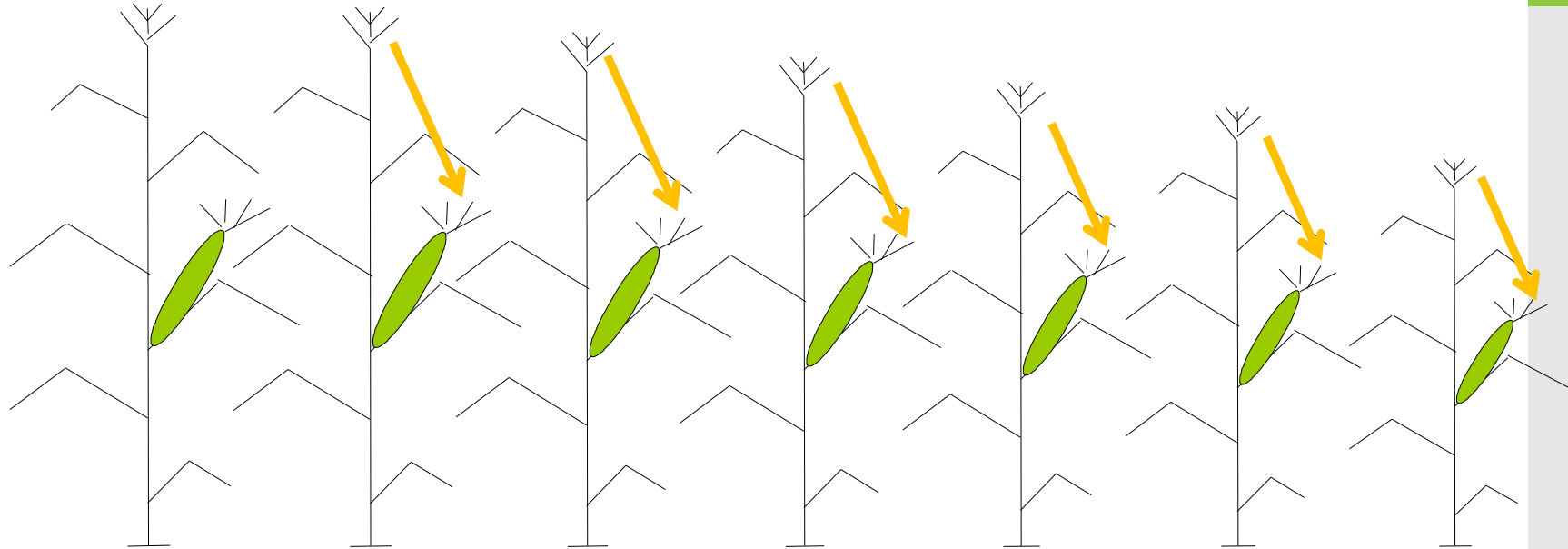


Inbred line development in landraces

Vijay Chaikam
Sarah Hearne



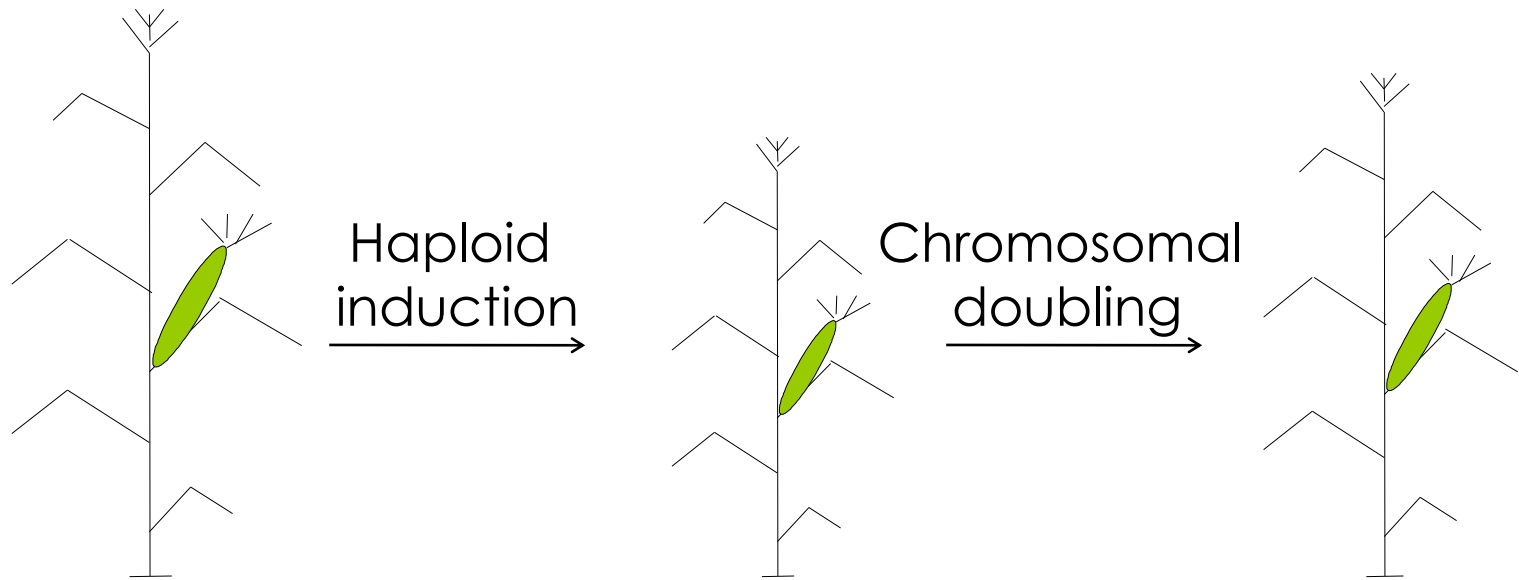
Inbred line development by recurrent selfing



Generation	Landrace	S1	S2	S3	S4	S5	S6
Homozygosity	50%	75%	87.5%	93.75%	96.875%	98.45%	99.23%
Months		6	12	18	24	30	36

- Need to start with **thousands of S1 families- Cumbersome and time consuming**
- **loss of advanced generation inbreds**
- Several attempts were futile resulting in very few viable and fertile inbreds (Schnell,1959; Pacheco et al,2002; Wilde et al, 2010)

Inbred line development using Doubled Haploid(DH) technology



Chromosome #	Landrace 2N	Haploid N	Doubled haploid 2N
Genotype	Aa, Bb, Cc	A, b, C	AA, bb, CC

100% homozygous inbred lines can be derived in two generations

DH Vs. recurrent selection in landraces

Possible to derive inbred lines from **few hundred plants** using DH

In haploids, recessive deleterious alleles are unmasked

- reduce the genetic load in one step
- no loss of inbred lines

Alleles present in heterogeneous landraces **are fixed in homozygous inbred lines in a single step**

Recent reports indicate possibility of developing enough number of inbred lines from landraces using DH

Wilde et al 2010

Strigens et al 2013

DH in improvement of landraces

DH Provides an efficient tool to **improve gene pool**

-eliminate unfavorable alleles and enrich favorable alleles

DH lines can be **evaluated in replicated trials** with high precision-not possible with landraces as such

Source material for breeding programs-**expanding genetic base** of germplasm

DH lines from Landraces represent **immortalized reproducible gametes**- Long term **conservation** without the risk of the genetic drift

DH lines from landraces are best suited for genetic studies and allele mining by sequencing

DH technology

Involves four steps

- Haploid induction
- Haploid identification
- Chromosomal doubling
- Production of seed for DH lines

DH technology

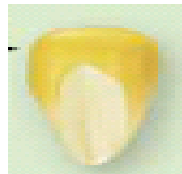
Haploid induction and identification



Inducer
 $R1-nj$



Donor
 r_1



F1
 $R1-nj/r_1$

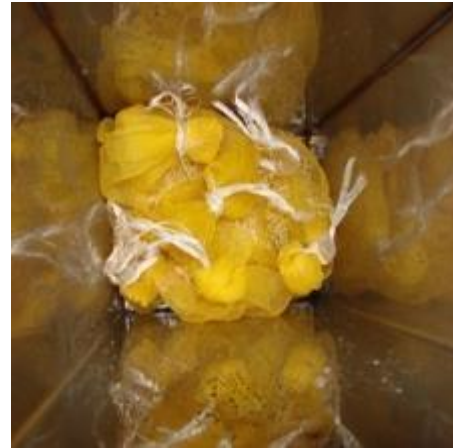


Seeds from induced ears:

- A: Normal kernels (diploid) from (unintended) outcrossing or color inhibition
- B: Normal kernels (diploid)
- C: Haploid kernels

DH technology

Chromosomal doubling

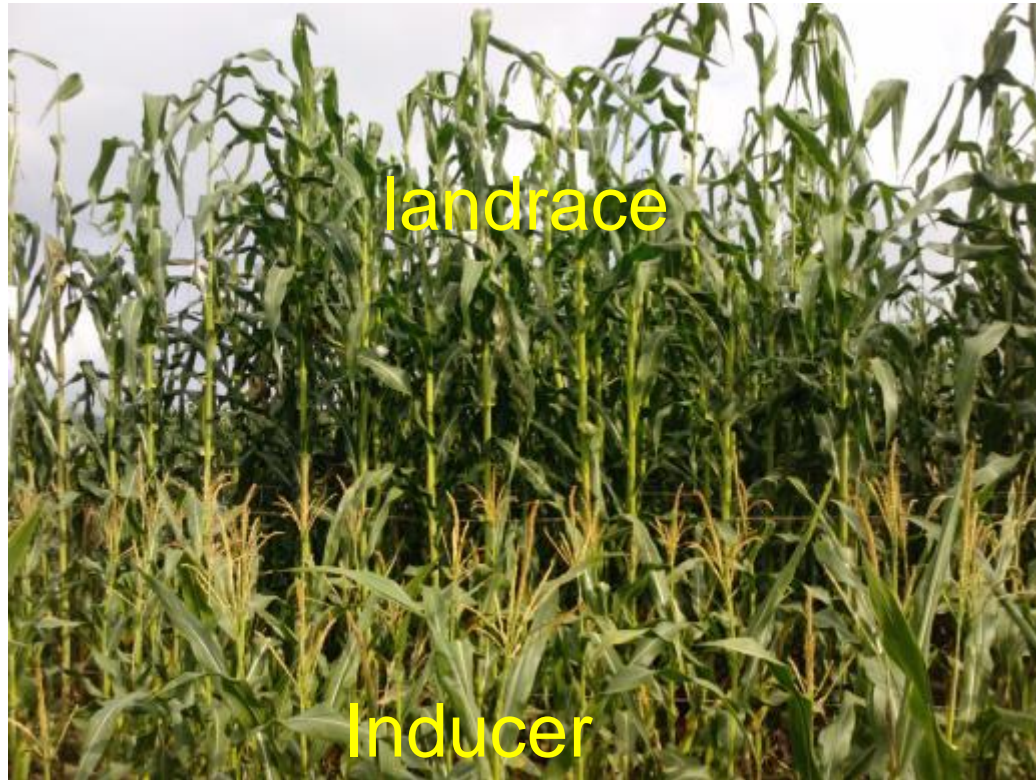


DH technology

Production of seed for DH lines



Challenges in haploid induction from landraces



- Difficult to pollinate
- Poor agronomic characters
 - root and stem lodging
 - susceptible to diseases(ear rots) and insects

Challenges in haploid induction from landraces

Landrace	Rows planted	Total seed	Yield/plant (no of kernels)	Total haploid seed	HIR(%)
CHIH 68	24	51849	115	2784	5
CHIHGP13	24	34585	77	2259	7
SNLPMEZC	24	12325	27	241	2
VERA133	24	21870	49	850	4
GUAT153	24	2021	4	26	1
OAXA290	24	4903	11	528	11
SINA102	24	14535	32	908	6
BRAZPB001	24	42080	94	1363	3
CUBA85	24	26902	60	1074	4



Poor yield from induction crosses-unable to get required numbers of haploids

Challenges in haploid identification



Germplasm	N	FULL EXPRESSION (%)	SEGREGATION (%)	COMPLETE INHIBITION (%)
Breeding populations	146	59.58	38.35	2.05
Landraces	155	3.9	69.48	27.27
Inbred lines	896	49.33	21.43	29.24

Challenges in haploid identification

Alternative marker systems:

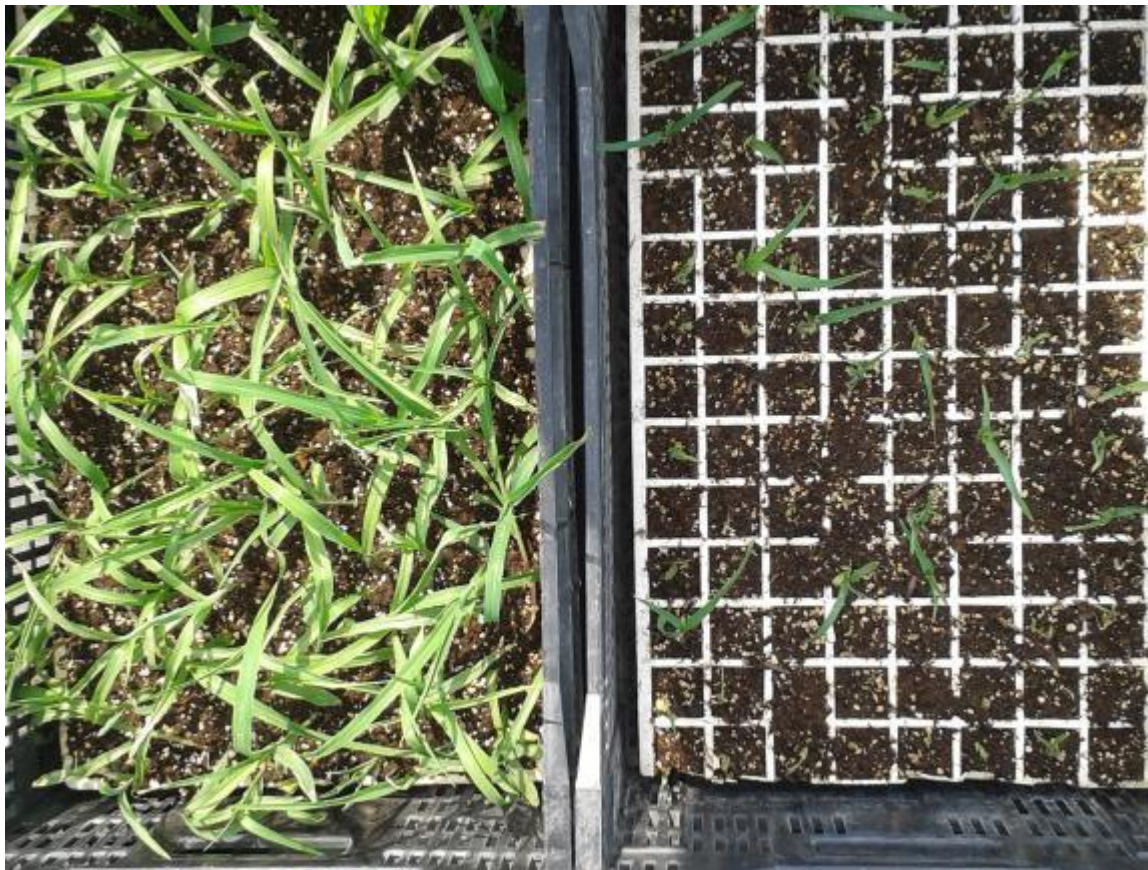
Purple/red colored root inducers, high oil inducers



Plants with white colored roots will be haploids

Challenges in Chromosomal doubling

seedling mortality



Breeding population

Landrace

Asynchrony



Success rates in chromosomal doubling with landraces

Season	# of accessions	# of haploid plants(treated) established	# of DH plants with seed	Doubling rate (%)
2013-winter-AF	24	804	10	1.24
2013-summer-celaya	11	108	23	21.29



Perspectives

Use of DH technology can overcome the difficulties in inbred development from landraces

Technical improvements in DH technology can lead to more efficient production of inbred lines from landraces

- New tropicalized inducers
- Alternate marker systems for haploid identification
- Locations with good agronomic conditions
- Better agronomic management

Even with limitations, DH technology offers fastest and cheapest way for producing inbred lines from landraces

Acknowledgements

SAGARPA

Leocadio Martinez

Luis Lopez Antonio

