to write better algorithms and apps specifically for agriculture

- We need to connect all those wireless data subscribers to their food supply

- We need to get farms connected in the way factories are (Buildings now have operating systems. Machines have their own networks)

The CS^2 undergrad program at U Illinois

- **Joint** degree offered by departments of CS and CPSC

- Students pay tuition at CS rate

- CS Core

- CPSC Core

- Equal weight

CS Core + Technical Track

- **7 Required** CS classes at 100, 200, 300 and 400 level

- Includes data structures, algorithms, programming languages and compilers

- **Technical track**: Also systems programming, computer architecture, two additional 400 level CS classes from approved list

CPSC Core and other requirements

- Genetics, Biotech & Genetic Engineering, Data Science, Stats, Weed Science, Entomology, Plant Path, Crop Management, Plant Breeding, Genomics

- Plus 3x MATH courses, Probability and Stats for CS, CPSC Professional Development, Writing and Public Speaking, ACES 101 and usual Campus reqs (eg Foreign Language).

Enrollment

- First BS students start in Fall 2018

- We're expecting 20-25 per year

- Professional MS degree – watch this space

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UNIVERSITY OF ILLINOIS

'CS + X' degrees add up on many different levels

By JULIE WURTH
jwurth@news-gazette.com

URBANA — Taylor Erwin has a unique résumé for a software programmer.

Yes, he has the requisite computer science training from the highly competitive CS program at the University of Illinois. But there's a "plus" to his degree.

Erwin graduated last May with a "CS + X" degree from the UI, a fairly new option for students who want to combine computer science with another field — in his case, linguistics, or the study of human language.

A "CS + linguistics" degree makes sense for those interested in artificial intelligence and how computers understand natural language (think Google searches, Siri or Alexa).

"I think I found something that combines my passions," said Erwin, 23, who has always loved languages.

Please see DEGREES, A-5

DEGREES: 'I found something that combines my passions'

Continued from A-1

The combined degree also set him apart from other applicants for his job as a software engineer at Qualtrics in Seattle, which designs survey platforms for companies.

"I did my best to try to communicate how the experience I had combining the fields would help me in industry," said Erwin, who grew up in Naperville.

The "CS + X" program — which just added two new options, in the Department of Crop Sciences and the School of Music — grew out of the incredible demand for computer science degrees and the increasingly vital role of data and computing in "just about every area one could imagine," said Lenny Pitt, head of the computer science department.

Ten years ago, about 12 percent of freshmen applying to the UI College of Engineering wanted to be computer science majors. Today, it's about 40 percent.

Computer science is now the second-largest teaching department on campus, behind math — surpassing chemistry, psychology and economics.

"Over 13 percent of applications to the university are for the computer science major. That's nuts," Pitt said.

To keep up, the department...

the College of Engineering came later, in 1972. And the UI added a computer science/statistics degree in 1988.

No 'watered down degree'

The idea behind "CS + X" is that students still get a strong foundation in computer science, so...

Matthew Hudson, a crop sciences professor who uses supercomputing and DNA sequencing to solve problems in plant, animal and human genetics, poses at the National Soybean Research Center in Urbana on Thursday. His research focuses on how crops are bred and on ways to treat and prevent plant, animal and human diseases.

Rick Danzl/The News-Gazette

Options aplenty

The University of Illinois computer science department, ranked fifth in the country, takes in more than 5,000 applications a year (average ACT: 33.5). Here's a breakdown of enrollment in the various CS options for fall 2017:

→ Computer science only: 1,122
→ Math and computer science: 306
→ Statistics and computer science: 203
→ CS + Linguistics: 69

next fall.

Pitt pointed to the technology behind music recognition apps such as Shazam or streaming systems like Pandora that analyze music to figure out what the user likes.

"When you listen to music, you listen on a digital device," he added.

...ics, and the other for technology in production agriculture.

Hudson works in plant genetics, where it takes a supercomputer to handle the enormous amounts of data in genome sequencing. He's also involved in a huge Department of Energy biofuels grant to genetically engineer grasses such as miscanthus, sorghum and sugar cane to produce fuel oil.

Even conventional plant breeding is becoming much more computer-intensive, Hudson said. Researchers are using automatic imaging and "machine vision" with cameras or MRIs to measure the shape and growth of plants and select desirable traits, he said.

On the farm, drones fly over fields to collect data on crops. Tractors have sensors that take images of plants and gather data on yield and soil moisture. Onboard devices help farmers determine exactly how much fertilizer or weed-killer is needed for every square foot of soil, optimizing production and protecting the environment from excess runoff that pollutes water supplies, Bollero said.

"The whole data-in-agriculture thing is just exploding right now, just because these capacities for mobile computing and wireless networking are available on farms in a way they weren't just a few years ago," Hudson said.

Thank you

- Fred Kolb, German Bollero (CPSC)

- Rob Rutenbar, Vikram Adve (CS)

New Developments in Herbicide Resistance and Management Strategies for Waterhemp and Palmer Amaranth

Dean E. Riechers, Professor
Department of Crop Sciences, University of Illinois at Urbana-Champaign

Abstract

Waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*A. palmeri*) are problematic weeds in areas of the U.S. that produce corn, soybean, and cotton, mainly due to their competitive ability, outcrossing nature, genetic diversity, and resistance to multiple herbicide sites-of-action (SoA). Additionally, extended emergence and the ability to produce thousands of seeds per plant allow waterhemp and Palmer amaranth to quickly infest fields if proper preventative or control measures are not taken. Early season competition in corn has the largest effect on yield; the potential for up to 50% yield losses exists if weed control measures are not implemented before V6. Utilizing preemergence (PRE) herbicides to provide residual weed control in corn and soybean during early crop establishment is advantageous in limiting crop-weed competition and reducing the number of plants for postemergence (POST) control.

Waterhemp and Palmer amaranth populations on most farms have resistance to at least one SoA. However, previous research by our weed science group reported multiple resistances to HPPD inhibitors and other POST herbicides in a waterhemp population from central Illinois (MCR), as well as in a waterhemp population from Champaign County (CHR) exhibiting multiple resistances to HPPD inhibitors, atrazine, and auxin herbicides such as 2,4-D. CHR and MCR have also demonstrated variable control with different acetamide herbicides applied PRE. Collectively, these findings indicate that waterhemp and Palmer amaranth populations in the U.S. possess multiple mechanisms conferring complex cross- or multiple resistance patterns.

Laboratory research at UIUC successfully determined underlying mechanisms and identified gene candidates conferring multiple resistances to mesotrione, topramezone, atrazine, imazethapyr and primisulfuron-methyl in MCR. Based on rapid atrazine metabolism, our recent research led to a unique diagnostic tool based on expression of a single *GST* gene to determine whether atrazine-resistant waterhemp possesses metabolic or SoA-based mechanisms. This *GST* can be used as a molecular marker to screen resistant waterhemp populations and, as technology advances, knocking out this *GST* could potentially reverse atrazine resistance. Research conducted at UIUC has revealed when and how resistance occurs in waterhemp and Palmer amaranth in an effort to gain insight into weaknesses that could be exploited for unique and innovative control measures. This new information is necessary to combat existing resistant weeds, prevent new resistant weeds from developing, and ultimately optimize crop yield.

NEW DEVELOPMENTS IN HERBICIDE RESISTANCE AND MANAGEMENT STRATEGIES FOR WATERHEMP AND PALMER AMARANTH

Dean E. Riechers, Professor
Department of Crop Sciences

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Outline of Presentation

- Biology of the dioecious amaranths = waterhemp and Palmer amaranth

- Genetic diversity favors development of herbicide resistant traits

- Integrated weed management systems based on research at UIUC

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Waterhemp
Amaranthus tuberculatus

Waterhemp Management Guide – 1997

ca. 1968

Waterhemp (*A. tuberculatus*) Biology

C4 dicot and dioecious; often confused with Palmer amaranth (*A. palmeri*)

Can be differentiated by their female flowers and petiole:leaf length ratio

Waterhemp has evolved resistance to **six different** herbicide sites of action, including numerous *multiple-resistant* populations (and individual plants!)

Waterhemp Control...

- sound familiar?

STAMPEDE   By Jerry Palen

"... then turn left. It's the place with the weedy-looking corn."

Amaranthus Identification

Common Waterhemp

Redroot Pigweed

Smooth Pigweed

Powell Amaranth

Palmer Amaranth

PA = stem longer than leaf blade

Pratt et al. 1999. Identification of the weedy pigweeds and waterhemps of Iowa.

Amaranthus Stems

<u>Smooth</u> stems

Common waterhemp

Palmer amaranth

<u>Hairy</u> stems

Redroot pigweed
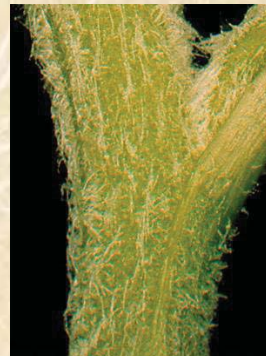
Smooth pigweed
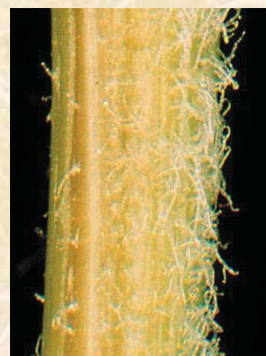
Pratt et al. 1999.

Palmer amaranth Biology

- C4 dicot

- Dioecious – male or female plants

- Inflorescence can be up to 1 meter long

- Produces 200 to 600,000 small seeds

- Multiple emergence events within season

- Distinguishing characteristics

  - long petioles and/or watermarks

- Reduction in corn yield 11-91% with a density of 0.5 to 8 plants per meter

  - 7-35% reduction when emerging after corn

COLLEGE OF
ACES Department of Crop Sciences

Velvetleaf Emergence
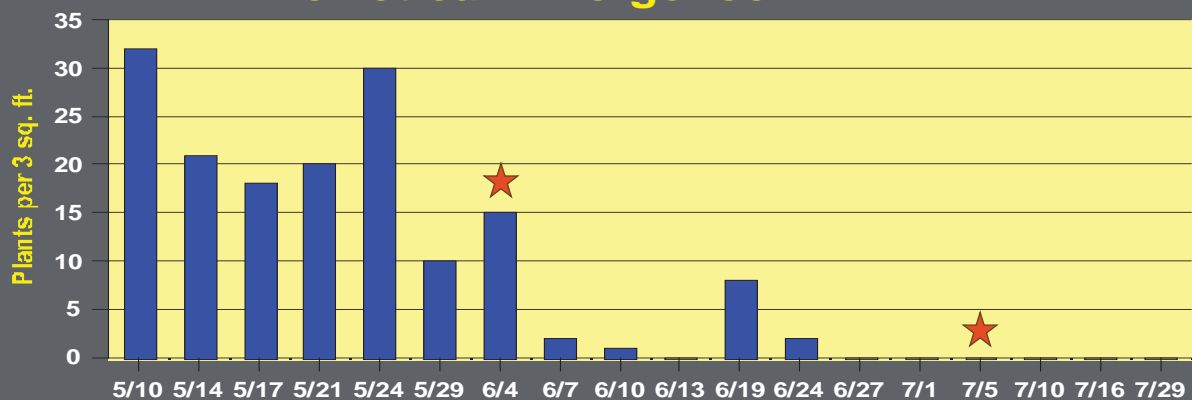
Waterhemp Emergence
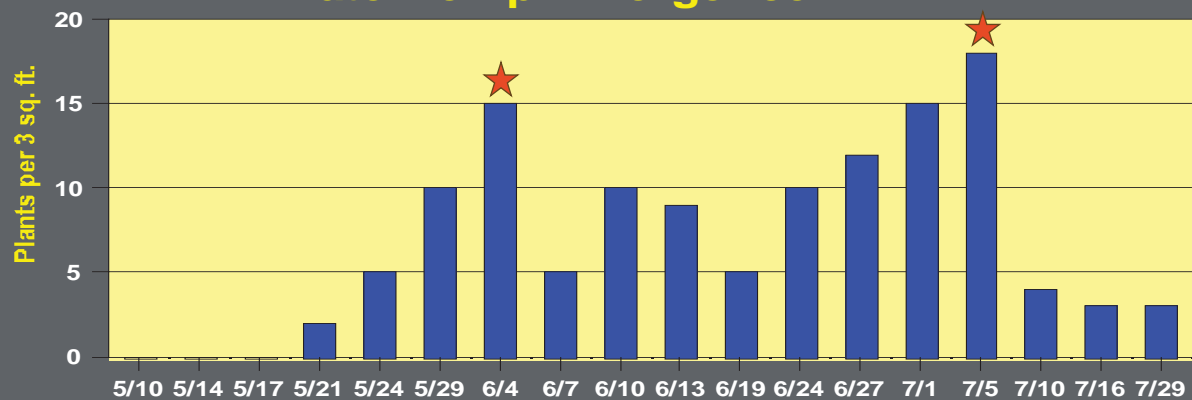
Current Status

Resistance to herbicides from 6 Sites of Action

- Microtubule Inhibitors
    - Preemergence herbicides
- PS II inhibitors (atrazine)
- HPPD inhibitors
    - Only *Amaranthus species* have documented resistance so far...
- ALS inhibitors
- EPSPs (glyphosate)
- PPO inhibitors

Amaranthus palmeri
Distribution in Illinois
2012–2015 sampling
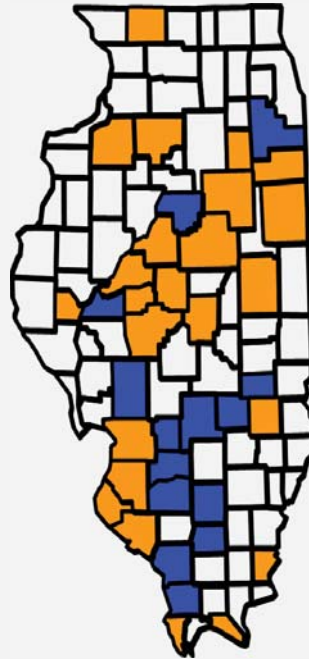
Palmer amaranth confirmed in counties colored orange or blue

Orange – counties with glyphosate-resistant Palmer amaranth
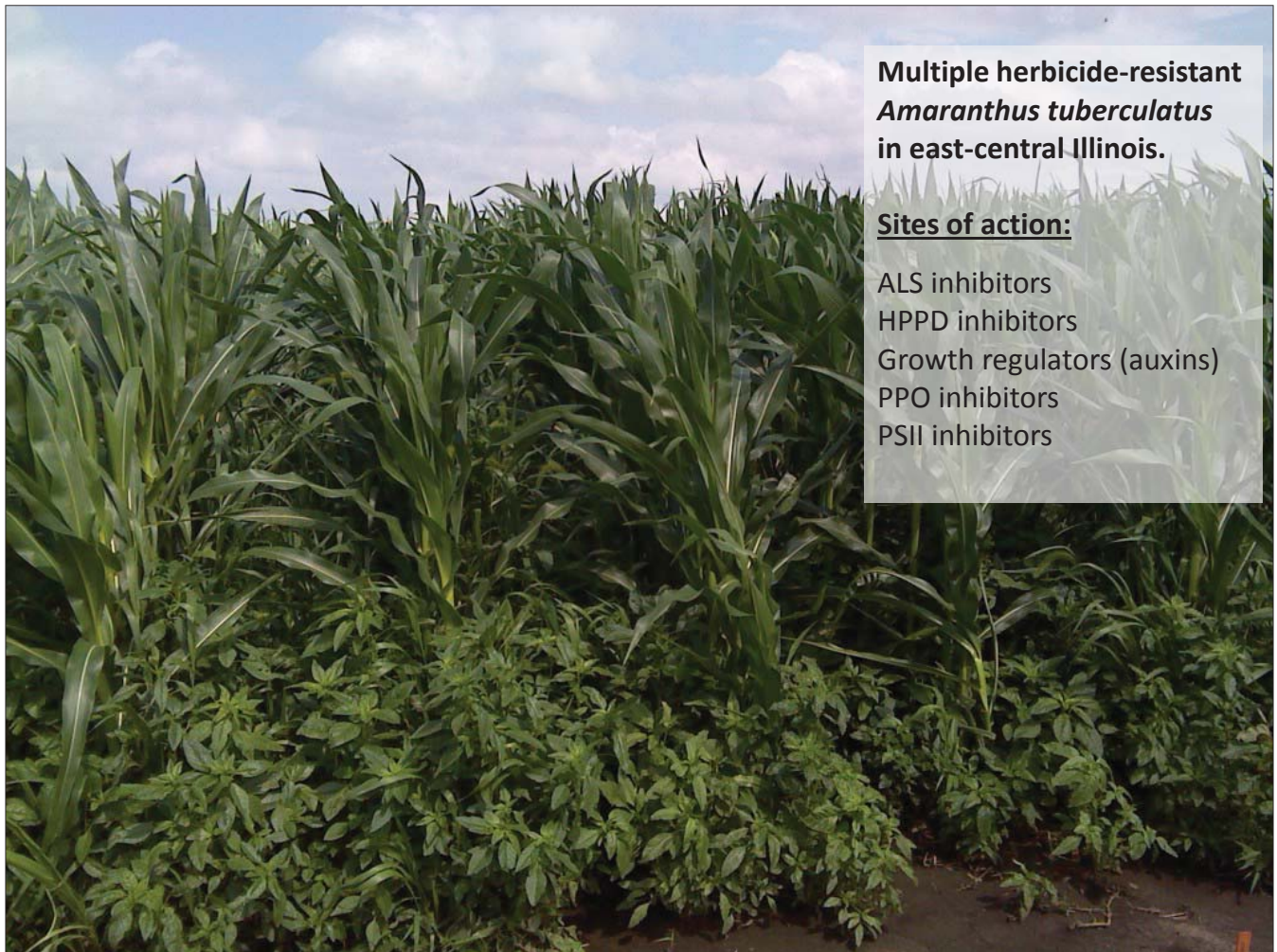
Multiple herbicide-resistant *Amaranthus tuberculatus* in east-central Illinois.

Sites of action:

ALS inhibitors
HPPD inhibitors
Growth regulators (auxins)
PPO inhibitors
PSII inhibitors

Metabolic herbicide resistance in dioecious *Amaranthus*

HPPD inhibitors
S-triazines (atrazine, simazine)
ALS inhibitors

JoVE video protocol
(excised leaf assay for metabolism)

http://www.jove.com/video/53236/measuring-rates-herbicide-metabolism-dicot-weeds-with-an-excised-leaf

mesotrione 420 g ha^{-1}
14 DAT

06.22.2010 14:3

Illinois HPPD-inhibitor Resistant Population

Year	Crop	Herbicides Applied POST*
2003	Seed Corn	mesotrione + atrazine
2004	Seed Corn	mesotrione + atrazine
2005	Seed Corn	mesotrione + atrazine
2006	Seed Corn	topramezone + atrazine
2007	Seed Corn	topramezone + atrazine
2008	Seed Corn	tembotrione followed by mesotrione
2009	Seed Corn	tembotrione followed by mesotrione

*S-metolachlor + simazine were applied each year before crop and weed emergence

Mesotrione (105 g ha^{-1}) - 14 DAT

new growth

WCS   ACR

MCR

Conducted by Nick Hausman

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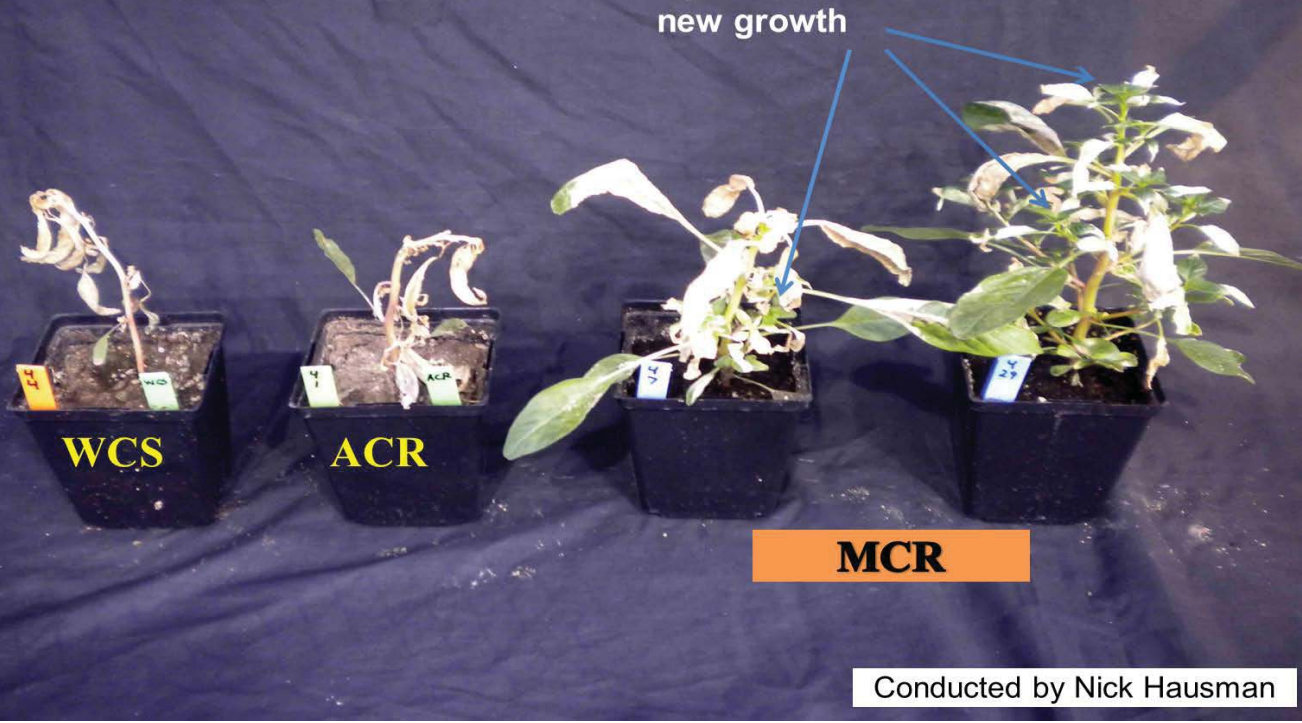
Atrazine resistance due to *metabolic detoxification*

Typical injury observed in resistant plants at 10,000 g/ha 12 DAT

Aatrex
residue

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Maize and grain sorghum, as well as fall panicum and wild-proso millet, are naturally tolerant to atrazine due to high levels of glutathione S-transferase (GST) activity.

Glutathione

GST
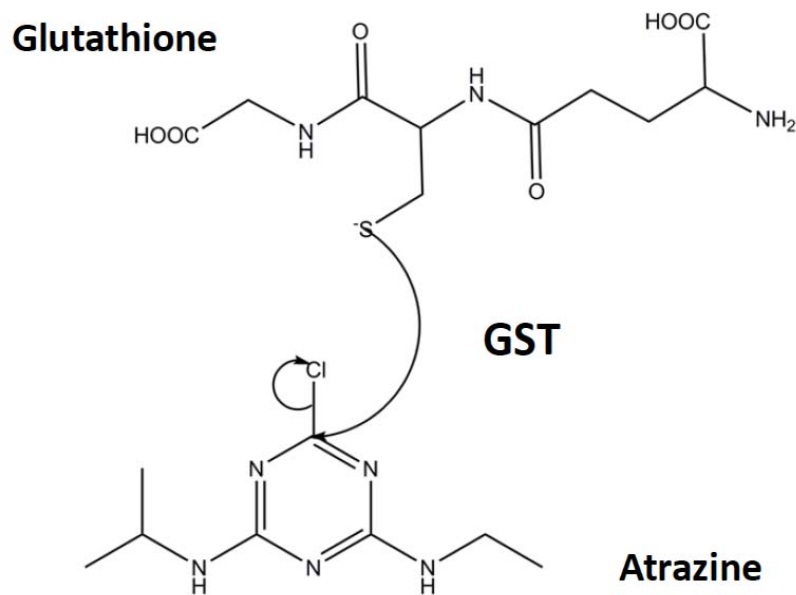
Atrazine

Visual injury following atrazine POST (12 DAT) 14.4 kg/ha

Research Article
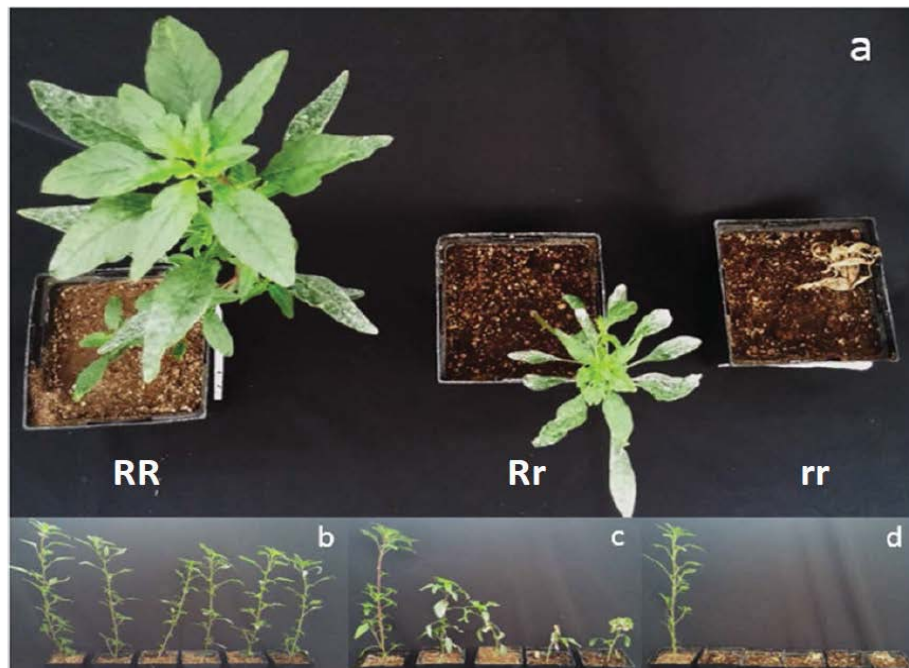
Biochemical characterization of metabolism-based atrazine resistance in *Amaranthus tuberculatus* and identification of an expressed *GST* associated with resistance

Anton F. Evans Jr, Sarah R. O'Brien, Rong Ma, Aaron G. Hager,
Chance W. Riggins, Kris N. Lambert, Dean E. Riechers ✉

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Metribuzin (Sencor, Tricor) is still effective on atrazine-resistant waterhemp
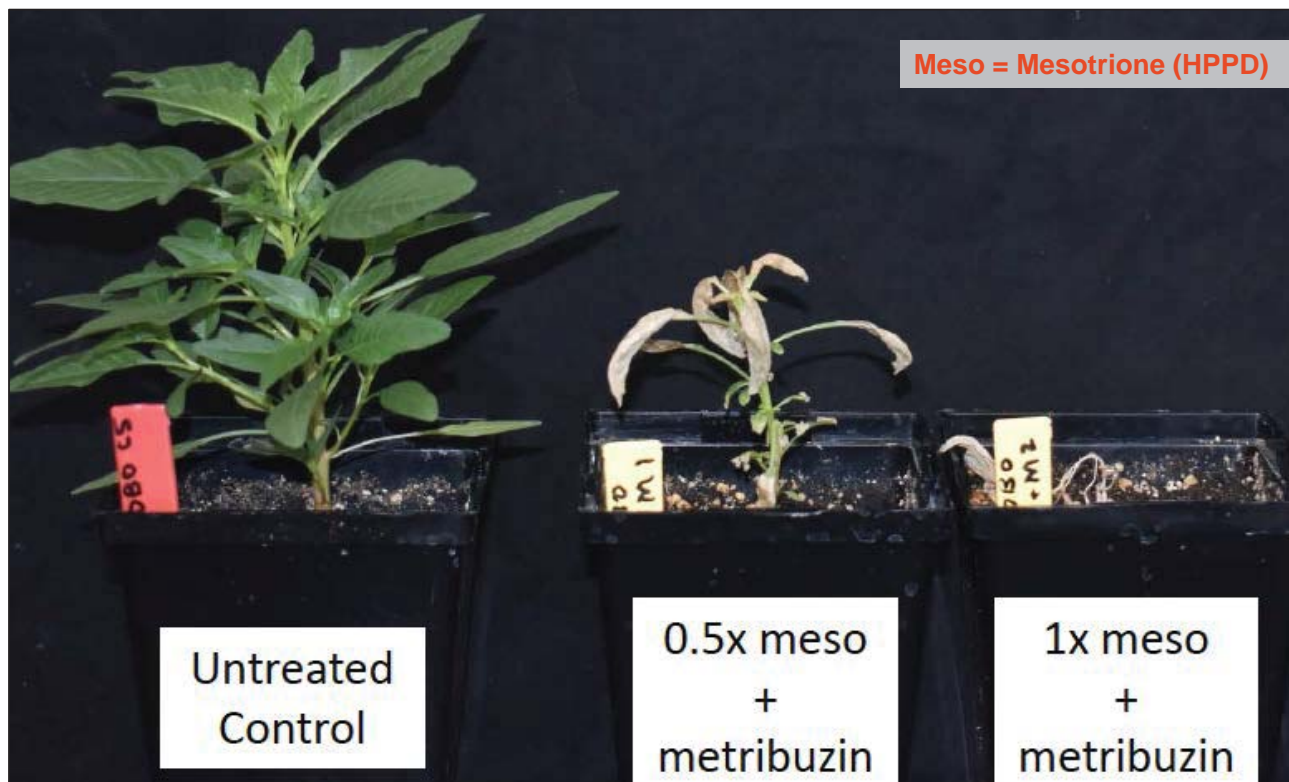
Meso = Mesotrione (HPPD)

Untreated Control

0.5x meso + metribuzin

1x meso + metribuzin

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Manage weed seedbanks
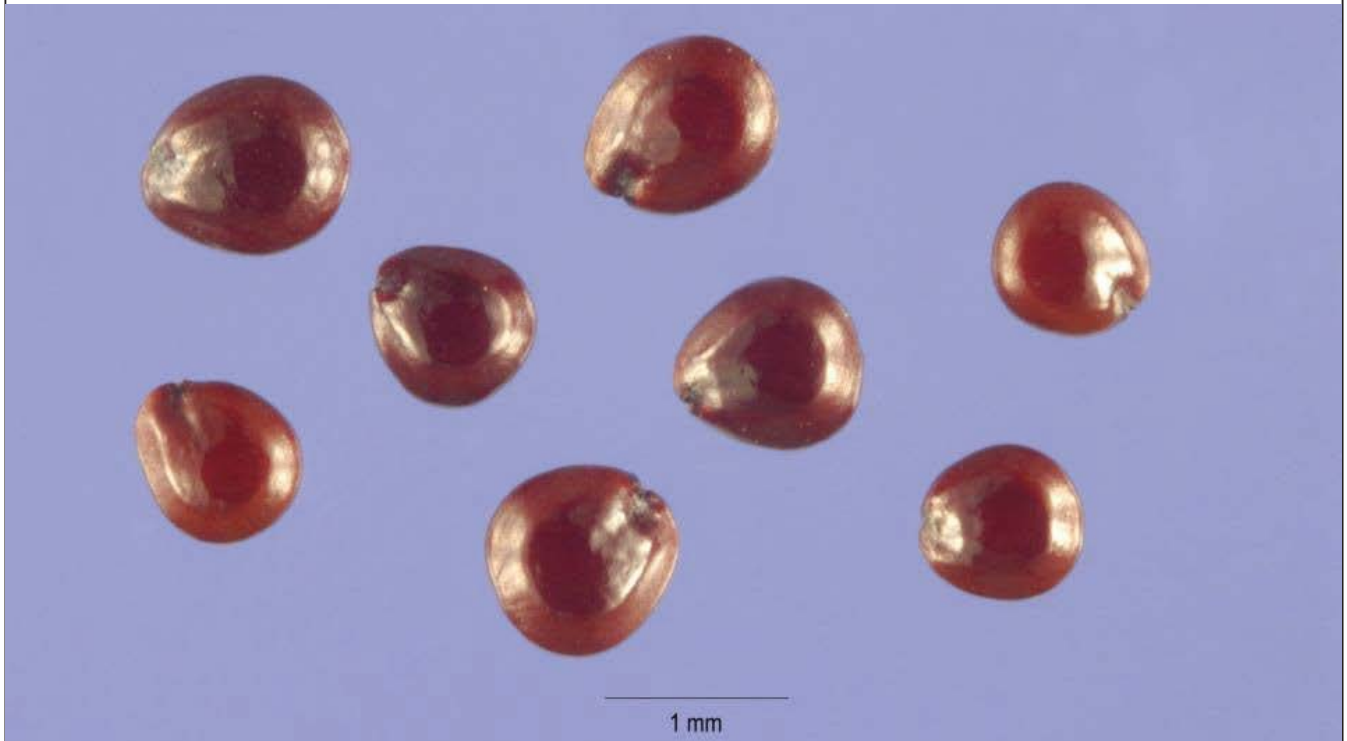
1 mm

JAN 27 2005

Combines are
one of the most efficient
weed seed dispersal
devices ever invented.

= standing weeds
with undispersed seed

2 m

Cousens and Croft, 2001

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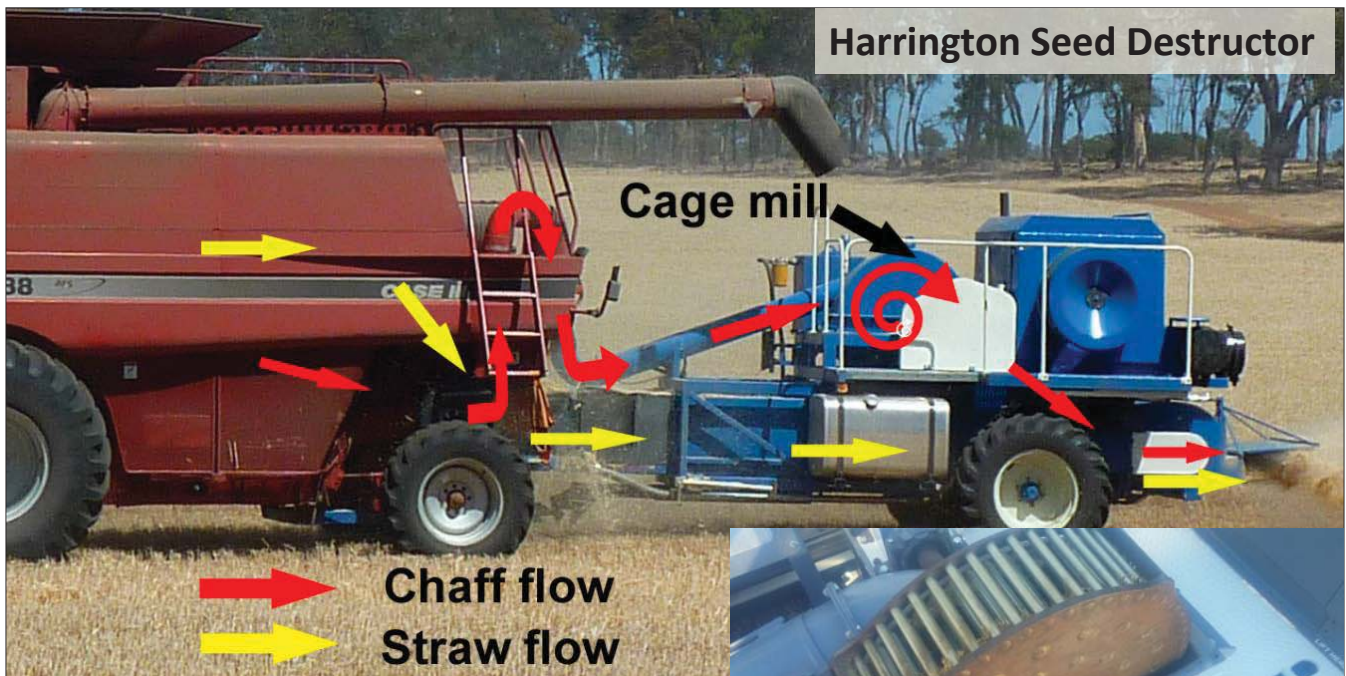
Harrington Seed Destructor

Cage mill

Chaff flow
Straw flow

Walsh et al. (2012)

Fuel use/hr: +0.5 gal (combine); 6-8 gal (HSD)
Weight: 12,000 lbs; tow hitch wt: 992 lbs
Engine: Cummins QSB6.7, 205 hp @ 1800 rpm
Cage mill: 188 hp @ 1400 rpm
Harvest speed: no restriction
Source: DeBruin Engineering, Australia

www.debruinengineering.com.au

HPPD-R waterhemp is not fire-resistant!

(Summer 2011 - greenhouse accident)

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Summary and Conclusions

- Dioecious *Amaranthus* species possess multiple mechanisms for herbicide resistance, including metabolic resistance that *mimics corn and cereal crops*

- Diverse metabolic enzymes (GSTs and P450s, others?) may be encoded by <u>single</u> or <u>multiple</u> genes

- However, metabolic resistance within the HPPD-inhibitor class in waterhemp can be **herbicide-dependent**
  - cross- or multiple resistance patterns

- Integrated management systems should be utilized
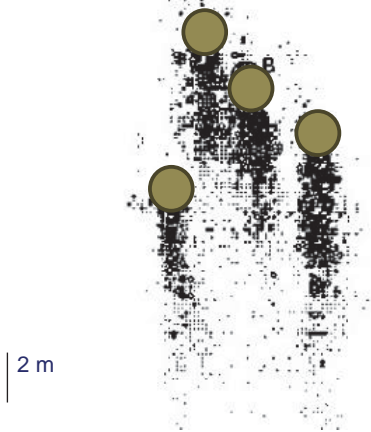
Acknowledgments

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THANK YOU!

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Validation and Implementation of Unmanned Aerial Systems in a Sorghum Breeding Program

N. Ace Pugh, David W. Horne, Geraldo Carvalho Jr., Lonesome Malambo, Anjin Chang, Xiongzhe Han, Jinha Jung, S. Delroy Collins, Sorin C. Popescu, Alex Thomasson, Dale Cope, Murilo M. Maeda, and **William L. Rooney**

Sorghum (*Sorghum bicolor*, L. Moench) improvement scientists have improved many key characteristics in the crop including biomass yield, disease resistance, and height; however, the rate of improvement is stymied by a phenotyping bottleneck. Unmanned aerial systems (UAS) serve as an attractive potential solution to this problem due to their high temporal and spatial resolution. To that end, several studies have been conducted to determine the ability of rotary-wing and fixed-wing UAS to accurately and efficiently phenotype several traits of interest. First, UAS were evaluated for their ability to estimate plant height in various ideotypes of sorghum. Correlations (*r*) between rotary-wing UAS and ground-truth measurements ranged from moderate to very high, dependent upon the ideotype of the measured germplasm. In addition, UAS could estimate similar amounts of genetic variance when compared to the ground-truth methodologies, and repeatabilities (*R²*) were very high. Another trait of interest was that of anthracnose (*Colletotrichum sublineolum*) resistance, wherein normalized difference vegetation index (NDVI) estimates were obtained from a fixed-wing UAS to estimate disease incidence and severity. The NDVI served as an excellent predictor of disease severity in later stages of growth, and showed stronger relationships to grain yield than traditional visual scores. Finally, a rotary-wing UAS system could predict biomass yield with a great degree of accuracy, and the relationship between the UAS measurements. An added advantage of UAS is that they enable high-resolution multitemporal growth and progress curves for height, biomass, and disease presence. Using growth curves, sorghum breeders could make novel determinations about their material and discover new phenotypes that were previously difficult to study.

A field-based high-throughput phenotyping system for tall crops

Maria Salas-Fernandez, Iowa State

High-throughput phenotyping (HTP) technologies have emerged as a consequence of the need to obtain data at large scale, to increase accuracy and repeatability, and to phenotype plants over time for complex traits that could not be characterized by hand. Field-based HTP efforts have focused on the use of unmanned aerial vehicles (UAVs) and the deployment of ground-based platforms carrying sensors or cameras with a top down view on short crops. We have created a novel field-based self-propelled platform equipped with high resolution cameras that was specifically designed for tall crops, to collect images with a side view. This technology has been tested in sorghum and used to obtain plant architecture parameters such as plant height, stem diameter and novel canopy descriptors. The accuracy of image-based algorithmically-derived data was demonstrated when compared with ground-truth measurements. The phenotypic data generated in this project has been used to discover genes/SNPs associated with variation in plant architecture traits and could be further utilized for the genetic improvement of sorghum. The platform and completely automated processing methods developed in our study are new tools for plant breeders and represent significant contributions to the emerging field of predictive phenomics.

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Phenotyping for Fungal Resistance in Corn

David A. Hubert
Senior Scientist - Plant Management and Phenotyping
BASF Plant Science, Research Triangle Park, NC

As ever larger and more complex plant populations are being created and utilized, the need for high throughput screening methods becomes greater. Given the size and cost of these experiments, small mistakes can lead to large consequences. BASF Plant Science is a leader in utilizing high throughput phenotyping for gene discovery and trait characterization. Our focus on imaging and sophisticated sampling has created many learning opportunities to refine techniques and streamline processes. We would like to share our general learnings in working with reverse genetic populations, image analysis, and turning a low throughput assay into a high throughput screen.

Infection by Fusarium species in corn is responsible for yield losses of several hundred thousand bushels of corn annually. Consequently, Gibberella and Fusarium stalk rot are two of the most important diseases in corn. Caused by *Fusarium graminearum* and *Fusarium verticillioides*, respectively, these important diseases are for several reasons possibly the most difficult diseases in corn to study. As such, they also provide an excellent illustrative example of how to move from a very low throughput assay into a high throughput screen.

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Phenotyping for Fungal Resistance in Corn

David Hubert
BASF- Bioscience Research

3/6/2018

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Outline

- About Phenotyping in BASF Plant Science

- Reverse Genetic Populations and Imaging

- Phenotyping for Fusarium Stalk Rot

- Alternative Bioassay for Maize:Fusarium Interaction

- Primary Bioasssay for Maize:Fusarium Interaction

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Facts about Bioscience Research

Approximately 1,100 employees

Biologists, chemists, agronomists, engineers, physicists, technicians and business functions

Four focus topics

Crop Protection R&D

White Biotechnology

Plant Science

Experimental Toxicology & Ecology

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Plant Science

Crop Protection R&D

Plant Science

White Biotechnology

Experimental Toxicology & Ecology

Plant Science aims for a better quality of life and an improved environment. We drive innovative solutions for agriculture, nutrition and industrial applications, creating value for BASF and customers.

Plant Science expertise in plant biotechnology is applied to understand crops and to enhance their performance.

Plant Science is focusing on four strong pillars: yield increase, omega-3 fatty acids, herbicide tolerance and fungal resistance. We collaborate with BASF's Operating Divisions or partners such as Monsanto and Cargill.

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About Phenotyping at BASF Plant Science

Focus on Gene Discovery and Better Understanding Traits

Strength in Image Analysis
- Easily automated
- Data can be analyzed many times as new information becomes available
- Highly quantitative for downstream and future analyses

Strong Interactions between Greenhouse and Field Research
Ensures relevance of greenhouse research and good use of resources

High-Throughput Phenotyping and Sophisticated Sampling
Multiple screens running simultaneously each with multiple phenotypes utilizing multiple species studying multiple traits

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Keys to High Throughput Phenotyping

Teamwork

Preparation

Defined Goals

Automation

Specialization

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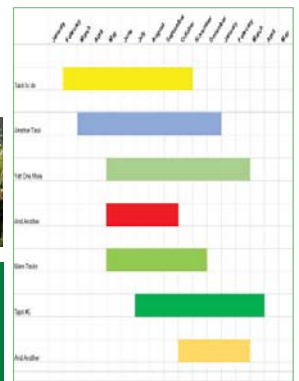
Phenotyping in Reverse Genetic Populations

Knowledge of Trait as the Source of Genetic Diversity

Types of Populations

- Transgenic lines
- TILLING
- Knock-outs
- Activation-tagging

- Non-Random

- Expensive
- Scalable
- Feedback
- Highly Knowledge Dependent
- Low genetic and phenotypic diversity

Characteristics

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Considerations When Working in Reverse Genetic Populations

Issues
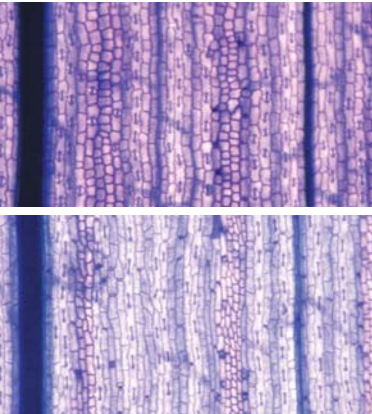
- Mutagenesis/ Transformation affects plant growth
- Hidden genetic contributions
- Positional Effects
- Strong Nursery Effects

What is Your Control?

- Wildtype
- Segregating Null
- Empty Vector
- Efficacious Gene
- Experimental Average
- Phenotypic Extremes
- Mock Treatment

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Important Considerations for Image Analysis

Set Your Camera on Manual

GIMP GNU IMAGE MANIPULATION PROGRAM

Use a Color Card

File Type is Important

- ■ Pixels = Data
- ■ Image Compression
  - ▶ Lossy v. Lossless
- ■ TIFF
- ■ RAW
  - ▶ Large File Size
- ■ Some PNGs
- ■ No JPEGs

Low Complexity

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Creating a High-Throughput Screen

Develop

- ▪ Qualitative to Quantitative
- ▪ Reproducible
- ▪ Efficient
- ▪ Relevant
- ▪ Identify Controls

Validate

- ▪ Stable
- ▪ Scaling
- ▪ Experimental Design
- ▪ Sample Size

Implement

- ▪ Consistency
- ▪ Necessary Adjustments
- ▪ Quality

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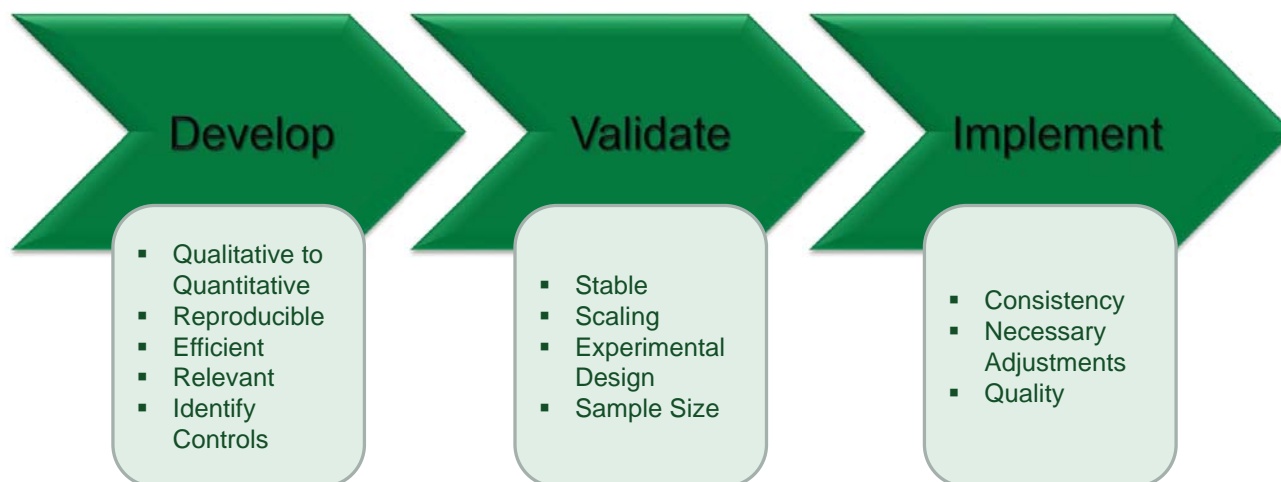
Gibberella and Fusarium Stalk and Ear Rot

Maize diseases caused by *Fusarium graminearum* and *Fusarium verticilloides* have big impacts

Disease	2012 Yield Loss (millions of bushels)
Fusarium seedling blight	37.5
Gibberella stalk rot	43.9
Fusarium stalk rot	124.6
Fusarium ear rot	91.6
Gibberella ear rot	38.7

- Yield loss
- Lodging
- Mycotoxin contamination

Estimated corn yield loss from diseases in the top 22 U.S. corn producing states and Ontario, Canada, in 2012
Mueller and Wise, Purdue Extension publication, 2014. BP-96-12--W

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Stalk Rot Assay- Practical Aspects for Measuring Infection
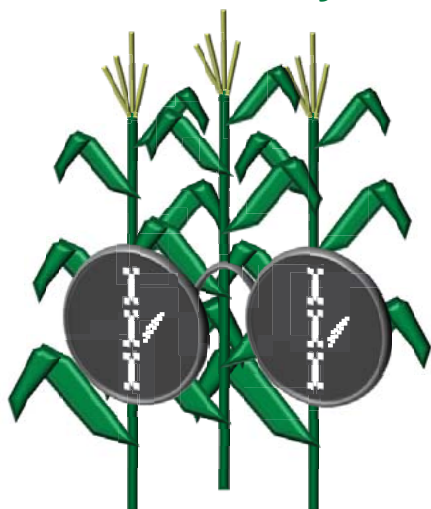
No Mock Controls

Requires Destructive Sampling

Planar Spread

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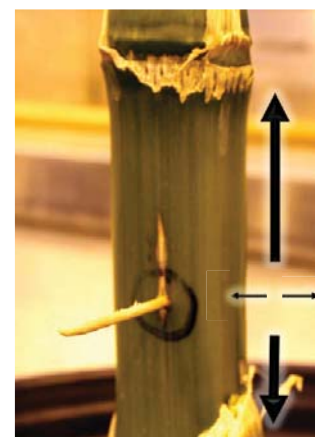
Stalk Rot Assay- Practical Aspects for Measuring Infection
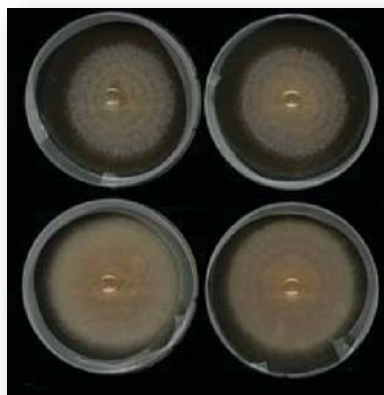
Higher Inoculum Titer
≠
More Symptoms

Nodal Plate Slows Infection Spread
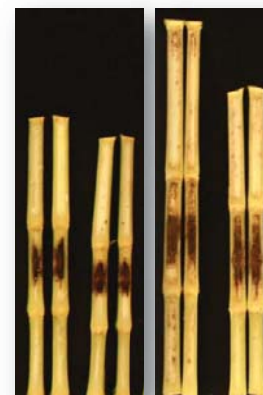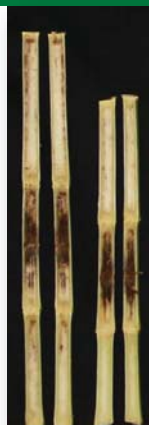
Developmental Regulation

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An In vitro Fungal Resistance Screen

Transgenic corn callus is formed

Transgenic corn callus is placed in multi-well containers

Spores are added and allowed to infect the callus

Supernatant is mixed with pH indicator and measured
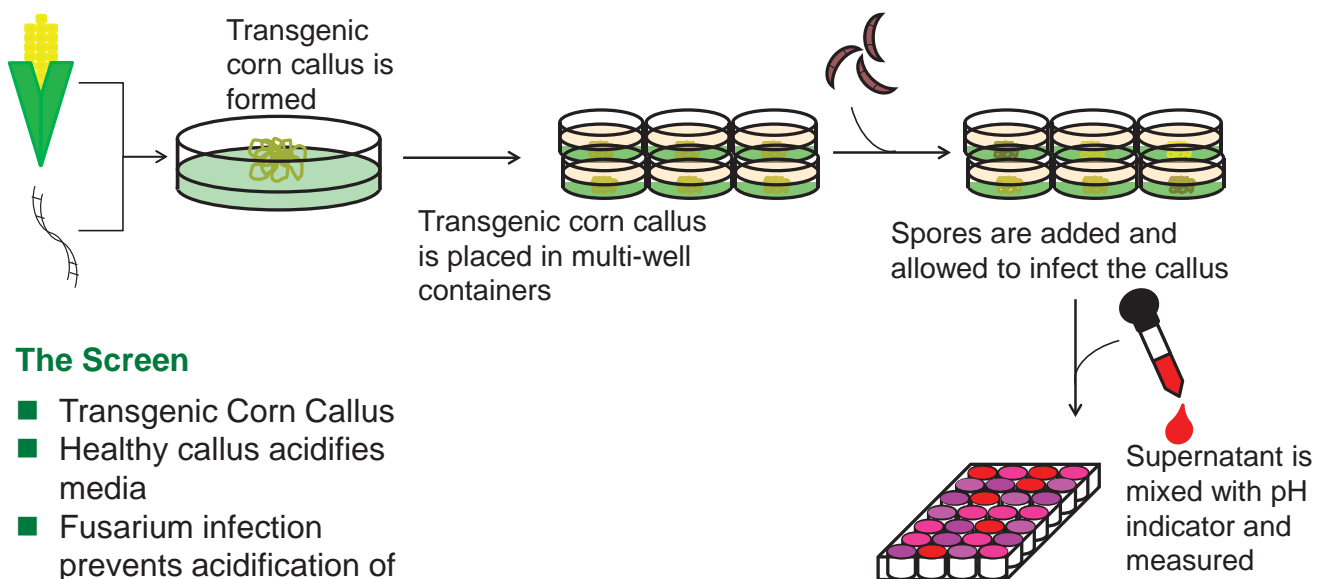
The Screen

- Transgenic Corn Callus
- Healthy callus acidifies media
- Fusarium infection prevents acidification of media
- Lower pH = Resistance

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pH Can Detect Pathogen Effect on Plant Tissue

Fusarium graminearum
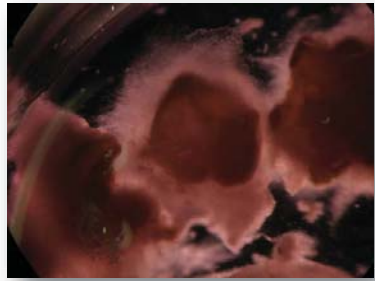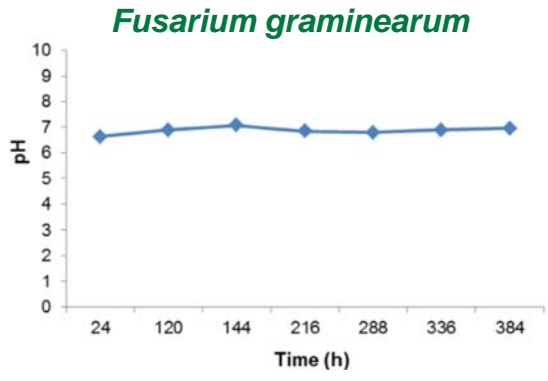
Empty Vector
Absorbance: 1.3976

Bax Inhibitor-like
Absorbance: 1.1279

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Differences in Fungal Growth Are Observed

Fg

EXTERNAL
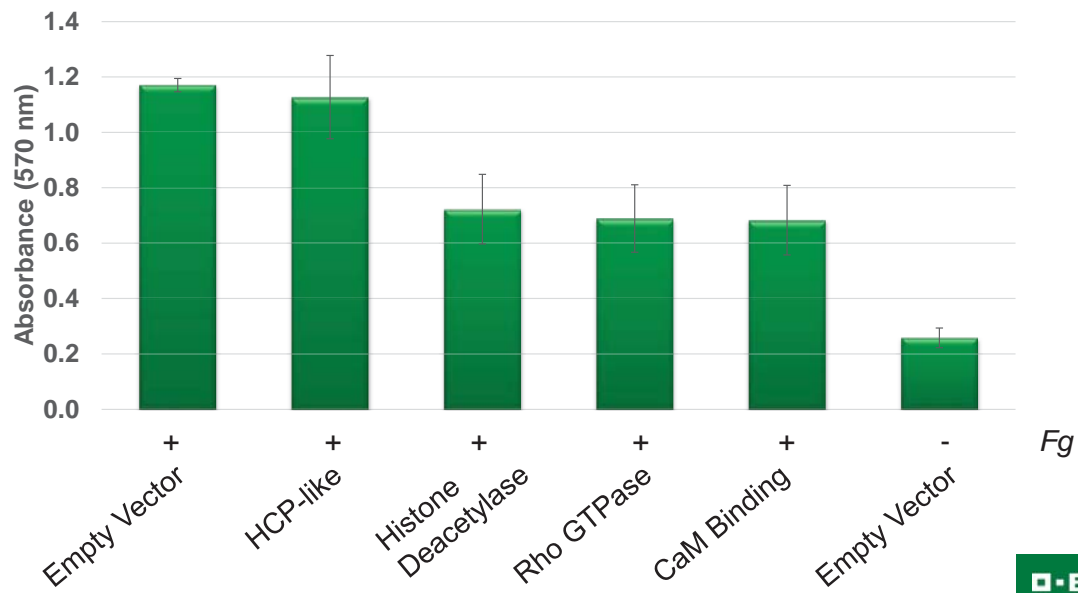
Using Multiple Phenotypes to Study an Interaction

The Screen

- Transgenic Hybrid
- Segregating Null Hybrid
- Infect at Tasseling
- After Imaging, Stalks are Dried
- Glucosamine and qPCR
  - Symptoms/Damage
  - Entire Fungal Biomass
  - Living Fungus

7-10 wks

2 wks

3 wks

3 wks

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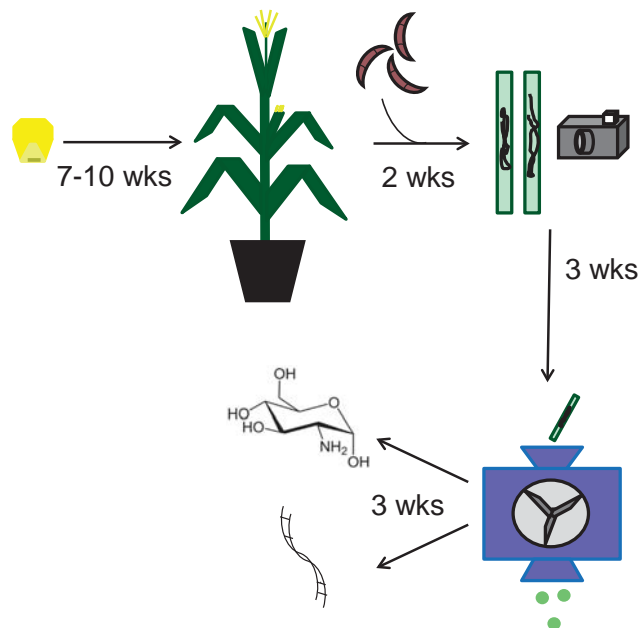
Creating an Efficient Screen

Infection Identification
- infection area per stalk
- infection color intensity

Barcode Identification
- assign barcode to stalks

Stalk Identification
- area per stalk

HALCON
the power of machine vision

File Edit Image Process Analyze Plugins Window Help

Angle tool

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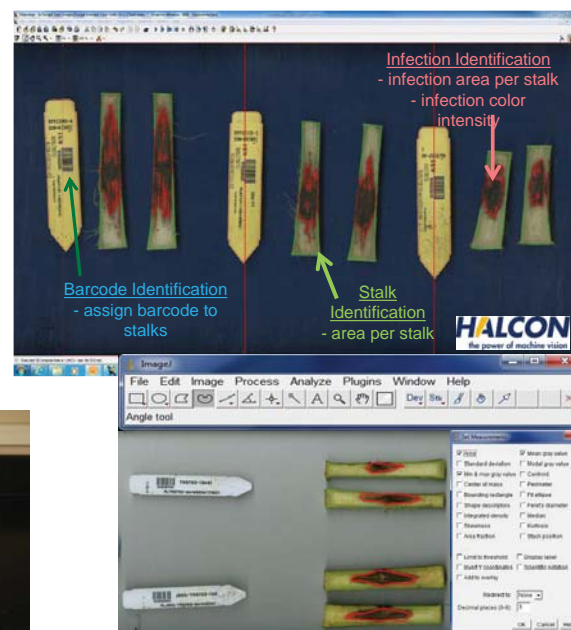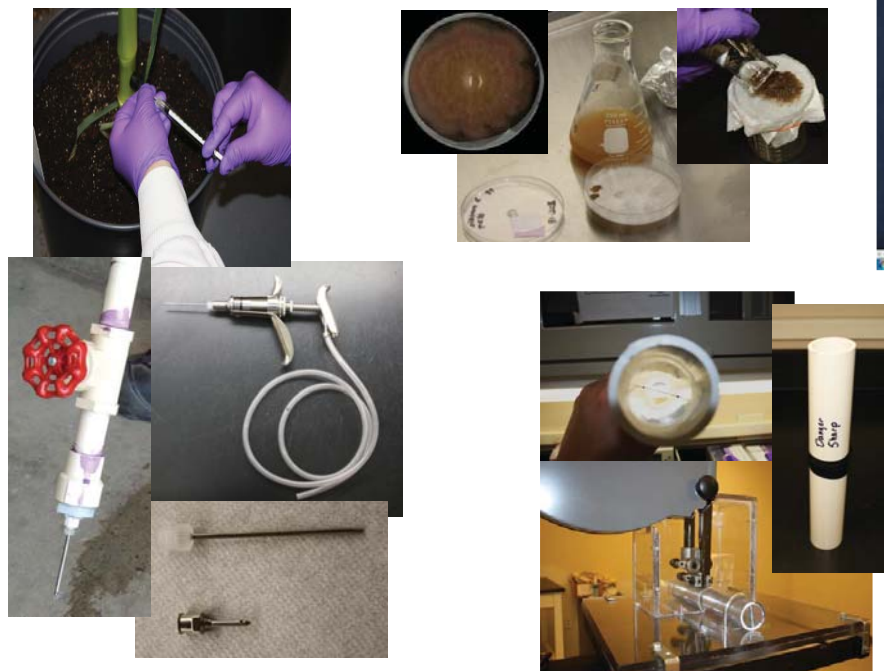
Different Phenotypes Give Slightly Different Results

qPCR

Glucosamine
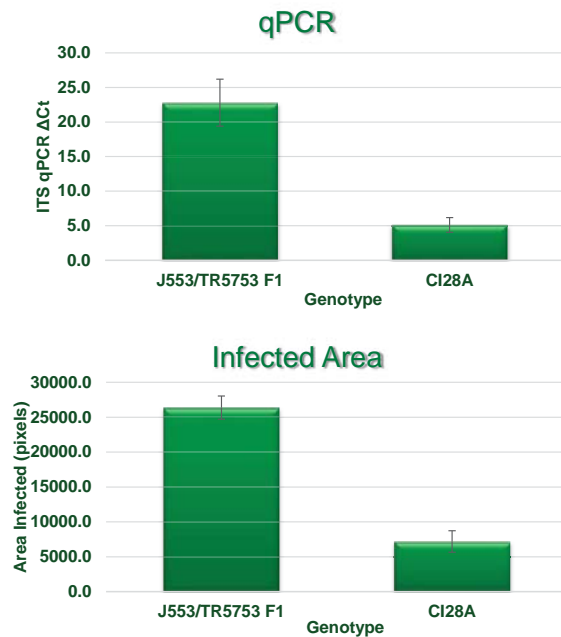
Infected Area

Fg	Glucosamine	ITS qPCR
% Area Infected	0.79	0.72
Glucosamine		0.62

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Lead Gene Performance

- Glutaredoxin identified through metabolic profiling
- Up-regulates pathway down-regulated by Colletotrichum stalk rot
- Efficacious against both Fusarium graminearum and Fusarium verticillioides
- Stronger phenotype in the field than the greenhouse
- Observable phenotype in GH and two years of field trials

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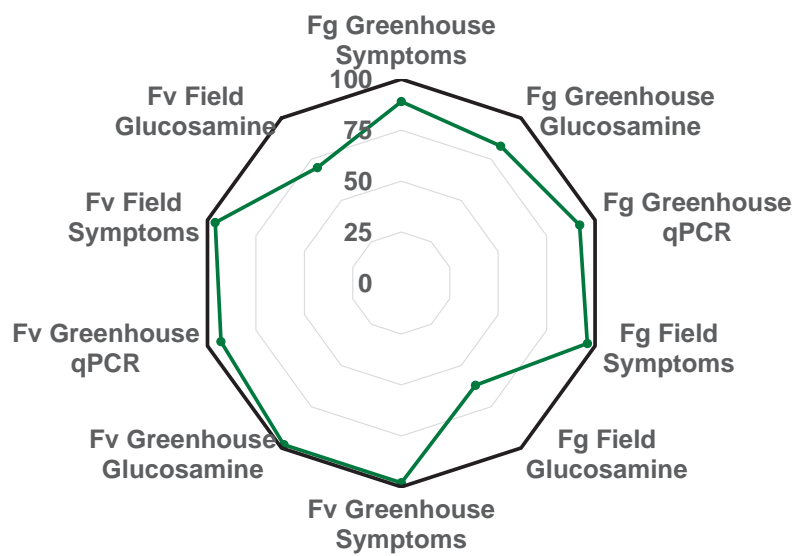
Take Home Points

- First identify a good phenotype, then figure out a way to collect it efficiently

- Proxy phenotypes like imaging can be an effective and efficient approach

- A collection of phenotypes can be more valuable than any one phenotype; the whole is not just the some of the parts

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Acknowledgements

BASF Plant Science Research

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- Dave Kendra

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- Scott Walker - BASF Agricultural Products
- Sam Anas – BASF Agricultural Products

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Presented by: Edward Ross, M.S. student in the Department of Crop Sciences – University of Illinois

Nitrogen (N) fertilizers are a major pollutant and input cost of maize (*Zea mays*) production, but their negative effects can be mitigated through the development of cultivars with higher nitrogen use efficiency (NUE). Yield increases due to N fertilizers are primarily attributed to increases in kernel number, a yield component that is determined early in kernel development. Responses to N at this early stage of development are difficult to investigate, due to the complex path of N within the plant and difficulties in precisely manipulating N supply at the developing kernel. To gain more control of N metabolites supplied to the developing kernel, an in vitro kernel culturing system was employed. Hybrid plants from crosses of B73 to Mo17, IHP1, and ILP1 were grown under variable N in the field. Developing kernels were dissected three days after pollination and placed in culture with variable N. B73 X Mo17 kernels were assayed with RNA sequencing and metabolite profiling of free amino acids. Trait and gene expression data were integrated using weighted gene correlation network analysis (WGCNA). A subset of gene modules was found to be highly correlated to free amino acid levels, either in cob or kernel tissue. GO term enrichment analysis of these modules indicates that their members are involved in carbohydrate metabolism, N metabolism, DNA packaging, and protein modification. Additionally, these modules contain genes orthologous to components of an N responsive transcriptional network identified in the Arabidopsis thaliana root. Alleles of these genes containing UniformMu transposon insertions have been obtained from the Maize Genetics Cooperation Stock Center, and are currently being introgressed into various backgrounds. Two of these backgrounds are the IHP1 and ILP1 inbred lines from the Illinois Long Term Selection Experiment (ILTSE) for kernel protein concentration, which represent the extremes of N utilization efficiency in maize.

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Presented by: Brian Rhodes, M.S. student in Department of Crop Sciences, University of Illinois

An important component to increasing crop productivity is improving Nitrogen Utilization Efficiency (NUtE). In maize this trait is measured as the ratio of grain yield to accumulated plant N. Enhancing NUtE offers substantial economic and environmental benefits, but little is known about the genetic mechanisms that govern variation for NUtE within maize populations. Our group has conducted high density genetic mapping for NUtE in a hybrid population developed from the intermated B73 X Mo17 recombinant inbred lines (IBMRILs), test crossed to the Illinois High Protein 1 (IHP1) inbred line, which has a superior capacity for N uptake but low NUtE. We identified 9 robust strong effect QTL for NUtE that range in size from 14-9030 Kb and aim to identify causal genetic variants. The largest effect QTL is localized to a 2 Mb region on chromosome 1 containing 23 annotated genes, including the high affinity nitrate transporter NRT1.1 B (GRMZM2G161459). The homolog to the maize NRT1.1 B in rice has been shown to contribute to the variation in nitrogen use between indica and japonica cultivars. A second QTL for grain nitrogen/protein concentration has been localized to a single HVA22-like candidate gene that likely regulates autophagy, a process important for nitrogen remobilization. In addition to analysis of mutant alleles and near-isogenic lines for the QTL interval, we have created transgenic maize inbred lines with grain specific expression of this candidate gene. Preliminary results show an increase in grain protein concentration in both the transgenic inbred background and in F1 ears following hybridization. Genome editing experiments are in progress to further verify the function of candidate genes within our NUtE regions. The results of this project will aid the development of maize hybrids that require lower nitrogen inputs and therefore would reduce costs for farmers and mitigate environmental and health effects associated with high ambient nitrogen levels.

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Tissue Culture and Genome Editing in the Illinois Long Term Selection Experiment

Stephen Jinga, Brian Rhodes, Christine Lucas, Stephen Moose
University of Illinois at Urbana-Champaign, Department of Crop Sciences

The Illinois Long Term Selection Experiment is a unique genetic resource for identifying and characterizing genes selected for nitrogen use and protein accumulation in maize. To facilitate study of gene functions, we aim to establish a CRISPR Cas9 mediated genome-editing system in these novel genetic backgrounds. A media regime has been developed for successfully regenerating fertile plants of both Illinois High Protein (IHP) and Illinois Low Protein (ILP). Currently, putative transgenic lines expressing the Cas9 protein have been recovered using NPTII as a selectable marker. These Cas9 positive lines will be used to make targeted mutations with this germplasm. We have also initiated experiments to edit the Prolamin Box Binding Factor (PBF), which regulates zein gene expression and shows changes in both allele frequencies and mRNA expression that are consistent with PBF being a target of selection for grain protein concentration. In addition to generating knockout mutations, we are also investigating the functional significance of variation in the length of an asparagine (Asn) repeat motif found at the C-terminus of PBF. This Asn repeat shares features with triplet repeat expansions studied in Arabidopsis and trinucleotide repeat disorders in humans such as Huntington's disease. It is hypothesized that variation in the Asn rich region of PBF could act as a sensor to control α-zein accumulation in response to incoming supply of amino acids, or possibly interacting with other transcription factors such as opaque-2. To target this Asn-repeat motif, single-guide RNAs were designed to create variation in Asn repeat length in conjunction with expressed Cas9.

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Response to Selection in the ILTSE and a population of epigenetic NILs

Nicholas Heller and Stephen Moose
Graduate Research Fellow, Department of Crop Sciences
University of Illinois at Urbana-Champaign

Advances in sequence technology have allowed incredible discoveries about the genomes of many organisms and elucidated relationships between gene and phenotype. These advances have allowed a deeper look into how variation in phenotype is created, selection for this variation is realized, and how phenotypes are inherited. However, many studies found that the heritability of many phenotypes is not fully explained by genomic DNA sequence, especially for quantitative, complex traits.

Here, I present some insights into the contributions to phenotypic response to selection in plants using a genetic system (utilizing the Illinois Long Term Selection Experiment, ILTSE), transgenic system (utilizing the red fluorescent protein driven by the *Floury2* promoter in the maize kernel), and an epigenetic system (utilizing variation created by the *mop1* mutation). Briefly, the ILTSE materials provide a unique resource because they have undergone continuous selection for the same trait for over 100 years and the last 50 years of seed is preserved. Reverse selection experiments are still underway to determine the plasticity of the populations' genomes after 50, 90, and even 100 years of forward selection. Finally, we utilize an inbred system to look more closely at the possibility that some of the response to selection is due to heritable, non-genetic factors.

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Analysis of habituation at the maize *r1* locus

Authors: Robert Lindsay[1], William Eggleston, Jr.[2]
1. Integrated Life Sciences, Virginia Commonwealth University
2. Department of Biology, Virginia Commonwealth University

Abstract:

A mutation of the maize *r1* locus in W22 with variable kernel color was used as the basis for directed selection to create sublines with kernel color ranging from colorless to nearly fully colored. These sublines were produced by 5-6 generations of selecting and planting the lightest and/or darkest kernels on selfed ears from the prior generation. Visual inspection indicates that there are multiple gradations in kernel color among the selected sublines ranging from nearly colorless to nearly fully colored, which is supported by quantification with light reflectometry. However, light reflectometry does not support the full range of color gradations discernable by visual means. Recombination studies indicate that the initial kernel color change was caused by a change in the 3` end (or beyond) of the *r1* gene controlling kernel color.  Initial sequence analysis of the 3` end of the *r1* in the progenitor, lightest subline, and darkest subline does not show sequence changes that could account for the change in seed color differences between the progenitor and two sublines in this region. The lack of sequence differences suggests that the change in kernel color between the sublines are due to epigenetic changes, rather than DNA sequence changes, and may result from a process known as "habituation." resulting from a lack of canalization at the *r1* locus. This idea of habituation, supported by methylation changes in the selected sublines holds promise for reducing the time required to isolate and develop crop phenotypes.

Determining the effect of the *sbe1* allele from *Z. mays parviglumis* on maize endosperm starch composition in an *ae1* background

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Starch is the main constituent of maize endosperm. Structurally, starch is divided between two main forms: unbranched (or less branched) amylose and highly branched amylopectin. Generally, amylose constitutes about 25% of maize endosperm starch. The amylose content in the endosperm is increased up to 50% when *ae1*, which encodes starch branching enzyme IIb (SBEIIb), is homozygous recessive. However, one variety of maize that is homozygous *ae1*, GEMS-0067, has up to 75% amylose in its endosperm starch. We have shown that this high amylose content is due to an allele of *sbe1*, which encodes for starch branching enzyme I (SBEI). The GEMS-0067 allele of *sbe1* translates into a protein with six amino acid polymorphisms relative to what is found in all Midwestern dents that have been surveyed. We have also found that the amino acid sequence for SBEI from GEMS-0067 is identical to what is predicted for *Z. mays parviglumis*. We are interested in whether the *sbe1* allele of *Z. mays parviglumis* has the same effect on maize starch composition as GEMS-0067. To test this, we will analyze the progeny of *Z. mays parviglumis*-maize hybrids. Instead of using a recessive *ae1* allele, we are employing *Ae1-5180*, which acts in a dominant fashion to eliminate SBEIIb. We will present our methods of analysis as well as data on developing markers to distinguish the *sbe1* and *ae1* alleles.

Keywords: Amylose, *ae1*, *sbe1*, *Z. mays parviglumis*, GEMS-0067

Mapping loci that modify the efficacy of *Teosinte crossing barrier 1*

Merritt B. Burch and Donald Auger

Teosinte crossing barrier 1 (*Tcb1*) is a genetic cross-incompatibility factor that is responsible for blocking non-self-type pollen in silks. Originally found in teosintes, *Tcb1-s* (*strong allele*) has been introduced into modern maize varieties conferring resistance to *tcb1* pollen. Previous studies using a similar cross incompatibility system, *Gametophye factor 1* (*Ga1-s*) suggest that the cell wall modification enzyme *ZmPme3*, a pectin methylesterase, along with multiple modifying QTL loci contribute to the effectiveness of silks at resisting foreign pollen types. In *Tcb1,* little is known about the genetic modifiers and, more importantly, what the underlying biological mechanism is for this cross incompatibility. Cross-incompatibility systems like *Tcb1* and *Ga1* can be beneficial to breeders and farmers when only certain pollen types are desired on specialty maize crops. It was observed that nearly all the F1's of various inbreds, including B73, crossed by W22 *Tcb1-s* demonstrate strong incompatibility with *tcb1* pollen. One exception was Mo17, whose F1s had weaker resistance. In this study we used recombinant inbred lines (RILS) from the intermated B73-Mo17 (IBM) population crossed with homozygous W22 *Tcb1-s* plants to test the efficacy of the various F1s at blocking *tcb1* pollen. The F1s were tested by first challenging the *Tcb1-s* silks with *R1 C1 tcb1* pollen and the next day pollinated the same silks with *r1 c1 Tcb1-s* pollen. The resulting ears were scored for the percentage of colored kernels. Six quantitative trait loci (QTL) were detected on chromosomes 1, 3, 5, and 7 that explained 28.9% of the phenotypic variability. Most modifying QTL loci showed simple additivity effects and epistatic interactions between loci. Further exploration into these genomic regions and the underlying candidate genes is underway, these results could shed light on the genetic and physiological mechanisms controlling *Tcb1*.

Interaction plot for csu207 and umc1752
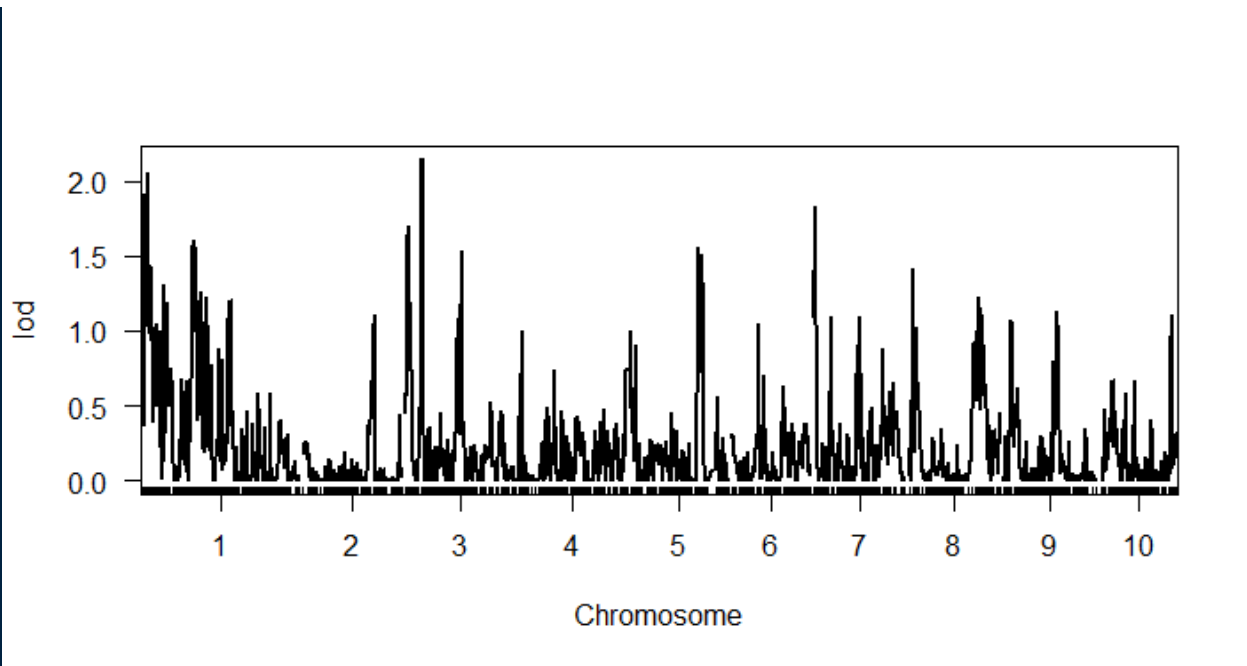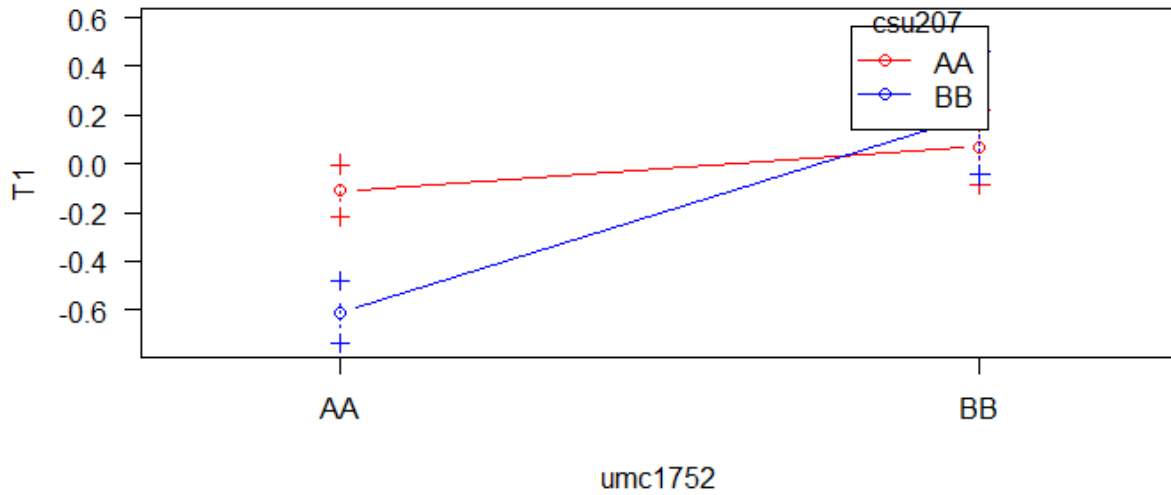

```
          fitqtl summary

Method: Haley-Knott regression
Model:  normal phenotype
Number of observations : 77

Full model result
----------------------------------
Model formula: y ~ Q1 + Q2 + Q3 + Q4 + Q5 + Q6

        df       SS       MS      LOD     %var Pvalue(Chi2)     Pvalue(F)
Model   6 14.06081 2.343469 9.359507 42.86583 1.113397e-07  4.084648e-07
```

```
Error 70 18.74110 0.267730
Total 76 32.80192


Drop one QTL at a time ANOVA table:
---------------------------------
        df Type III SS    LOD  %var F value Pvalue(Chi2) Pvalue(F)
1@28.0   1       2.0279 1.7179 6.182   7.575        0.005   0.00753 **
1@324.0  1       0.4868 0.4288 1.484   1.818        0.160   0.18186
3@21.0   1       1.2024 1.0397 3.666   4.491        0.029   0.03762 *
3@107.0  1       1.9384 1.6456 5.909   7.240        0.006   0.00891 **
5@426.0  1       1.2296 1.0626 3.749   4.593        0.027   0.03558 *
7@4.0    1       2.5974 2.1702 7.918   9.702        0.002   0.00267 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Deployment of a High-Throughput Plant Height Mapping System on Genomes to Fields Germplasm

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A remote sensing system for crop height measurement was developed using a 360-degree 2D laser scanner and onboard computer mounted on an unmanned aerial vehicle (UAV). Algorithms for data processing and visualization were developed to process the dense data generated by the Light Detection and Ranging system (LiDAR) on the UAV. This system was deployed on a two acre field of various maize inbreds and hybrids in nitrogen use experiments, including the Genomes to Fields germplasm. Plant heights for each plot were generated from the resulting spatial point clouds. Across the field, the plant heights determined via remote sensing show an average R^2 value of 0.87 when compared to manual plant height measurements. In the Genomes to Fields experiment plant heights were taken at tassel height and at the point of flag leaf attachment. The comparison of these to the LiDAR data will be used to determine where on the plant the LiDAR system is recognizing a point for the spatial point cloud. Future research can determine if late season fly-overs to develop a spatial point cloud of a field post-leaf senescence could provide a high-throughput method of measuring ear height in addition to plant height. This UAV-based system can cover a two-acre field in just eight and a half minutes, providing a truly high-throughput and non-destructive measure of plant height that can be used throughout the growing season and across locations.

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