

Approaches and Technologies for Challenges in Agriculture: A View from a Texas Maize Breeding Program



TEXAS A&M
AGRILIFE
RESEARCH

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Maize in Texas

2013 Region	Acreage planted	Average yield
High Plains	951,000 acres (855,000 harvested)	204.8 bu/acre
Rest of Texas	1,479,000 acres (1,234,000 harvested)	89.6 bu /acre

Texas 2010 – 300 million bushels = \$1.5 billion (12th in Country)

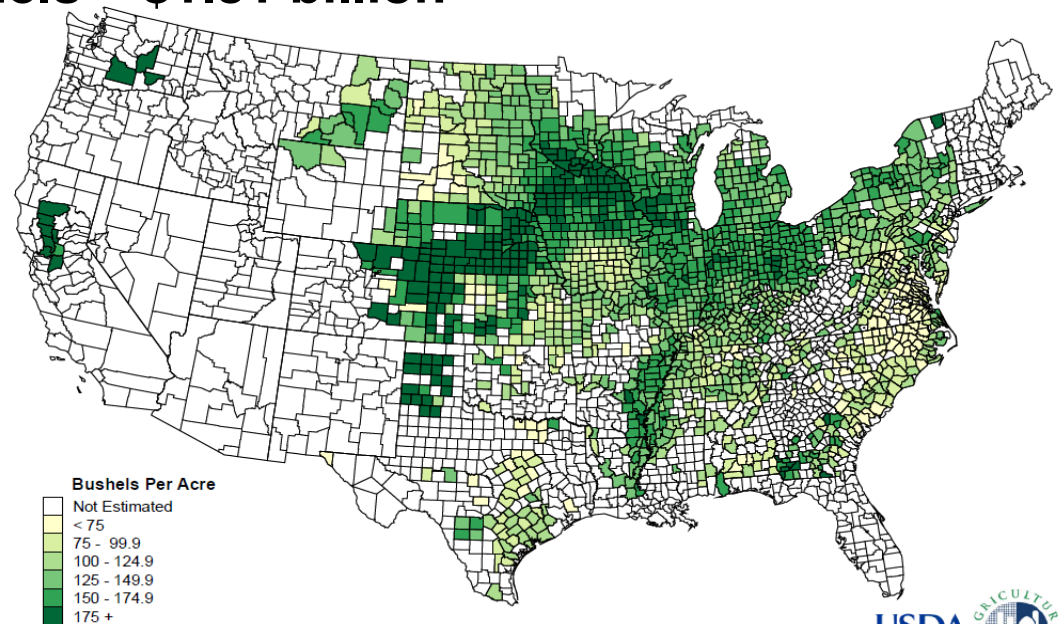
Texas 2011 – 136 million bushels, **heat and drought** = \$0.9 billion

Texas 2012 – 202 million bushels = \$1.46 billion

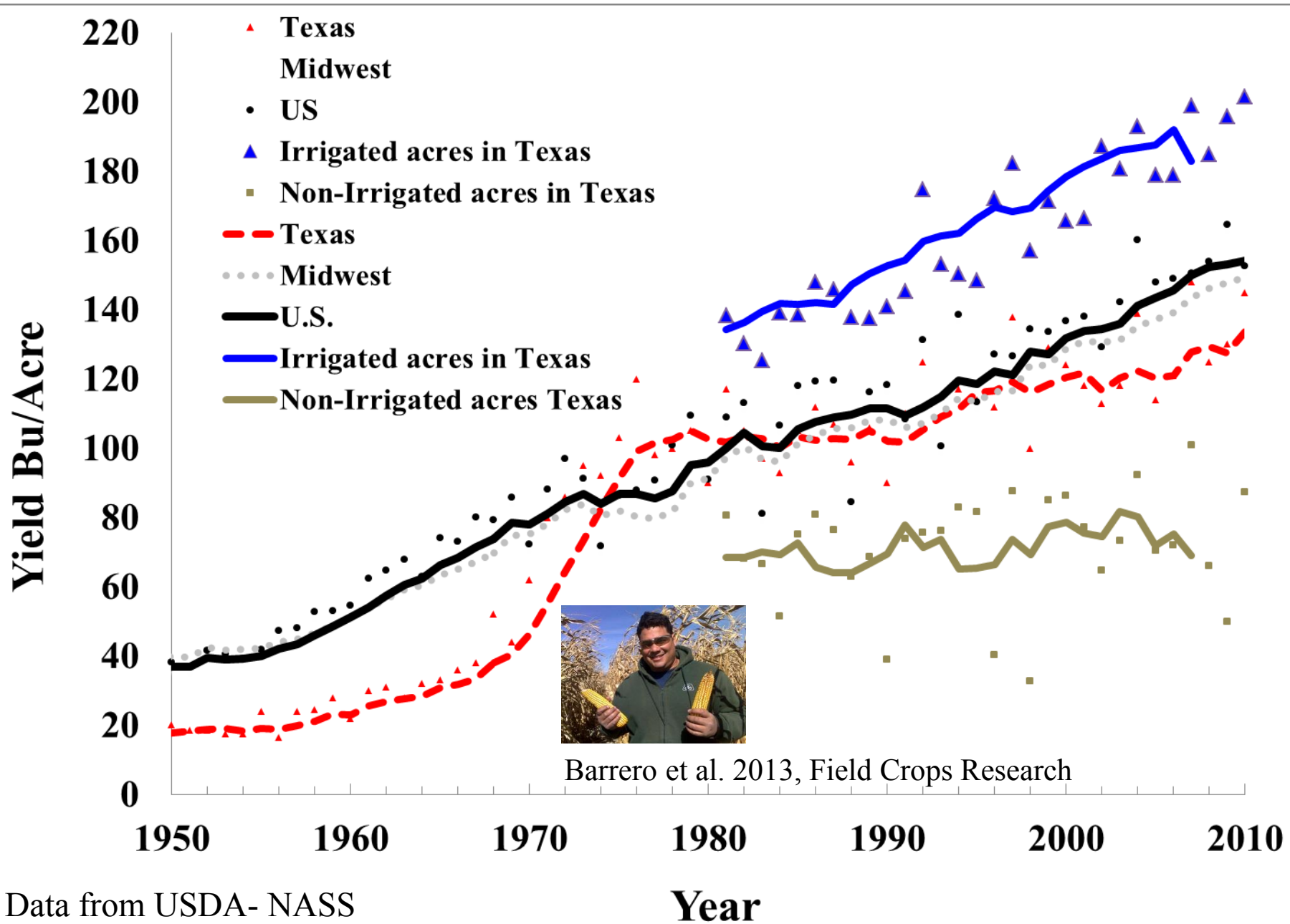
Texas 2013 – 283 million bushels = \$1.36 billion

Texas 2014 – 295 million bushels = \$1.31 billion

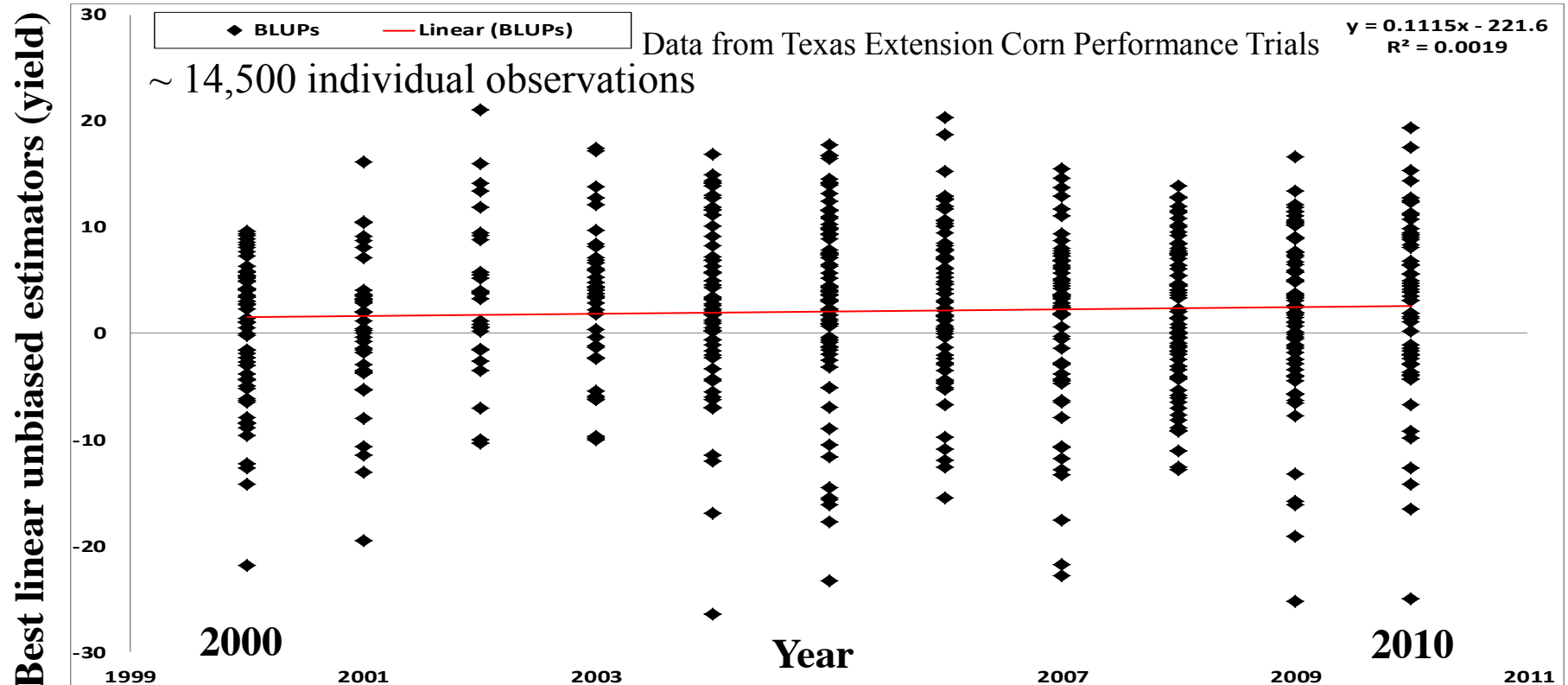
Texas Acres	2,430,000
Acres per bag of seed	~ 3
Cost per bag of seed	~ \$150
Total \$ on seed	~ \$121 million



Texas maize production



Meta-analysis - minimal genetic yield gain in **commercial** varieties grown in Texas over the last 11 years



Hybrid	BLUP	SE	Year	Locs
TG895W	-37.38	7.088	2007	1
TRX01601X	-25.03	6.892	2010	2
F3025	-19.52	7.002	2001	3
DKC6469GENVT3P	19.35	8.548	2010	1
6361RB	20.3	3.928	2006	10
5202B	20.99	7.037	2002	3



Barrero et al.
2013, Field
Crops Research

Agronomic traits are more highly correlated with yield in the lower yielding Texas environments

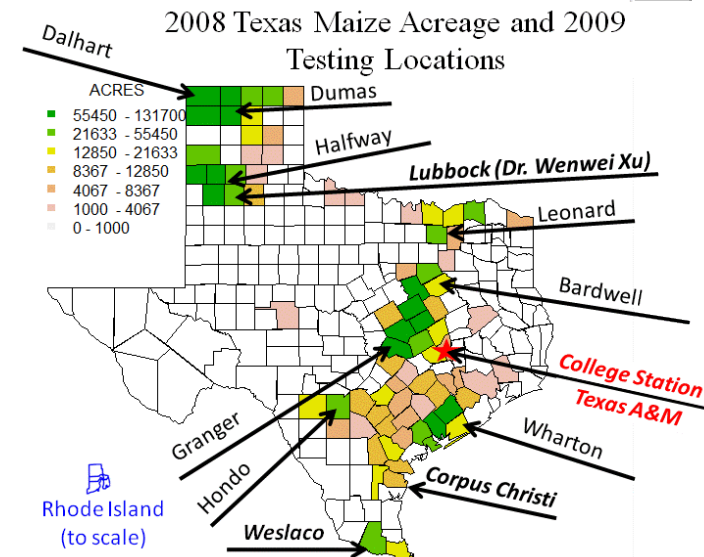
	Grain yield (tons/ha)			
	Texas	High Plains	Rest of Texas	Dryland
Plant height (cm)	0.61***	0.19***	0.46***	0.45***
Ear height (cm)	0.56***	0.03NS	0.40***	0.35***
Days to silk	0.13***	-0.25***	0.05***	-0.08***
Plant density (plants/ ha)	0.66***	0.44***	0.51***	0.36***
Lodging (% plants/ plot)	-0.16***	-0.24***	-0.15***	-0.21***
Moisture (%)	0.55***	0.04*	0.28***	0.30***
Test weight (kg/hl)	0.33***	0.04NS	0.45***	0.50***

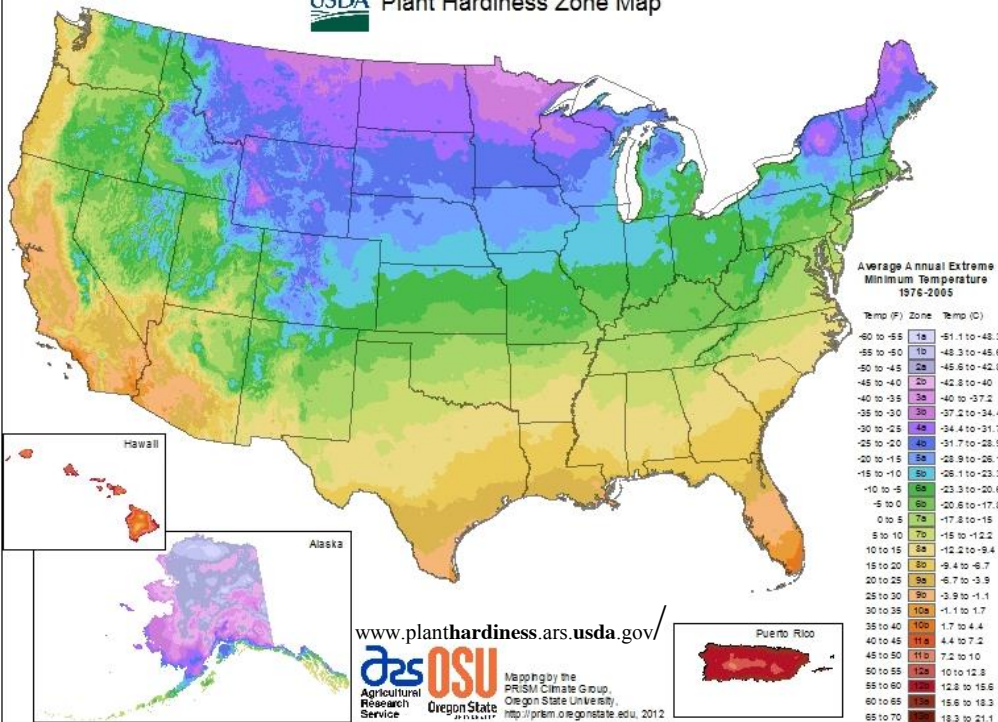
~ 14,500 individual observations on each trait

~ 1,000 commercial hybrids



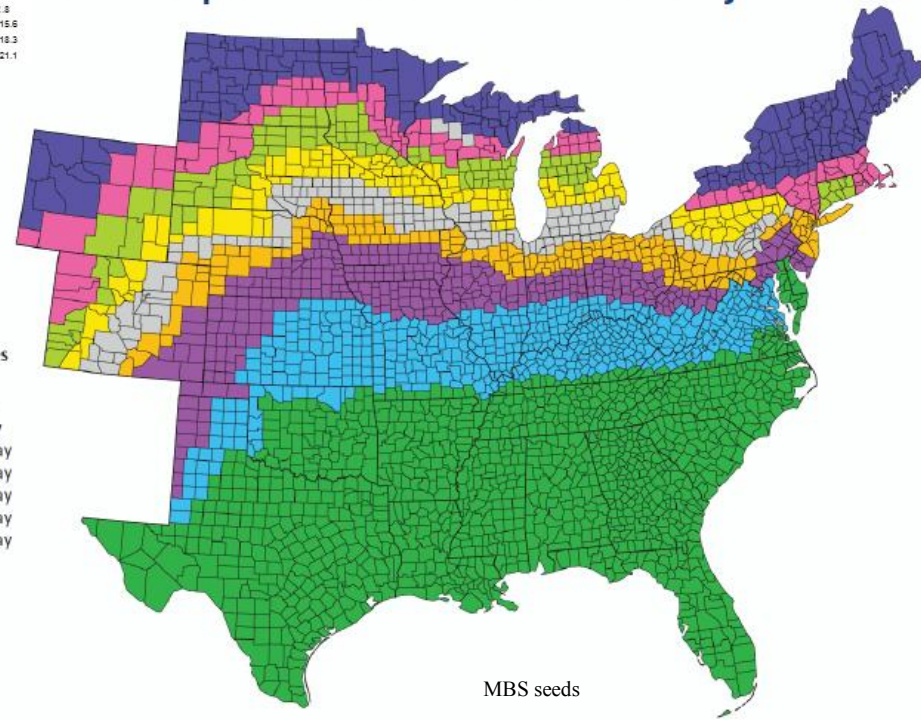
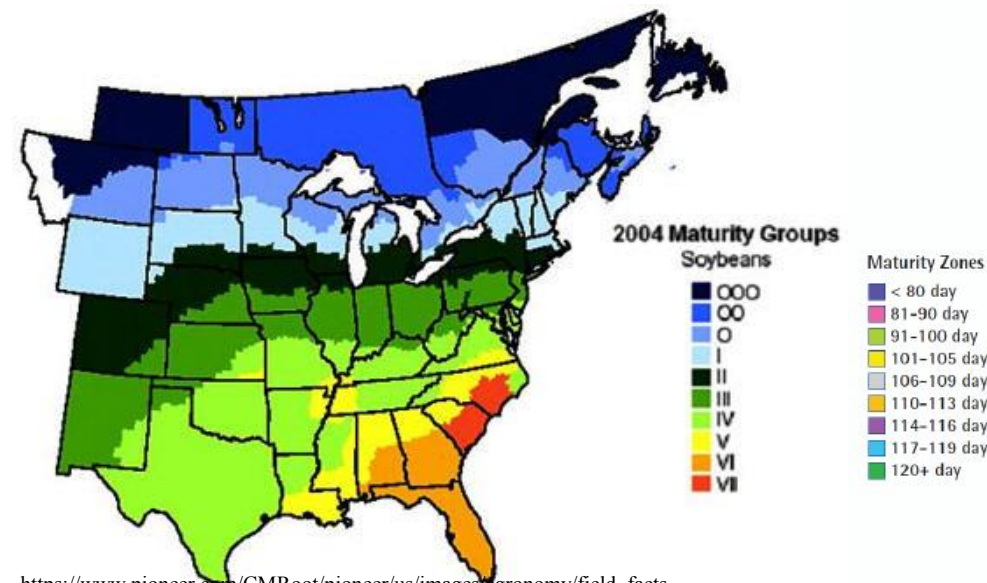
Barrero et al.
2013, Field
Crops Research





Midwestern bias observed between USDA heat zone map and maize relative maturity zones

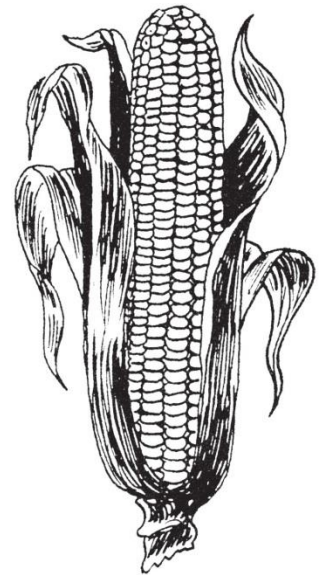
Corn Adaptation Zones – Relative Maturity



MBS seeds

Why do we need a public breeding program on corn? Aren't the companies doing this?

- **Not targeting Texas or the south - a unique adaptation (aflatoxin, heat, drought, etc.)**
- **Unique traits / exotic introgression – colored corn, QPM, perennial corn, high biomass corn**
- **Graduate student training**
 - Primarily in industry
- **New breeding methods**
- **Long-term high risk research**
 - perennials



**Most commercial
inbred lines
are not adapted to
Texas heat and
drought**



Tx777

**Tx777 x
LH195RR2**

LH195RR2



Tx782

LH287

**Tx782 X
LH287RR2**

2013 inbreds and hybrids demonstration



LH195

**LH195 x
Tx775**

Tx775



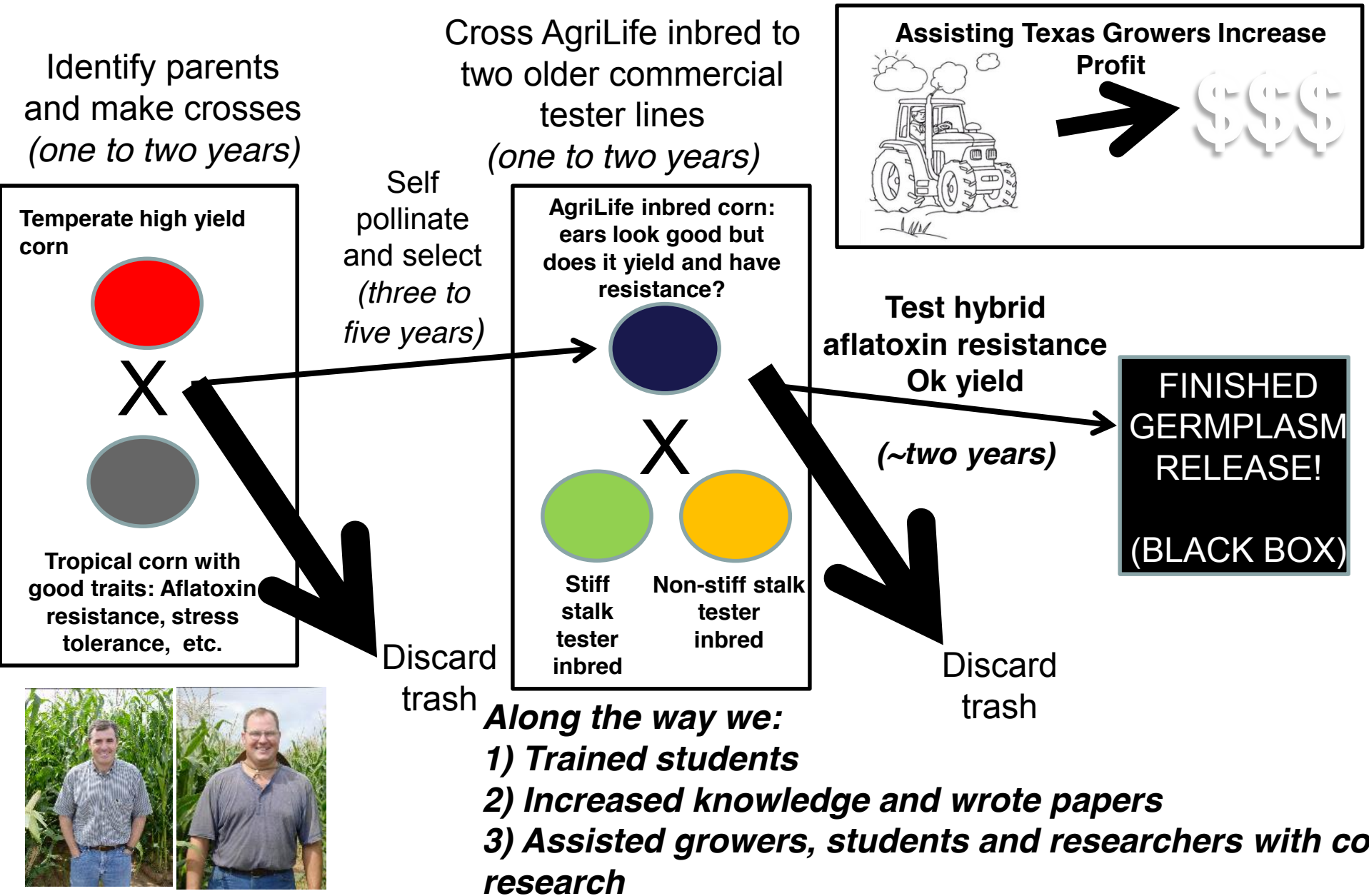
Tx773

**Tx773 x
TR7322**

TR7322



Previous phase of public corn breeding at Texas A&M (the last seven to eleven years) – *Corn breeding at A&M started in 1927*

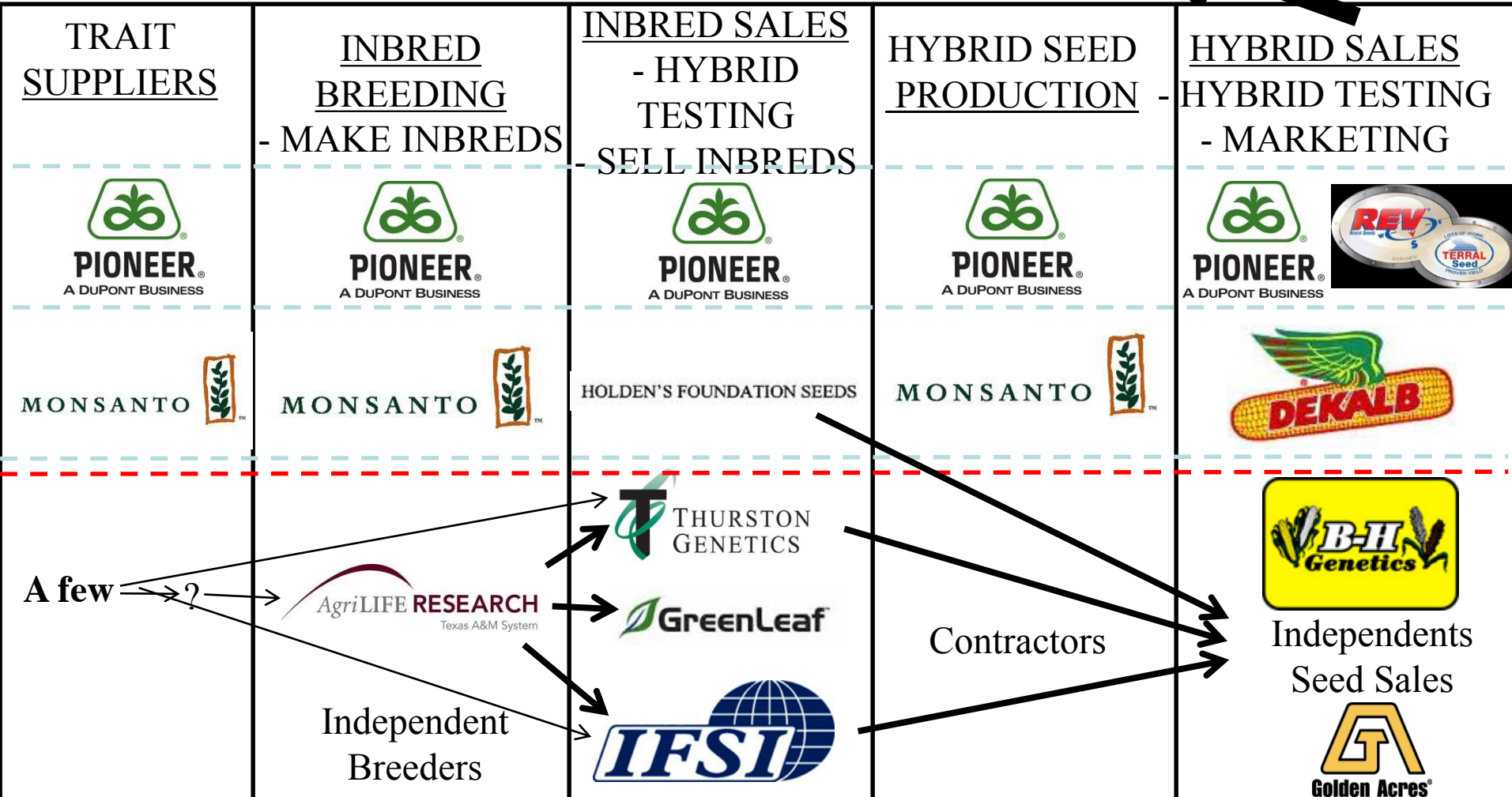
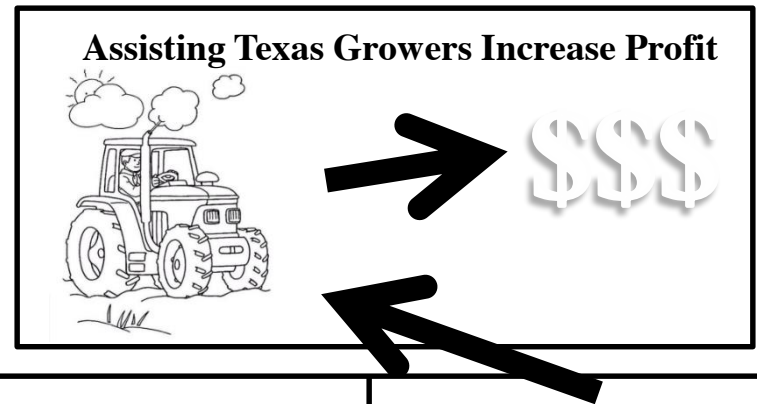


Increasing focus on best yielding lines

	2010	2011	2012	2013	2014	2015
Summer Testers	2	4	15	17	12	5
Summer TAMU lines	646 + 2 ISO	665	278	67	37 + 2 ISO	7 +2 ISO
Winter Testers	2	9	10	19	14	-
Winter TAMU lines	364 + 2 ISO	70	25	33	32	-

Only get yield data in time to make decisions for winter nursery the following year (maybe)

Today's corn seed chain and where public breeders fit (a personal opinion)



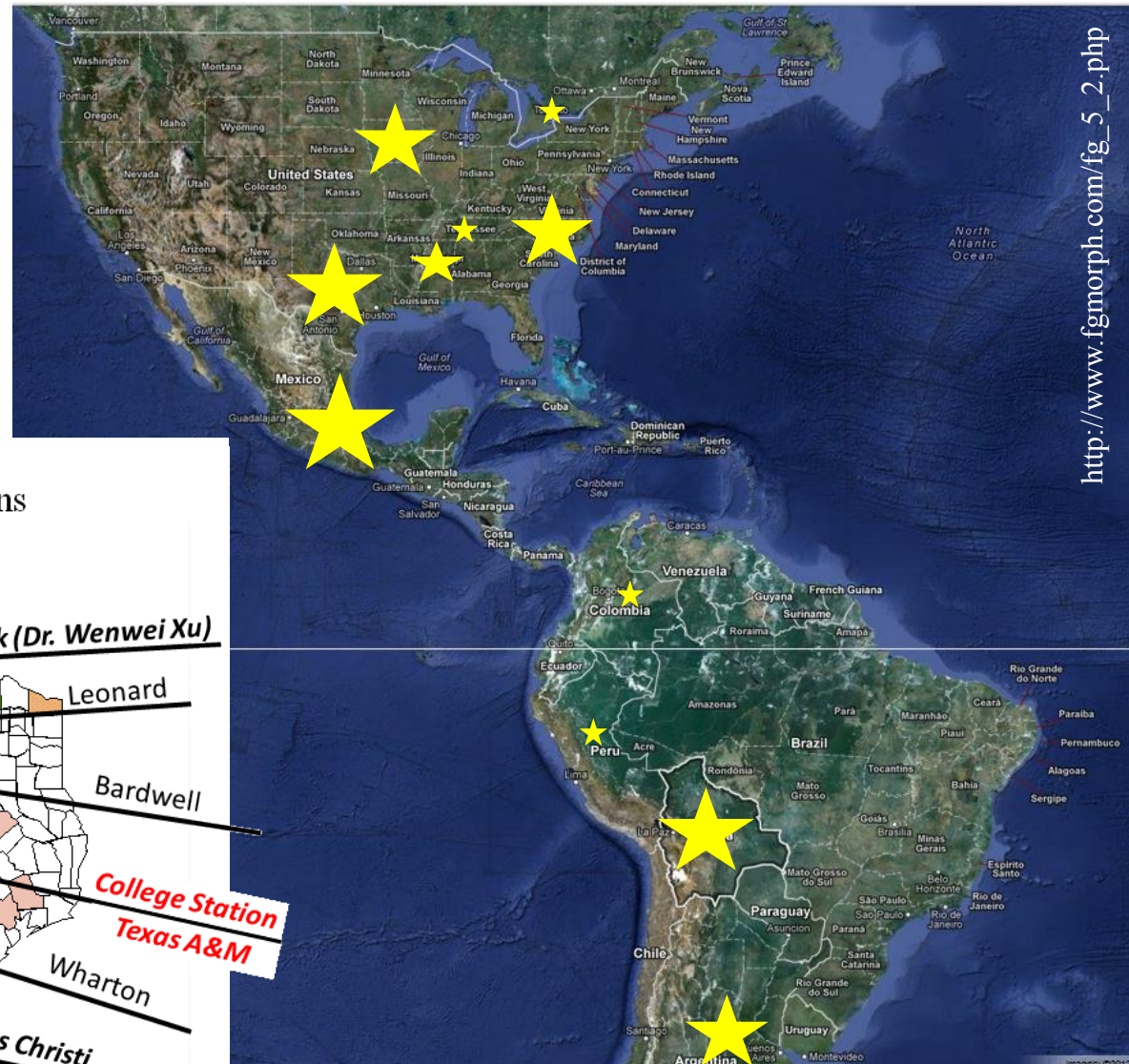
723 hybrids tested in 2012 across six environments

Rank	Hybrid	Bu/ Acre above mean	Std Error	P
1	Tx777 X SS2	51.5	± 11.3	****
2	Tx954 X SS5	49	± 16.8	**
3	Commercial Hybrid #09 (TX company)	46.8	± 5.4	****
4	Commercial Hybrid #08 (TX company)	46.2	± 13.7	***
5	Commercial Hybrid #02 (TX company)	45.1	± 4.8	****
6	Tx114 X Tx120	44	± 14.5	**
7	Commercial Hybrid #04 (National company)	43.3	± 6.7	****
8	Commercial Hybrid #05 (National company)	42.3	± 13.7	**
9	NSS1 X Tx150	40.7	± 11.3	***
10	Tx773 X NSS2	38.1	± 8.9	****
11	SS1 X Tx149	37.5	± 9.6	***
12	DKB 64-69	37.2	± 6.1	****
13	Tx777 X NSS2	37.1	± 9.6	***
14	Tx775 X NSS2	37	± 14.5	*
15	SS1 X Tx904	36	± 14.6	*
16	Commercial Hybrid #11 (TX company)	35.9	± 6.3	****
17	Tx150 X SS4	35.5	± 10.2	***
.	.	.	.	.
29	TX740 X SS3	31.9	± 8.9	***
.	.	.	.	.
50	Commercial Hybrid #06 (TX company)	26.5	± 6.4	****
.	.	.	.	.
56	Commercial Hybrid #10 (National company)	25.6	± 13.7	NS
57	(LAMA2002-22-3-B-B1-B-B/LAMA2002-10-1-B-B-B-B)-2-3-B-2-1 X SS2	25.4	± 12.4	*
58	Commercial Hybrid #07 (TX company)	24.9	± 13.7	NS
60	TX740 X NSS2	12.6	± 16.8	NS

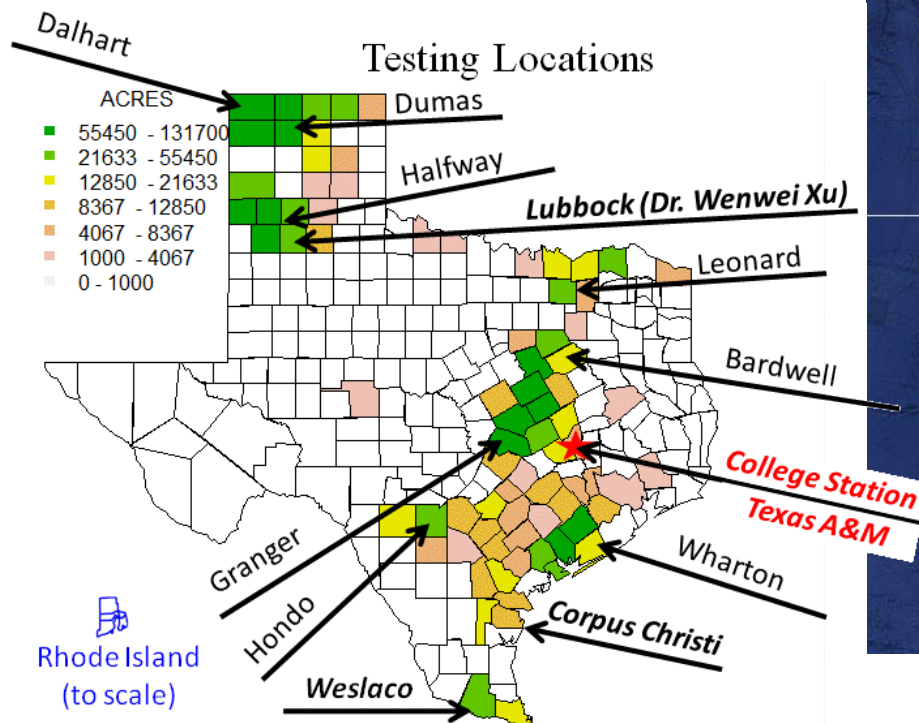
Table 1: Grain yield (Bu Ac⁻¹) trial results over 15 trials in 2013.

2013 SERAT yield data from North Carolina out of 37 hybrids including 7 commercial checks

Important origins of germplasm to the TAMU Maize Breeding Program



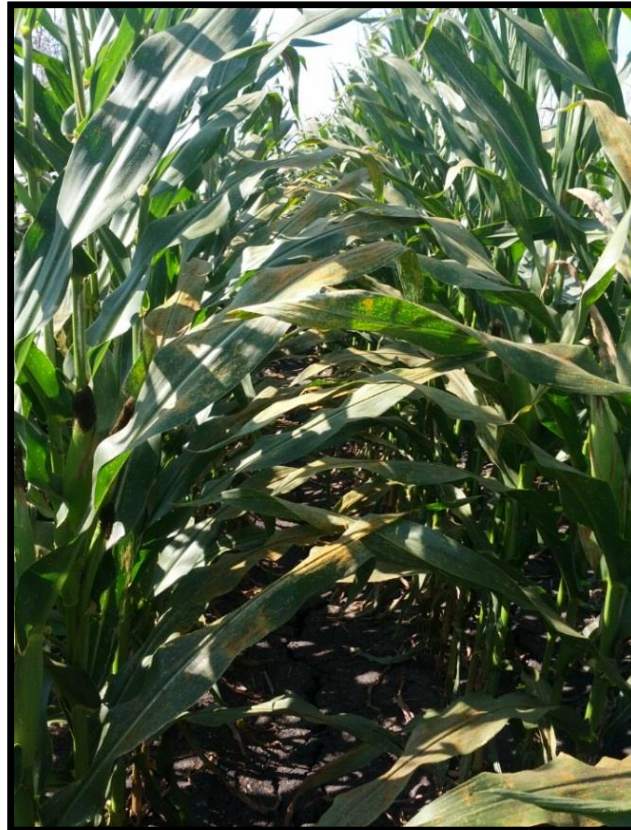
http://www.fgmorph.com/fg_5_2.php



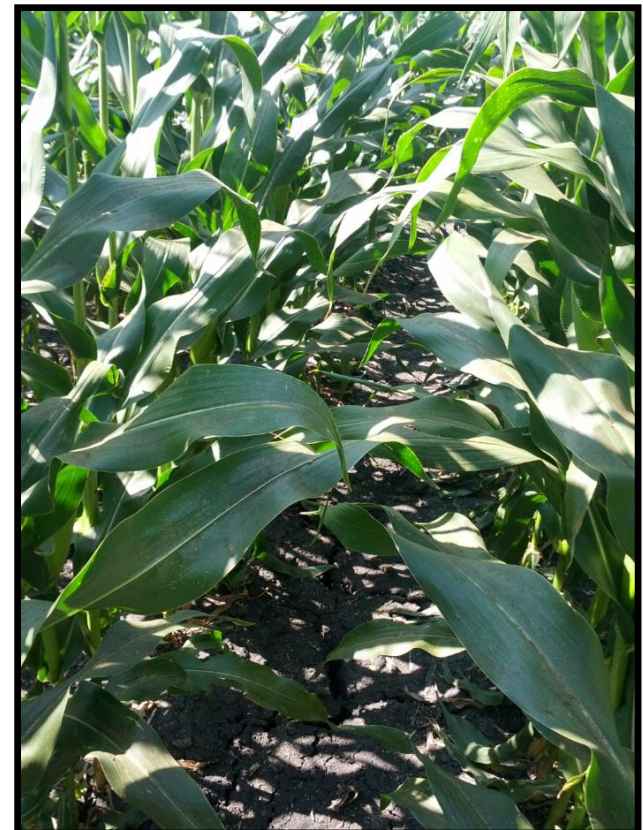
Southern rust resistance

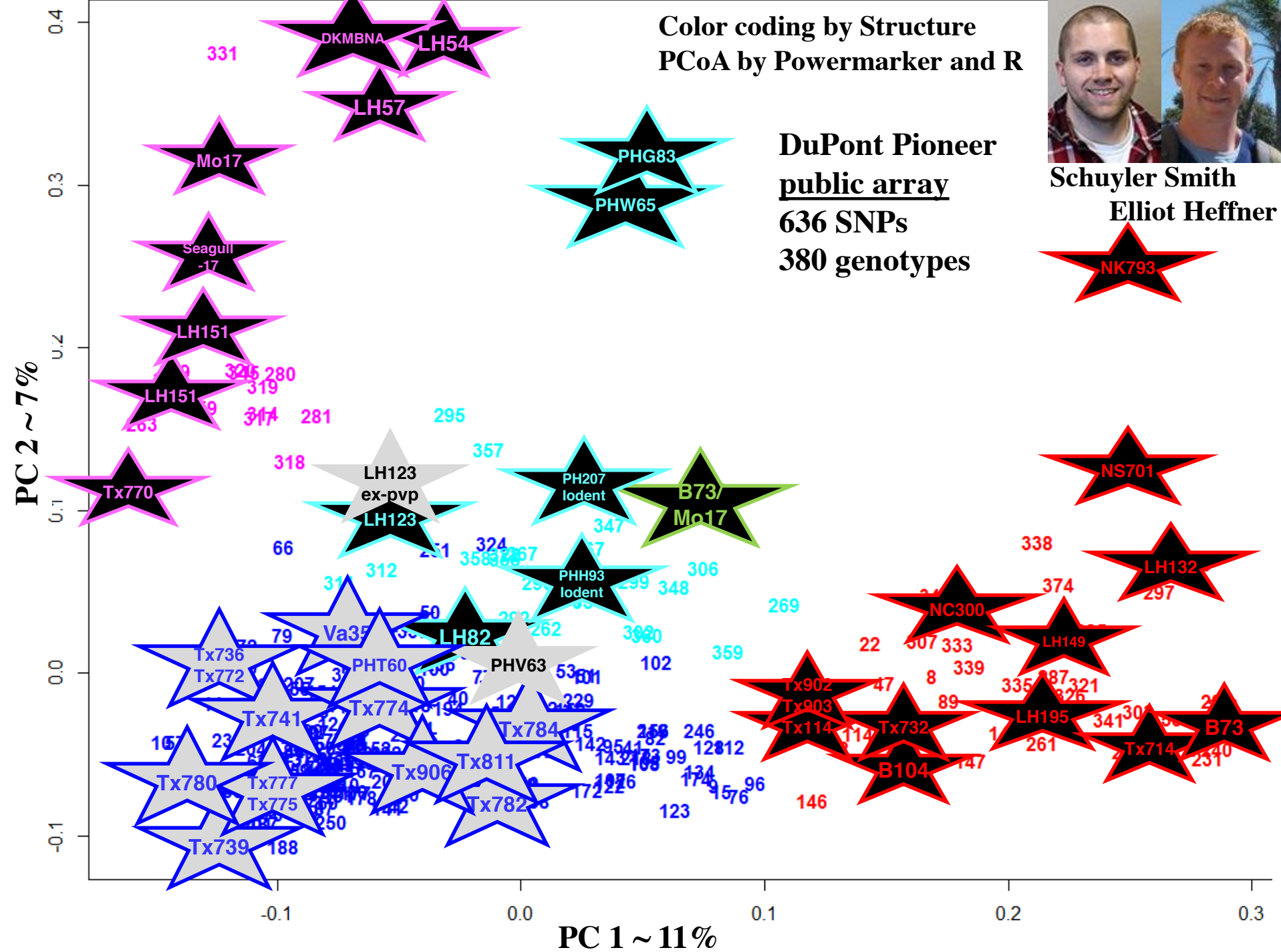


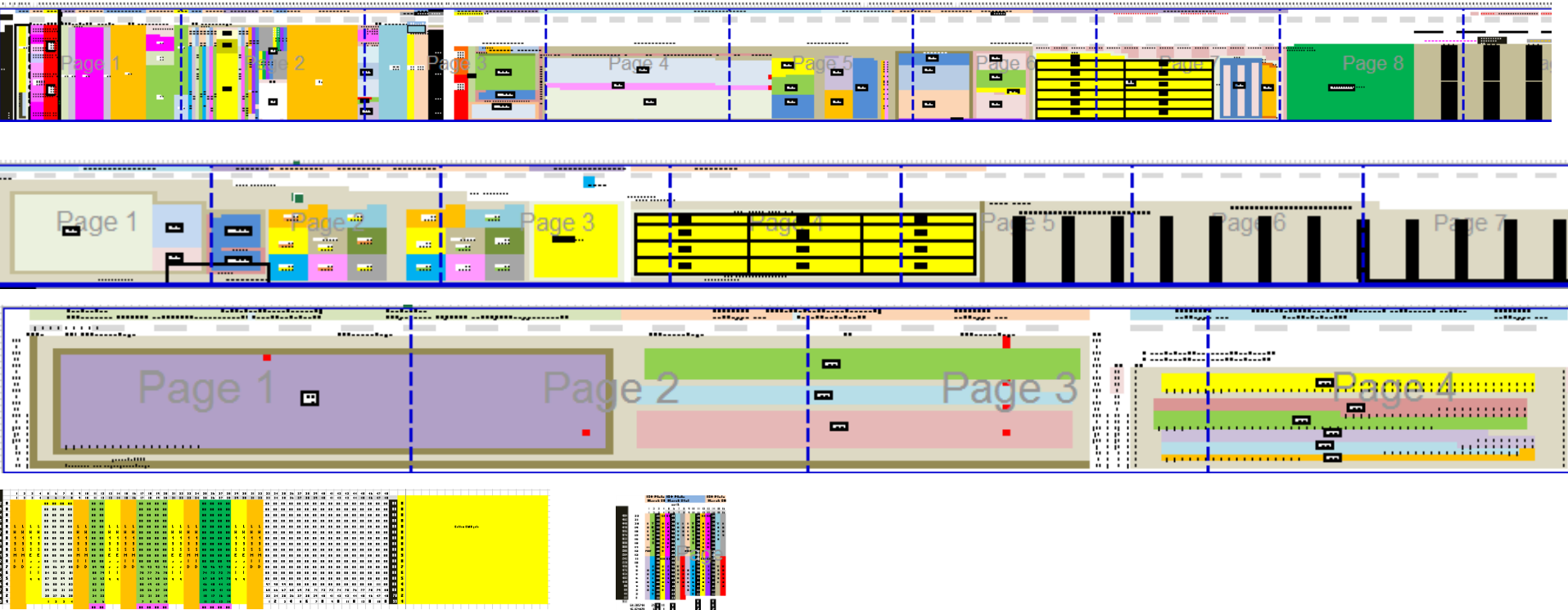
**Commercial hybrids
from 100 %
temperate material**



**TAMU Hybrids
with ½ tropical
background**







Summer yield trials

- Generally seed limited
- 2 row- 2rep; 1 row - 2rep; 1 row 1rep
- Generally 8 to 12 commercial checks per test
- ~20 acres in College Station into ~ 20 tests
- Late planting, limited irrigation treatments
- Combine harvested
- Aflatoxin testing
- Row / column effects are often highly significant

Summer nursery

- ~7 acres in College Station into ~ 20 groups
 - Yellows
 - Whites
 - Coloreds
 - Genetics
 - Student projects
- 2 isolation blocks, but very hit or miss
- Perennial breeding requires planning

Corn breeding program timeline

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Prepare Nursery		CS	CS					WE				
Plant Nursery			CS					WE				
Pollinate						CS				WE		
Harvest								CS				WE
Process Seed	WE	WE						CS	CS			
Prepare Yield Trials	WE		CS									
Plant Yield Trials		WE	CS	CS								
Agronomics	CS			CS	CS	CS	CS					
Notes				CS	CS	CS						
Inoculate AF					CS							
Harvest						WE		CS				
Grind, NIRS, Aflatest									CS			
Analyze Data												

CS = College Station, TX

WE= Weslaco, TX

Downtime!

Information management

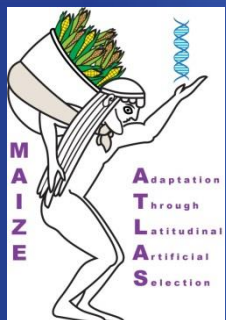
- In **2012** –
 - Over 4679 Summer yield trial plots:
 - Yield
 - Stand count
 - Flowering time
 - Height
 - Other traits
 - Some with subsampled grain
 - Summer nursery >6007 seed stocks
 - Weslaco winter nursery (2011) 3584 seed stocks
 - Genotyping results >2000 plants
- Multiplied by 5-10 years of seed in cooler
- Still no single data format we are really happy with



Unadapted germplasm to the Midwest (IA)

Still green!

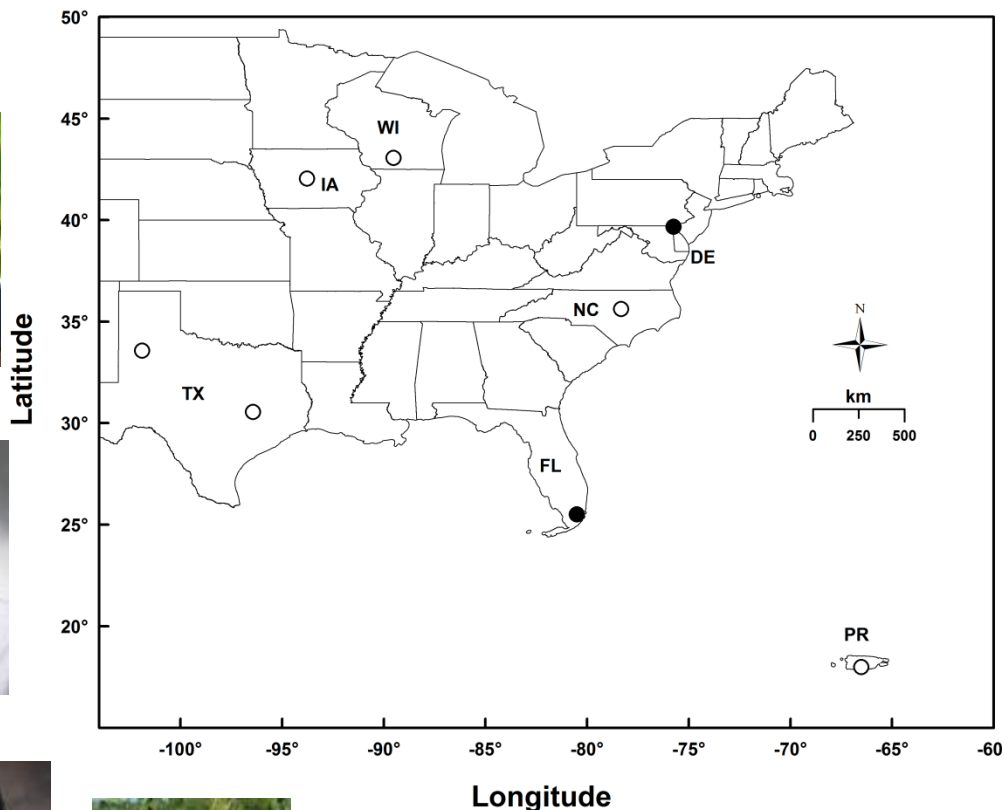




Maize ATLAS



Adaptation Through Latitudinal Artificial Selection



Design,
Simulations
Analysis



FT-NIRS

<http://maizeatlas.org/>



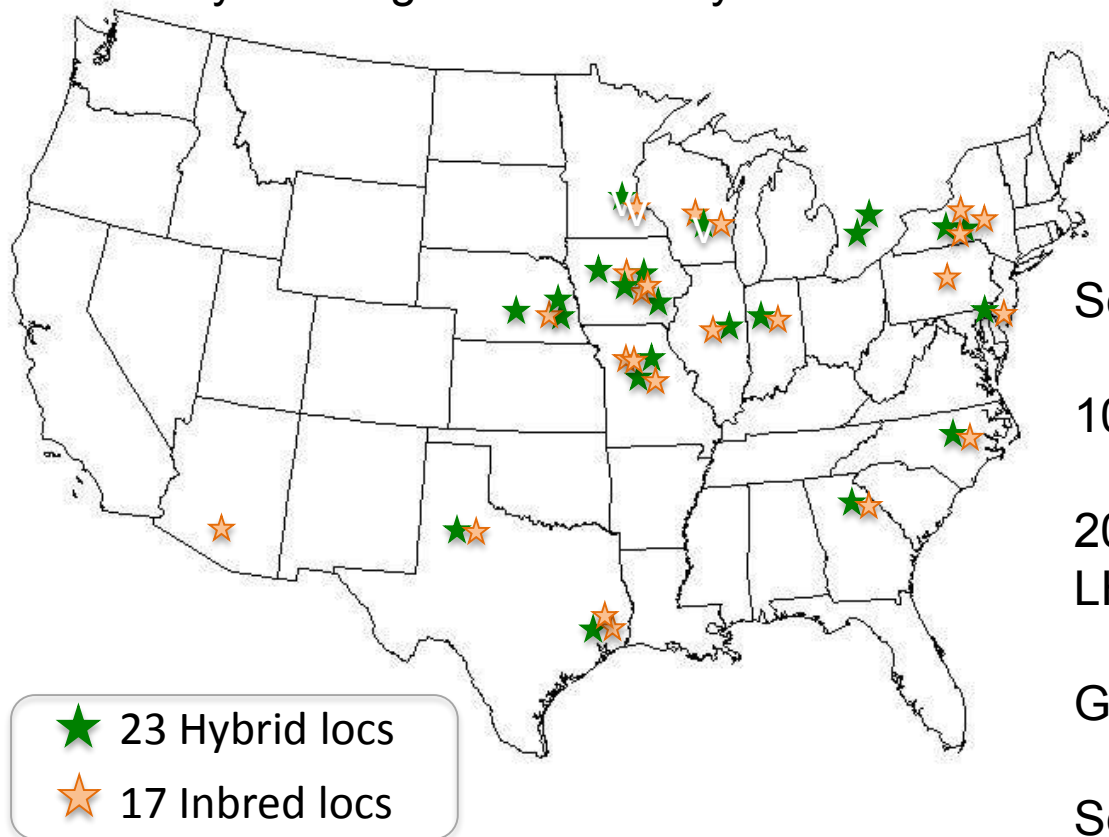
United States
Department of
Agriculture

National Institute
of Food and
Agriculture

G2F: Maize G X E Project

Goal: To Enhance ability to predict plant performance and a deeper understanding of relevant biology

Apply the maize genome sequence, multi-location phenotypes and environmental data to identify useful genetic diversity



Build and strengthen the community of maize breeders and researchers

Set of ~250 hybrids, 2 reps per loc.

10 hybrids common across locations

2014 testers are LH198, LH185, LH195, PB80 and CG102

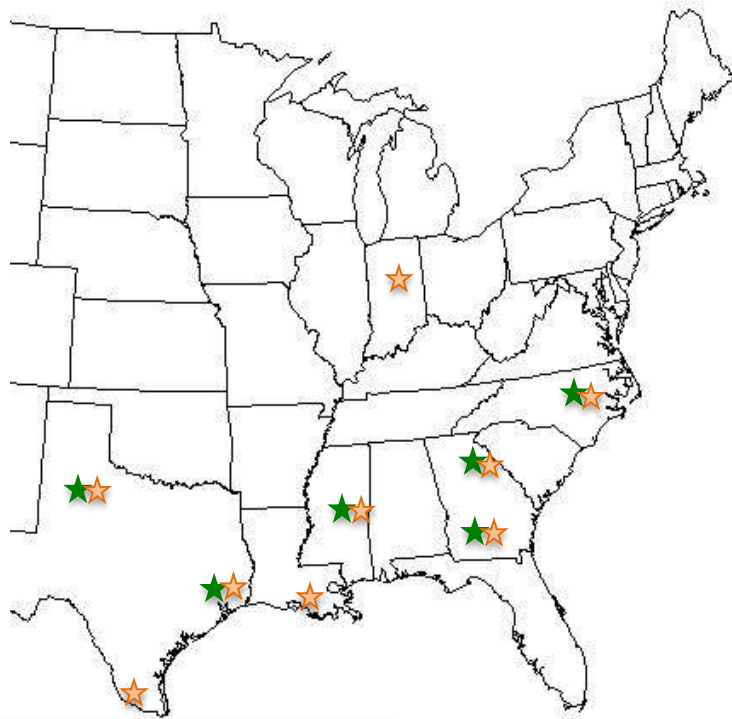
Genotyped using GBS

Set of 31 inbreds

Courtesy of Dr. Natalia de Leon, and the work of many researchers throughout the US

Southeast Regional Aflatoxin Trials (SERAT)

Goal: To determine the multi-environmental adaptation and phenotype of breeding material for aflatoxin resistance, with high yield and associated agronomic traits.



- ★ Current locations
- ★ Past locations

- * Started in 2003
- * Set of ~32 hybrids, 3 reps per loc.
 - Investigators enter ~ 7 hybrids
 - ~4 checks
- * Inoculated for *Aspergillus flavus* (various methods)
- * Measure yield, aflatoxin and other traits

Current project expansion (USDA and AMCOE)

- * Inbred screening
- * Interaction of resistance genetics and atoxigenics
- * Meta-analysis of historical data



NC – Dr. Krakowsky

GA – Drs. Ni, Guo, Scully

MS – Drs. Williams, Windham, Warburton

Tx1 – Drs. Xu, Odvody

Tx2 – Drs. Isakeit and Murray

Companies:

BH Genetics – Dr. Raab and Arnold, Pioneer,

Monsanto – Dr. Gorman

AMCOE



United States
Department of
Agriculture

National Institute
of Food and
Agriculture

Aflatoxin contamination

- Produced by fungus *Aspergillus flavus* L.
- US federally regulated at 20ppb
 - Above 500ppb corn must be destroyed!
- Can lead to acute death
 - Kenya 2004: 125 people died, 192 poisoned (levels to 2,000ppb)
 - US 1998: 25 dogs died eating levels of 100-200ppb for 3 months
- Potent chronic carcinogen, leads to stunting, and other health problems in humans and animals
 - 1.7cm decrease in height for highly exposed children in Benin
 - Higher rates of liver cancer in China
 - Varying sensitivities among species
- \$14 - \$250 million loss from mycotoxins in 2008



Clean feed



**Aflatoxin
contaminated feed**

Challenges in developing lines with good aflatoxin resistance and yield under stress



*How do we
separate these
various
correlated
traits ?*

- Husk Coverage
 - Long, cover tips
 - Tight and thick
- Maturity
 - Flowering time
 - Days to maturity
- Kernel hardness
- Ear nod (ear droop)
- Earworm resistance
- **Drought tolerance**

*How do we
make gains
from selection
on this many
traits ?*



Inoculating yield trials with colonized kernels

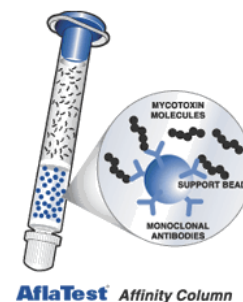


Harvesting yield trials



Fourier Transformed
Near Infrared
Reflectance
Spectroscopy
FT-NIRS
(Thermo Antaris II)

Vicam AflaTest
immunoaffinity columns



Multi-parent populations to pyramid aflatoxin resistance sources and yield

- Develop 4-way and 8-way cross populations from known sources of resistance (Warburton et al.)
- Select inbred lines from these populations across different environments and investigators
 - Phenotypic for yield
 - Using markers for aflatoxin (Warburton)



← 4-Way Cross Populations; Ne=200+

- a (Tx740/Mp313E)//(Tx772/Mp715) fourway 3 sib mated
- b.(Tx772/Mp313E)//(Tx740/Mp715) fourway 3 sib mated

8-Way Cross Populations (3); Ne=100+→

- c ((CML 108/Mp715))//(A6 /Tzi 8)///((Tzi 18/Ki3)//(NC334/Hi27))
- d. ((CML 108/NC334)//(Tx740/CML 348))///((Tzi 18/Mp313E)//(CML 311/Mp715))
- e. ((Tzi 18/CML 69)//(Tzi 8/Mp313E))///((CML 108/A6)//(CML 311/Mp715))



Hybrid crossing scheme

Tx714 (~~zmlox4-10::mu~~/zmlox4-10)



Pollen ~~zmlox4-10::mu~~

2010
2011



Tx714
inbred

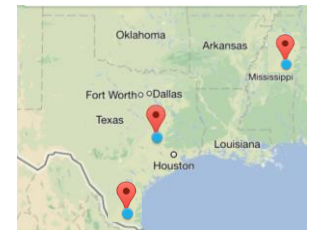


Inbred females
357 diverse lines

Tx714 (~~zmlox5-3::mu~~/zmlox5-3)

Pollen ~~zmlox5-3::mu~~

2010
2011



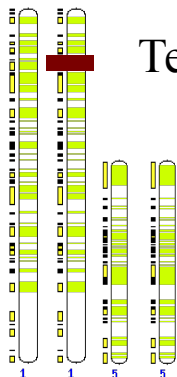
College Station,
TX
Weslaco, TX



Hybrids (**ZmLOX4**/~~zmlox4-10::mu~~)

A. flavus inoculated

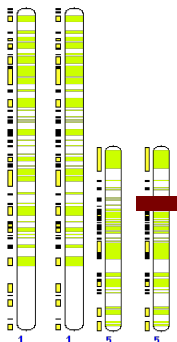
Test aflatoxin resistance



Hybrids (**ZmLOX5**, ~~zmlox5-3::mu~~)

Limited and full irrigation

Test drought tolerance



2011, 2012 Yield trials:
3410 plots in College Station, TX
580 plots in Mississippi State, MS

Testers used:

Va35 [(C103 X T8) T8] – **NSS** – Warburton et al. 2013, Crop Science

TX714 [(K55/3*B73)-B-B-B)/B73]-1-B-B – **SS** – Barerro et al. 2015 PLoS ONE

Pleiotropic effects of loci – agronomics and yield

QTL variant	Bin	Chr.	B73	Mo17	Effect	Description
Grain yield						
1	7.04	7	C	N	5-7 bu/ac	Leucine rich repeat
2	2.03	2	N	A	3 to 8 bu/ac	PUT-2-171a-Zea_mays-13770
3	9.06	9	N	A	3-5 bu/ac	ATP-dependent CLP protease
Plant height						
1	7.04	7	C	N	2 inches	Leucine rich repeat
4	3.05	3	A	C	1 to 3 inches	Chromatin assembly factor I
3	9.06	9	N	A	~1.5 inches	ATP-dependent CLP protease
Days to silk						
1	7.04	7	C	N	1.8 days	Leucine rich repeat
5	8.05	8	C	N	1 day	Protein tyrosine kinase motif

CHR	MAF	FDR_adj_P	Log10	Effect	R2 (%)
CS11-WW ^a (log10 [aflatoxin + 10])					
4	0.32	0.2	5.48	-0.03	5.27
CS12-WW ^a (log10 [aflatoxin + 10])					
4	0.32	0.23	5.43	-0.07	5.69



Barerro et al.
2015 PLoS
ONE

Great....more significant QTV SNPs....

But how do you validate and use them?

- Confirm with other studies
- Develop iso-lines?
 - Labor/ time,
 - Genetic background / context dependent,
 - Relevance?
- Develop a bi-parental population relevant for other reasons?
 - Which parents to cross?
- Screen germplasm collections for individuals with the SNPs of interest (Romay et al. 2013)
 - >4000 lines!
 - ~700,000SNPs!

Romay et al. *Genome Biology* 2013, **14**:R55
<http://genomebiology.com/2013/14/6/R55>



RESEARCH

Open Access

Comprehensive genotyping of the USA national maize inbred seed bank

Maria C Romay¹, Mark J Millard^{2,3}, Jeffrey C Glaubitz¹, Jason A Peiffer⁴, Kelly L Swarts⁵, Terry M Casstevens¹, Robert J Elshire¹, Charlotte B Acharya¹, Sharon E Mitchell¹, Sherry A Flint-Garcia^{2,6}, Michael D McMullen^{2,6}, James B Holland^{2,7}, Edward S Buckler^{1,2,5*} and Candice A Gardner^{2,3*}

Validating these SNPs in three bi-parental linkage populations

Population	F _{2:4} N=	SNP1	SNP2	SNP3
LH82 X (LAMA2002-12-1-B-B-B-B/LAMA2002-1-5-B-B-B-B)-3-2-B-1-B3-B)	178	X	X	
Tx740 x NC356	110 (55)	X	X	
Ki3 x NC356	239 (72)	X	X	X

Ear height SNP2 $P < 0.03$

Level	LS Means	N =
X:X	22.5	156
X:Y	21.5	144
Y:Y	21.2	182
Pop effect	P < 0.0001	482 (45 missing)

Pop. 3 Plant height SNP1 $P < 0.01$

Level	LS Means	N =
X:X	62.5	56
X:Y	61.2	64
Y:Y	59.2	86
Pop. 3 only		206 (33 missing)

Pop. 3 Plant height SNP3 $P < 0.003$

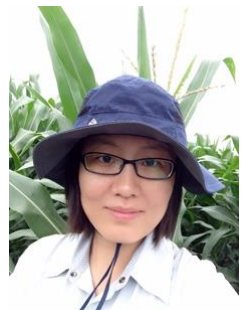
Level	LS Means	N =
X:X	59.0	70
X:Y	63.2	60
Y:Y	61.4	87
Pop3 only		217 (22 missing)

Good results considering

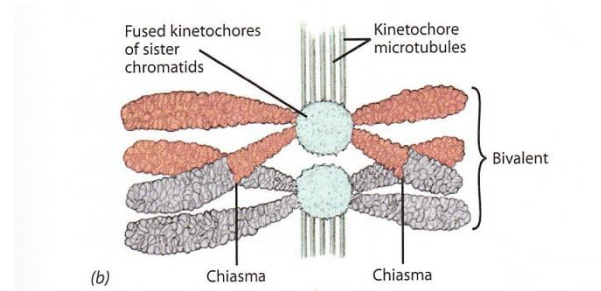
- Off season nursery
- Lines per se and not hybrids
- Not yield

Genotyped with KASPAR assay's

Ms. Yuanyuan Chen PhD student



In Plant Breeding and Genetic Linkage Mapping effective recombination is more often becoming the limiting factor



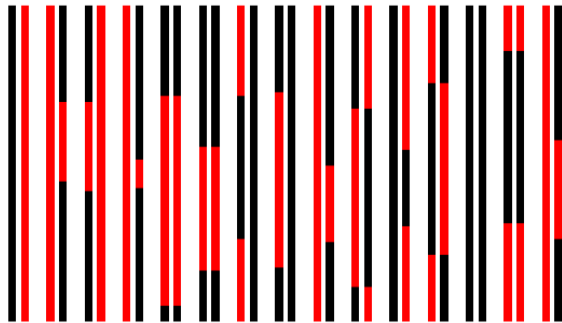
Raven, 1999.

Effective recombination < Total recombination

Introgression of gene we would still expect linkage drag.

Not near “gene” resolution for map based cloning

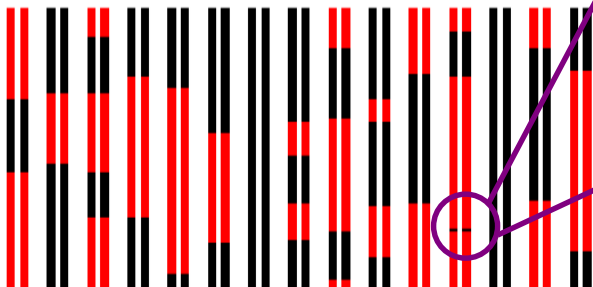
F₁



Self to homozygosity

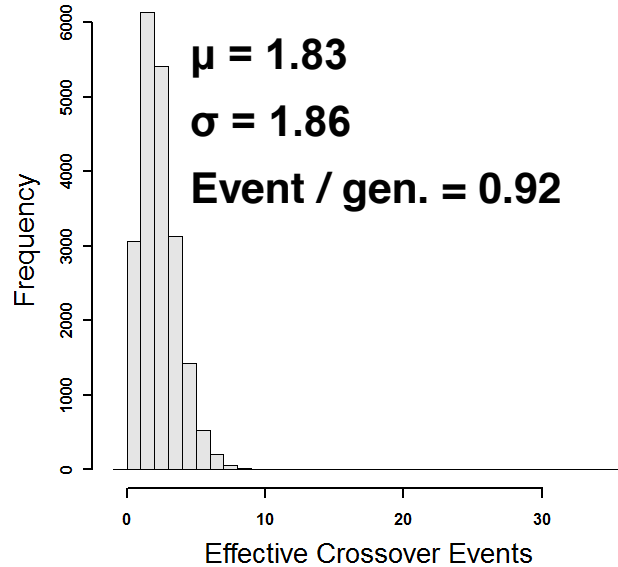


RIL's

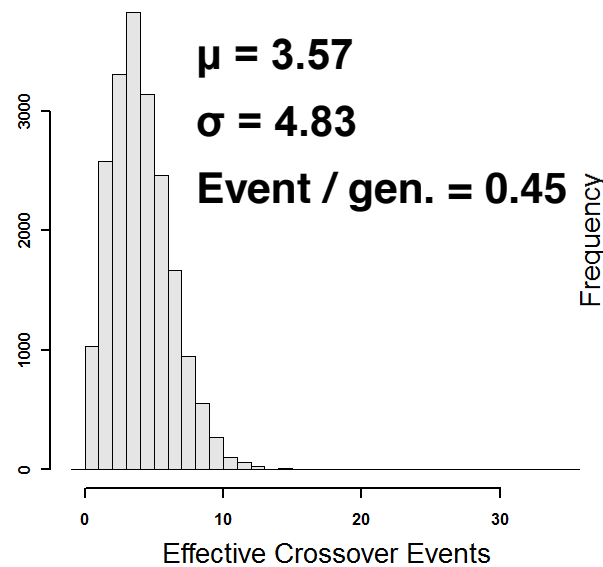


Predicted distributions of effective recombination events under different population development scenarios

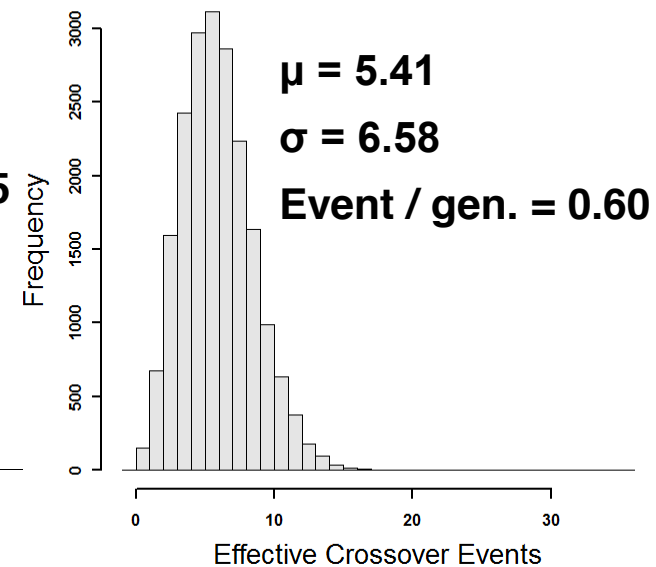
A3 - Biparental cross to F2



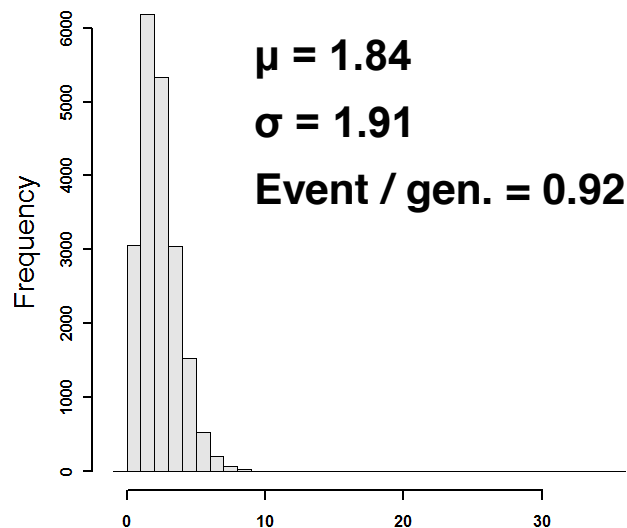
B3 - Biparental cross to F8 RILs



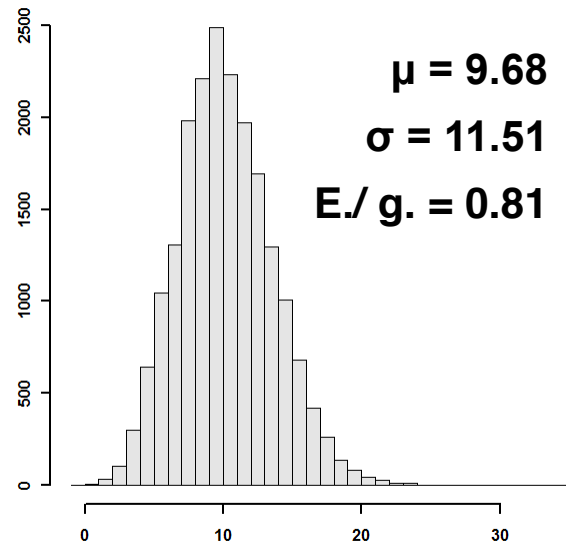
D3 - Dihybrid cross to F8 RILs



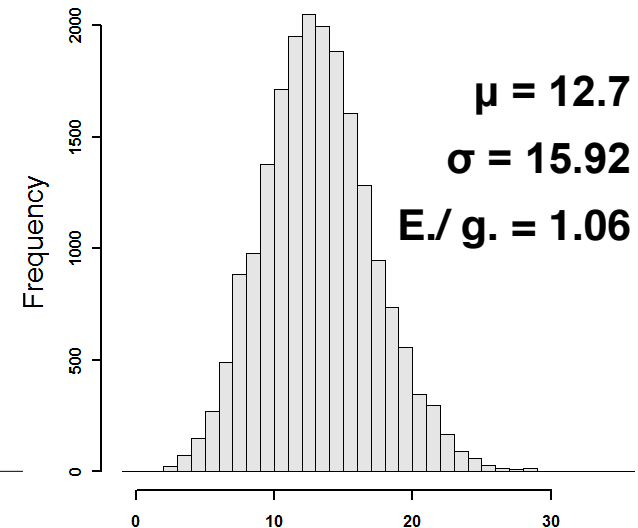
G3 - Double Haploid population from F1



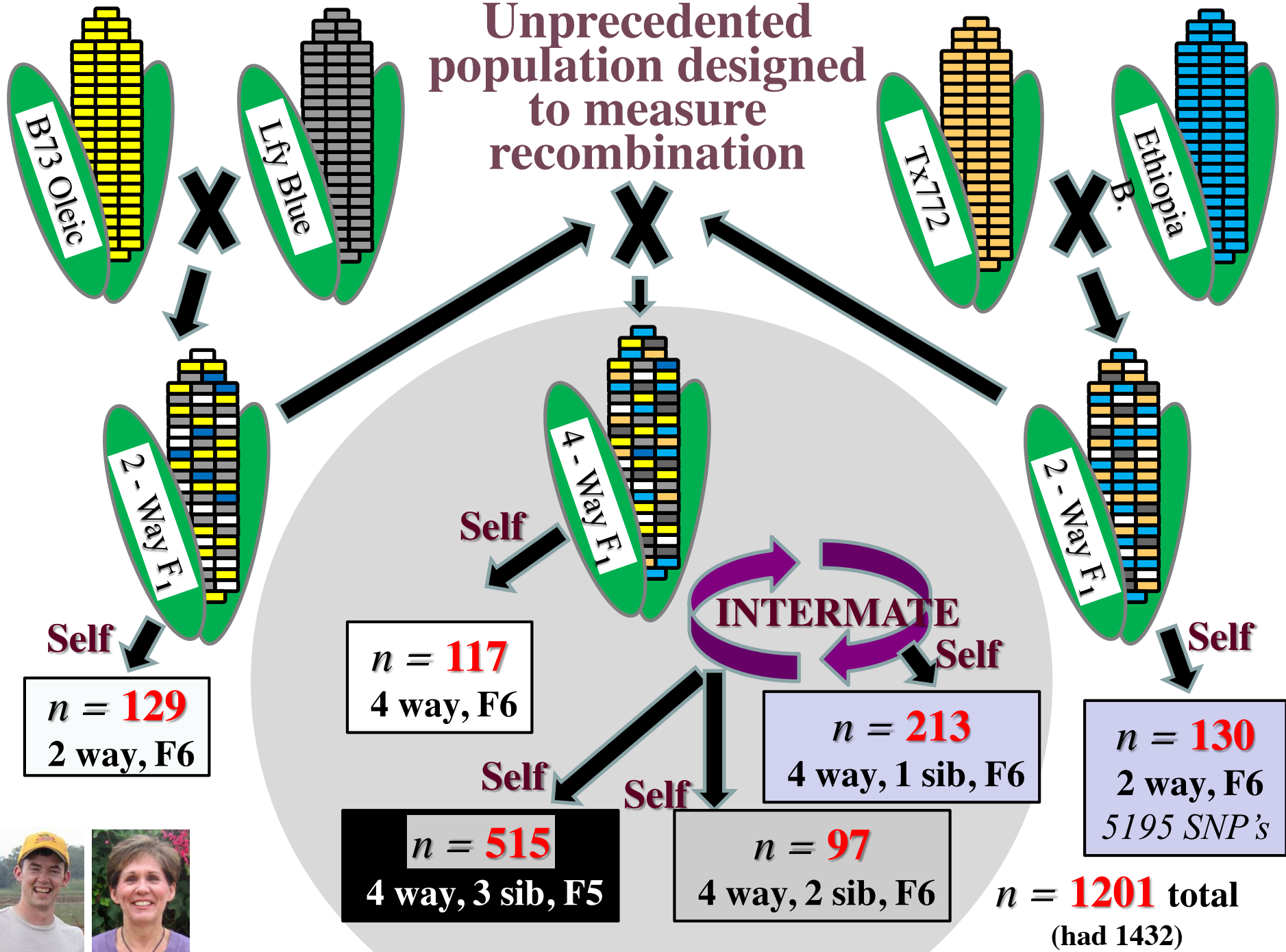
C3 - Biparental cross >4 sib >F7 RIL



E3 - Dihybrid cross >3 sib >F8 RILs



Unprecedented population designed to measure recombination



Marker results

107,308 total markers

B73 x Tx903

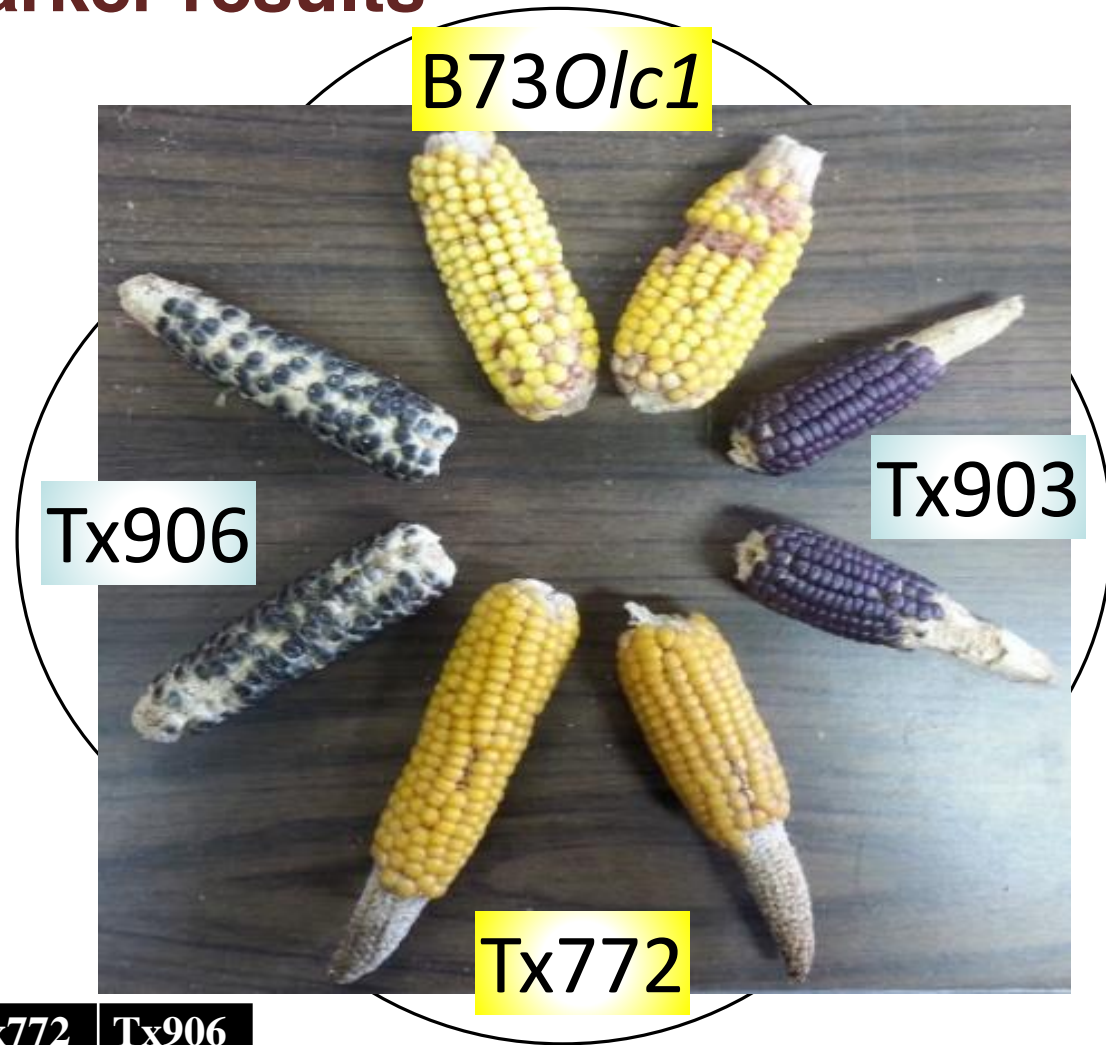
44,581 markers

Tx772 x Tx906

73,717 markers

Polymorphic in both

11,149 markers

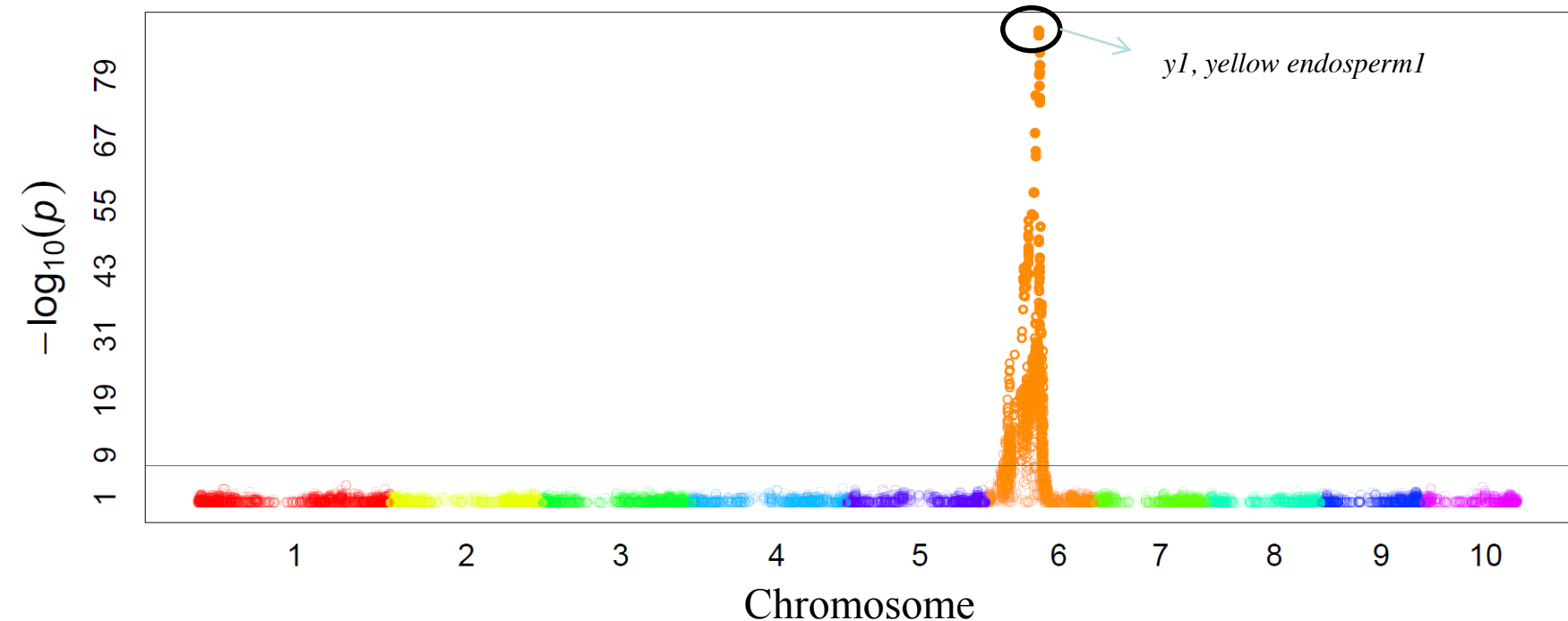


	Ref.	B730lc1	Tx903	Tx772	Tx906
Reference	100%	98.5%	64.7%	40%	44.9%
B730lc		100%	64.7%	40.1%	44.9%
Tx903			100%	40.9%	42.9%
Tx772				100%	40.7%
Tx906					100%

107,308 markers * 1207 lines
 = 129,000,000 marker data points
 @ \$0.0004 per data point

Huge power to detect QTLs with high resolution

Manhattan plot of all 4-parent individuals (899 observations); ~130,000 SNPs



- Gene region of 3,731 bp: 82,017,148 – 82,020,879
- Yellow endosperm detection was very strong, and in correct place.
- Peak logarithm of odds (LOD) value of 87.20.

Kernel color distribution

% blue	% white	% yellow	% seg.
23.1	31.3	33.0	12.6

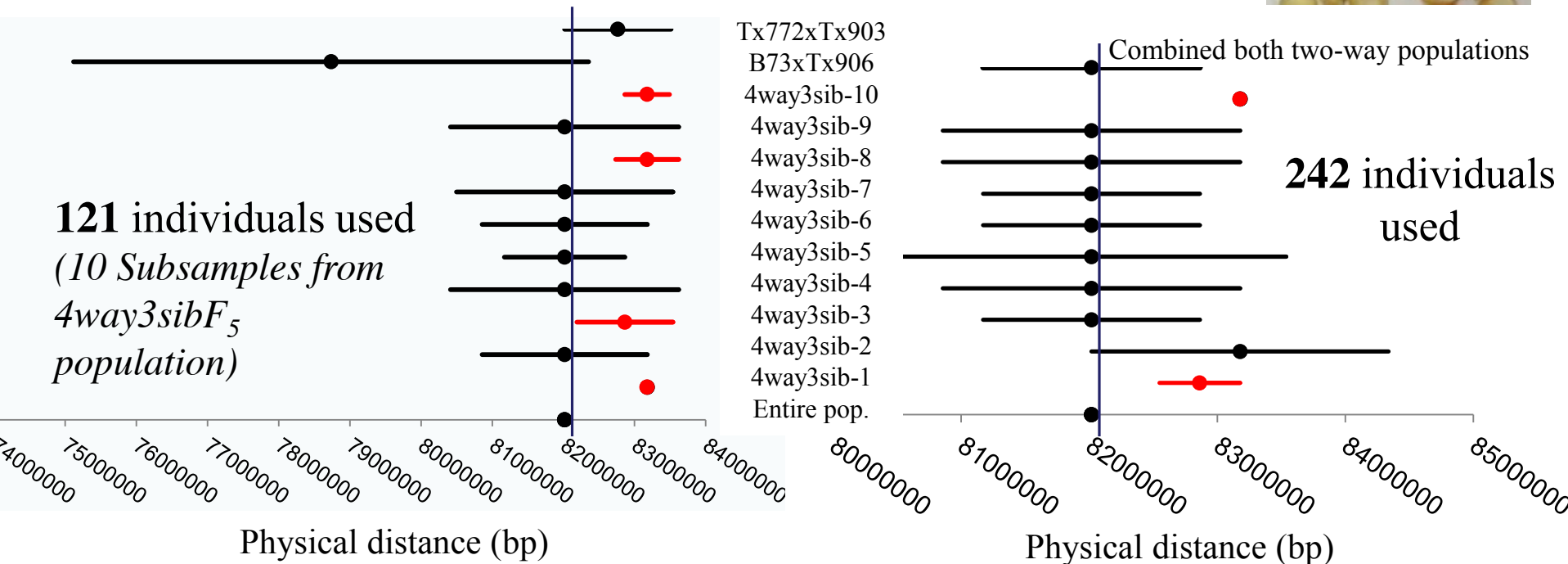
Blue kernels were dissected to determine endosperm color



yellow endosperm1 (y1) on Chromosome 6



Group	n	Chr.	Peak position (bp)	LOD	Confidence interval distance (bp)
all subpops	1141	6	82,017,348	107.08	54
all4ways	899	6	82,017,348	87.2	54
4way3sib	488	6	82,017,402	48.95	54
4way2sib	89	6	83,621,389	11.89	2,712,138
4way1sib	203	6	82,017,348	26.85	7,303,989
4way0sib	109	6	83,621,056	14.74	757,405
B73xTx903	121	6	78,735,091	16.90	3,618,539
Tx772xTx906	121	6	82,764,656	14.81	747,254
all2ways	242	6	82,017,348	29.41	845,669





Z. mays
Commercial
Hybrid



Z. diploperennis
shattering



Z. Mays
X
Z. Diploperennis
F1



Lines derived
from *Z. mays*
X
Z. diploperennis
X
Shavers
populations ~F4



Weslaco, December 2013
Note differences in roots

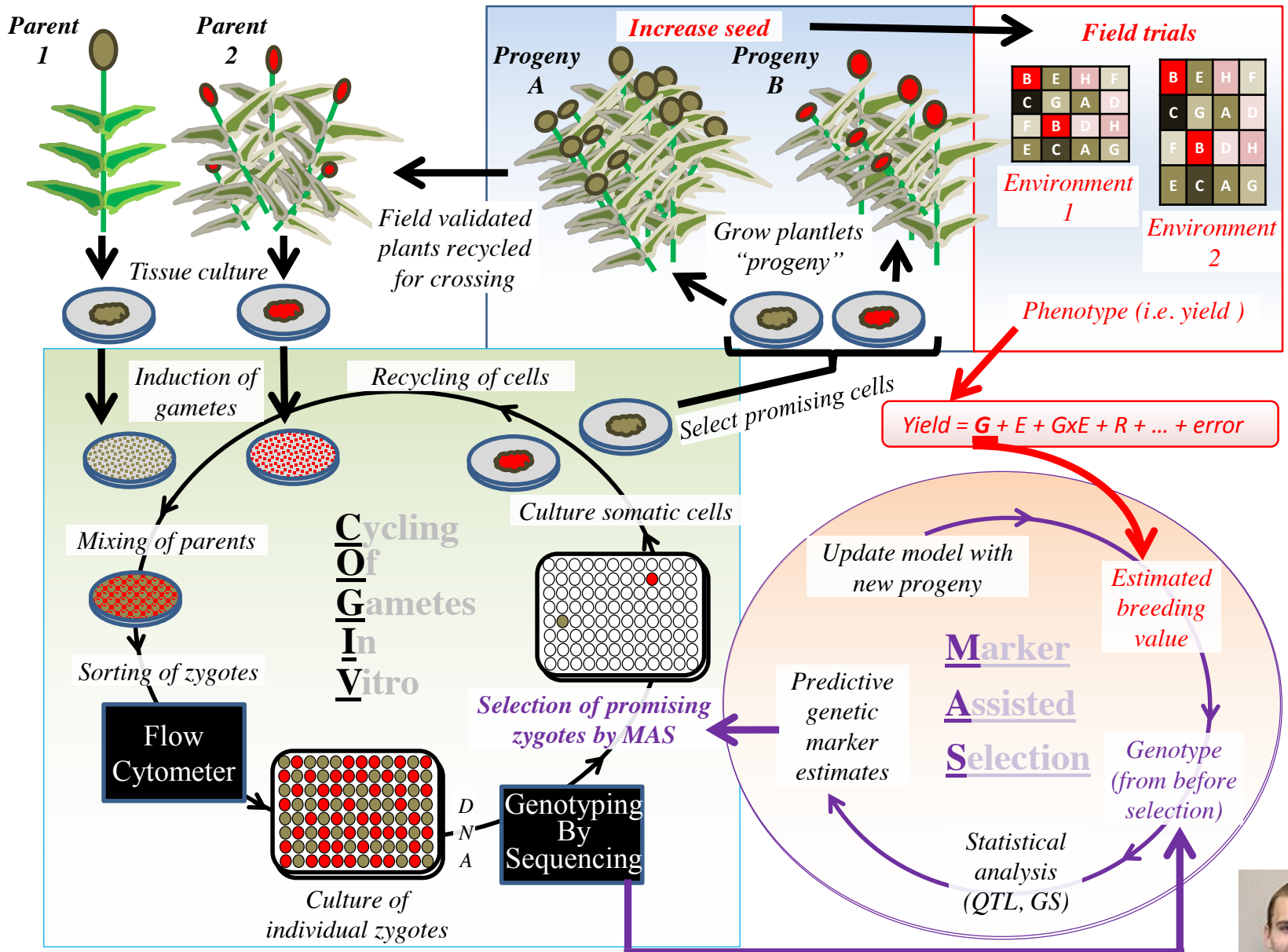
Z. diploperennis

**‘Perennial’
crosses**

Z. mays



Cycling of gamete in vitro (COGIV)





- **Undergraduates & high school students**



- **Graduate students, technical support, and visiting professors**



Acknowledgments - Funding

These projects were supported by the Agriculture and Food Research Initiative

Grant no. #2010-85117-20539 USDA-NIFA Plant Breeding and Education Program

Grant no. #2011-67003-30342 USDA-NIFA Climate Change Mitigation and Adaptation in Agriculture Program

Grant no. #2014-68004-21836 USDA-NIFA Food Security

Eugene Butler Endowed Chair in Agricultural Biotechnology

Texas A&M Agrilife Research

Texas Corn Producers Board

USDA-Hatch

USDA-SCA

Texas AgriLife Research

Monsanto Fellowship

Pioneer Hybrid Fellowship

USDA-APHIS



United States
Department of
Agriculture

National Institute
of Food and
Agriculture



Acknowledgments

Predecessors

Dr. Kerry Mayfield
Dr. Javier Betran
Dr. Tony Bockholt
Frank Foight

Critical assistance

Texas A&M Weslaco Center staff
Beto Garza and the Weslaco Special Projects unit crew
Al Nelson and the Brazos Bottom Farm crew
David Baltensperger and the Department of Soil and Crop Sciences

Collaborators and Contributions

Dr. William Rooney (TAMU) and the Sorghum Project
Mr. Dennis Pietsch and the Crop testing program
Dr. Tom Isakeit (TAMU) and laboratory

Dr. Wenwei Xu (USDA)
Dr. Mike Kolomiets (TAMU)
Dr. Gary Odvody (TAMU)
Dr. Marilyn Warburton (USDA)
Dr. Paul Williams (USDA)
Dr. Gary Windham (USDA)
Dr. Randall Wisser (UD)
Dr. Patricia Klein (TAMU)
Dr. Matt Krakowsky (USDA)
Dr. Lloyd Rooney (TAMU)
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Collaborators and Contributions

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