A Novel Method for Simplified Hybrid Breeding

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ŌTĀKOU WHAKAIHU WAKA

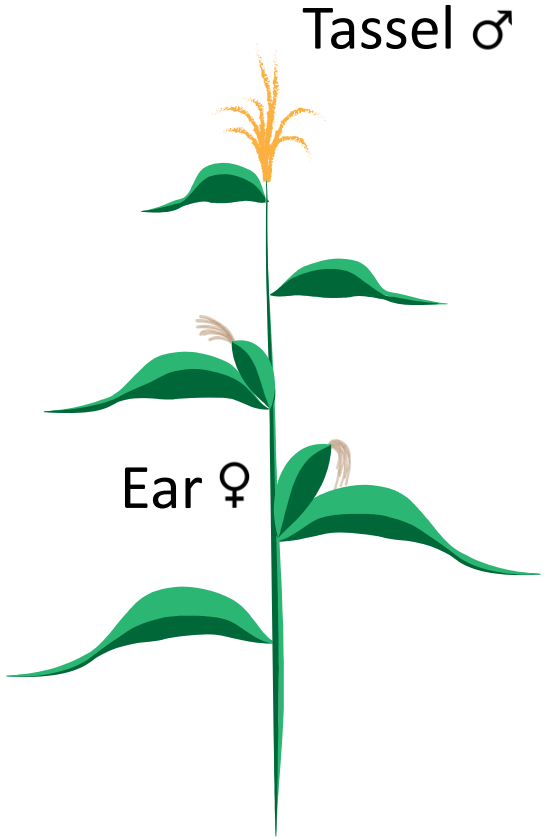
F1 Hybrid breeding

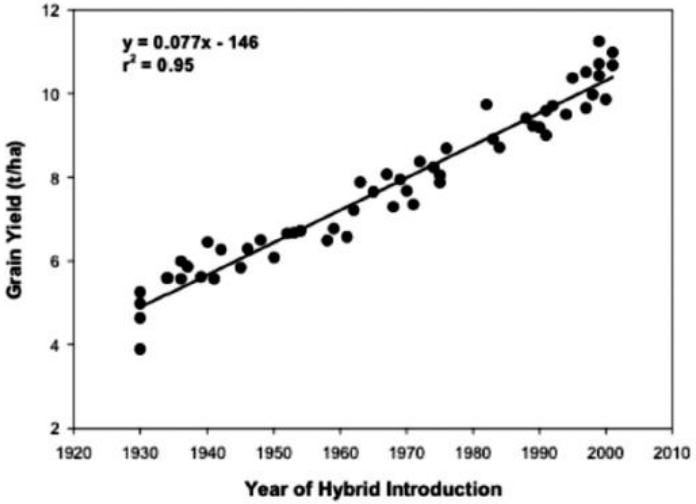
- Widely used in Maize
  - Ease of self-pollination
  - Ease of emasculation

- Difficulties for potato:
  - Self incompatibility
  - Small/bisexual flowers

- Solving two problems at once

  Combined **I**nbreeding **A**nd **O**utcrossing



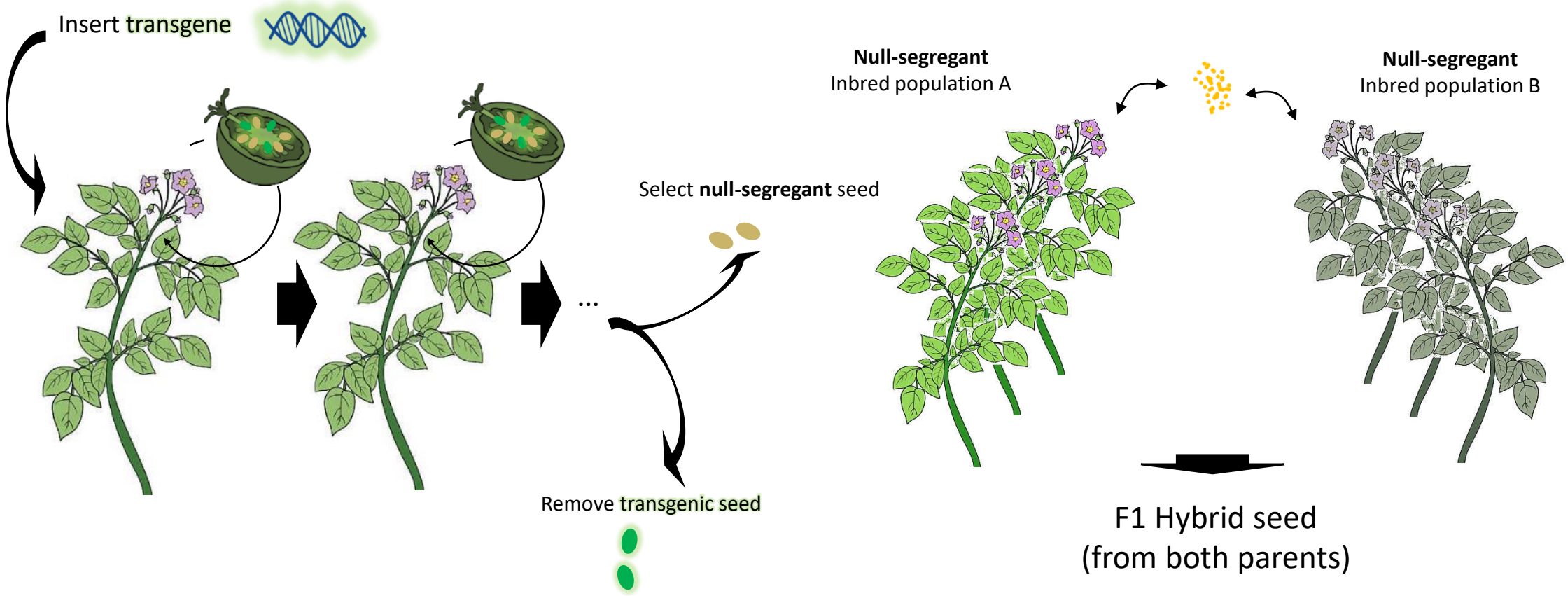


Duvick, 2004

Combined Inbreeding And Outcrossing (CIAO)

Self-fertile while inbreeding

Genetically-enforced
outcrossing for F1 hybrid production



The CIAO Transgene

Contains Three Key Components:

Self-fertility-inducer (SFI)

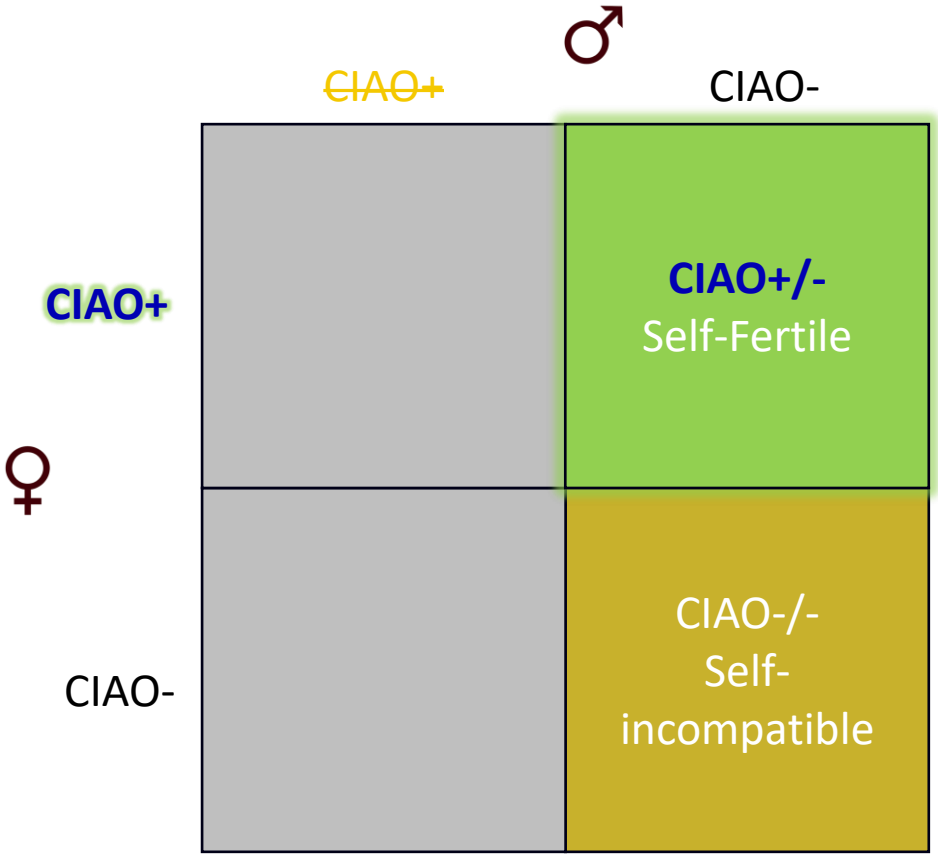
Pollen-Lethal-Cassette (PLC)

Seed-fluorescent-marker (SFM)

SFI enables inbreeding while transgene is present

PLC maintains CIAO construct as hemizygous

SFM allows selection of CIAO+ or CIAO- seed every generation



Arabidopsis proof-of-concept

- Used Self-Incompatible *A.thaliana*
  (expressing SRKb/SCRb from *A.lyrata* (SI))
- Introduced CIAO transgene and combined lines
- CIAO transgene designed to target SRK_b

CIAO-/-
(white seed)

CIAO+/-
(red seed)

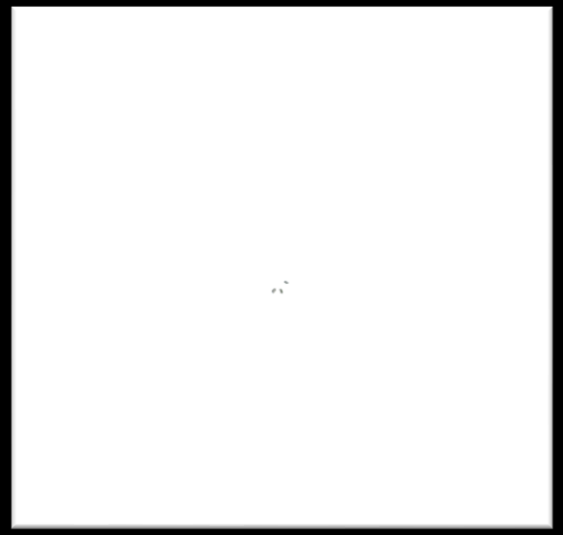
Seed production

CIAO-/- (Null segregants) produce little/no seed

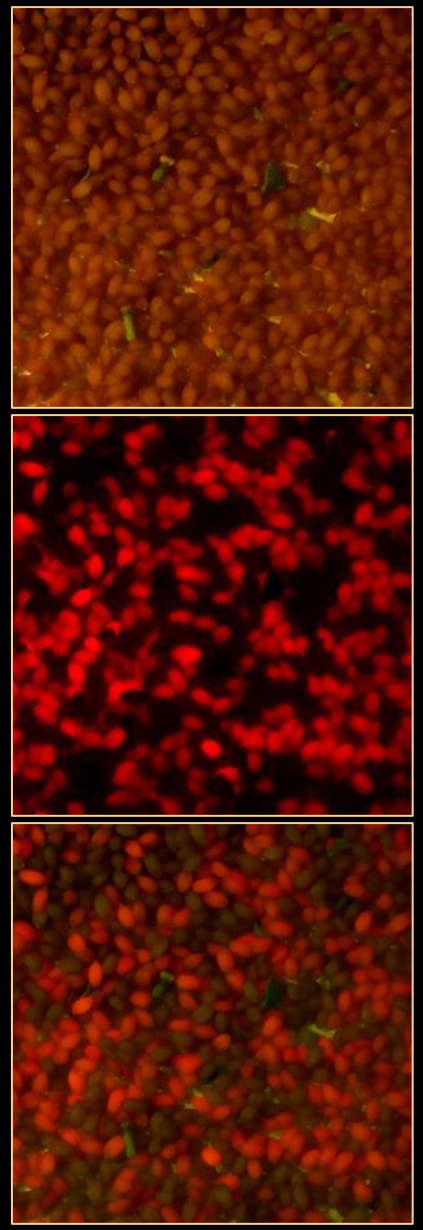
CIAO+/- (hemizygotes) produce high levels of seed

50% of Seed from CIAO+/- is red

CIAO-/-



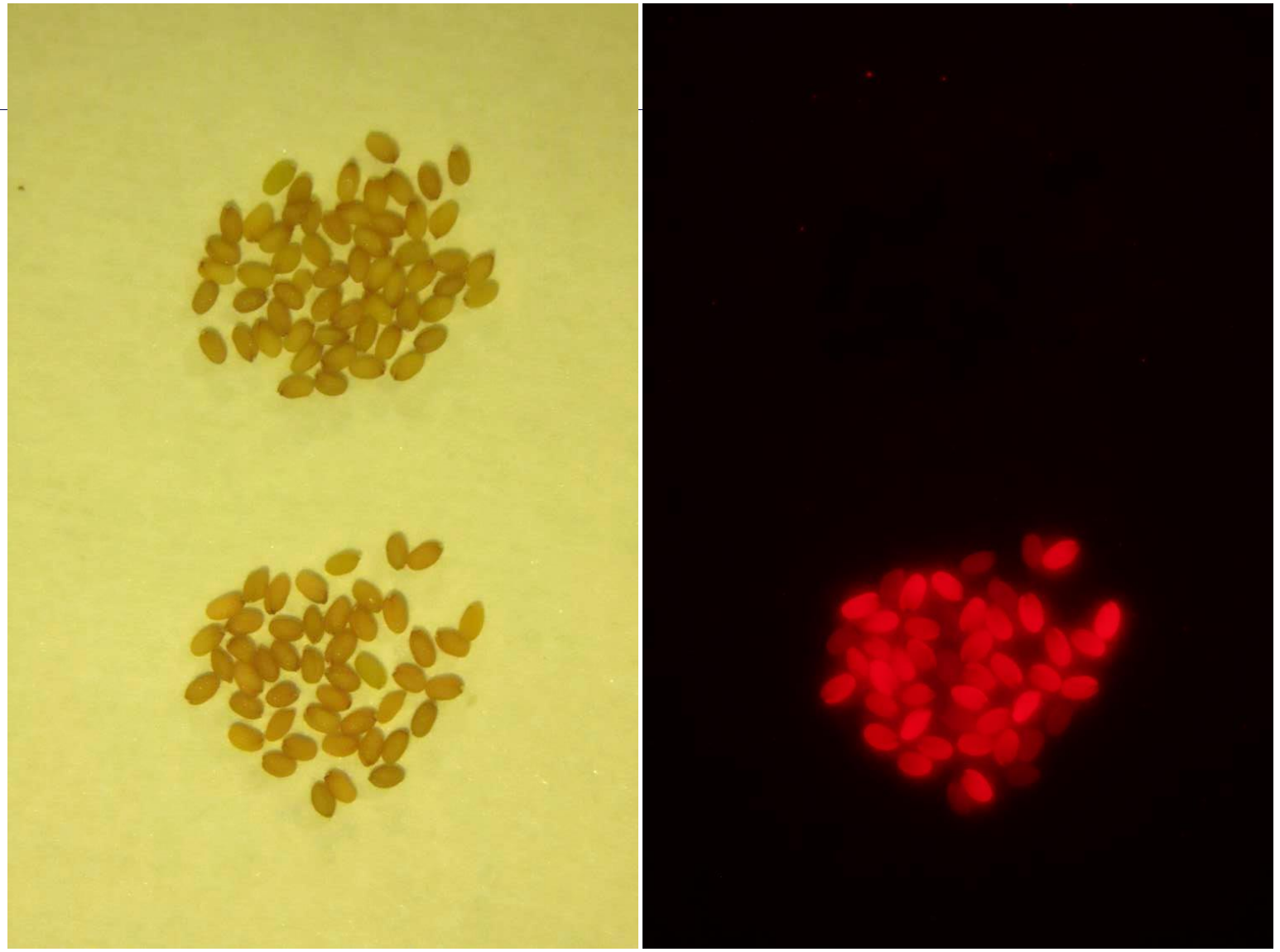
CIAO+/-



Seed Sorting

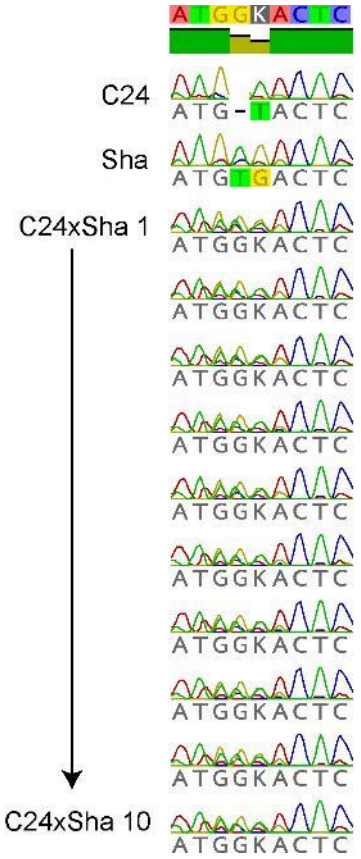
Seed can be sorted based on fluorescence

Mechanical sorting is possible



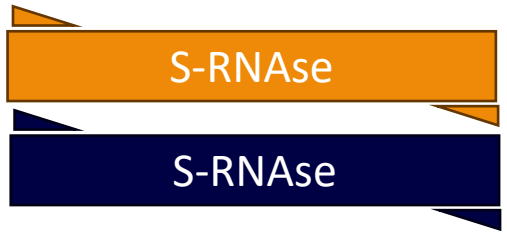
Genetically-enforced outcrossing

- The SI mechanism remains active in the null-segregant seed/plants
- Outcrossing is enforced by the SI mechanism
- No emasculation is required



Proof-of-concept in Potato

- Tailored CIAO machinery to three diploid varieties:
  - Aztec Gold
  - Scapa
  - Andean Sunside
- Tested transgenics for:
  - Self-fertility
  - Seed fluorescence
  - CIAO-transmission through pollen



Sequence S-RNAse

Allele-specific multiplex knock-down

Wild-type (no berries)

CIAO+ (inbred berries)

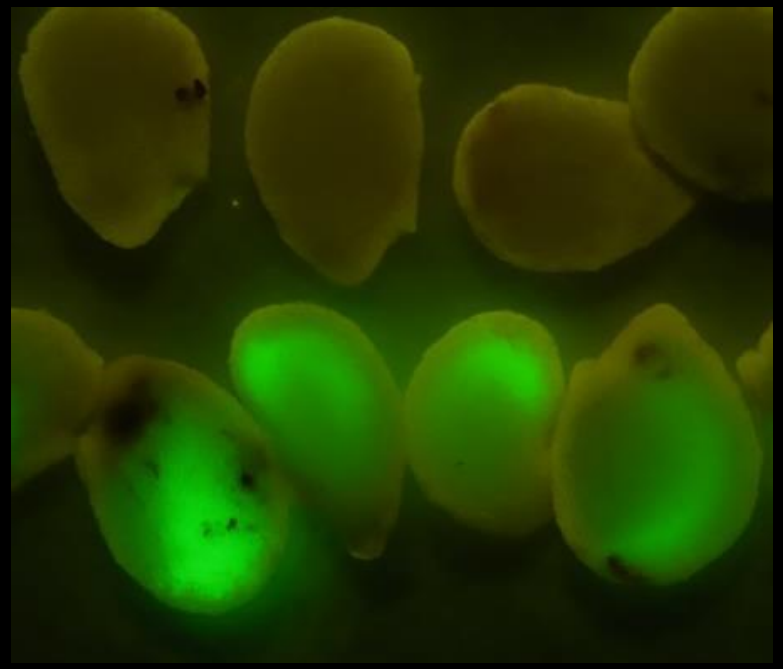
Potato seeds

Crossed in both directions to determine CIAO transgene transmission

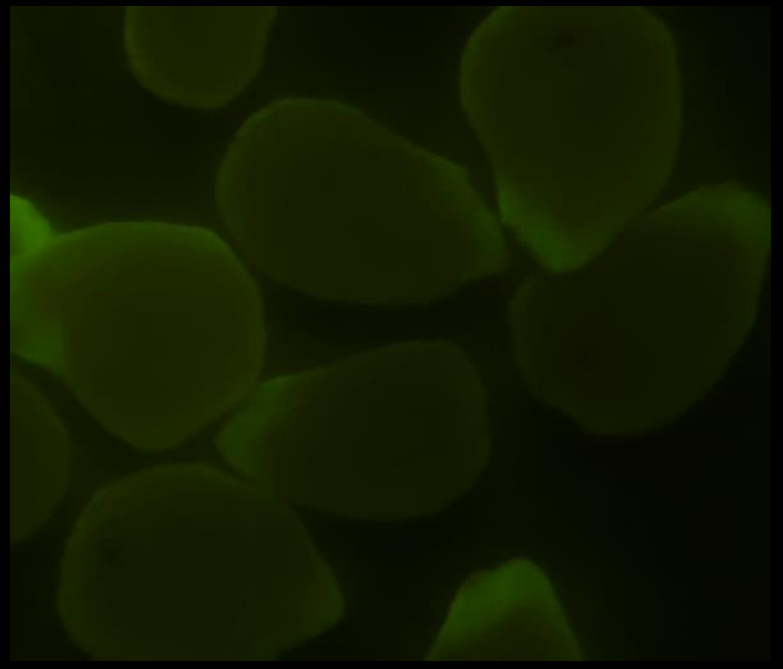
No transmission through pollen

(PLC active)

CIAO+/- Female



CIAO+/- Male

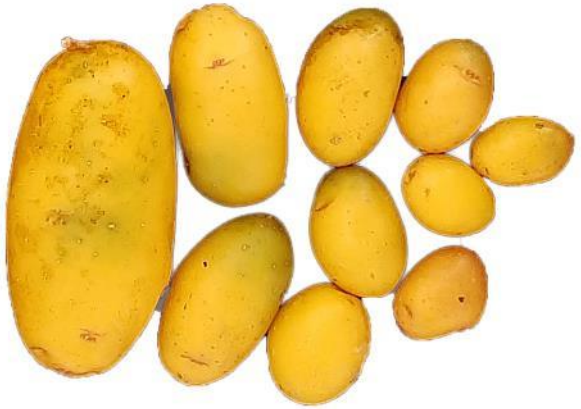


Future Directions

- Improved CIAO lines with good self-fertility (pollen and berry production)
- Test genetically-enforced outcrossing in potato
- Partnerships/Funding/R&D

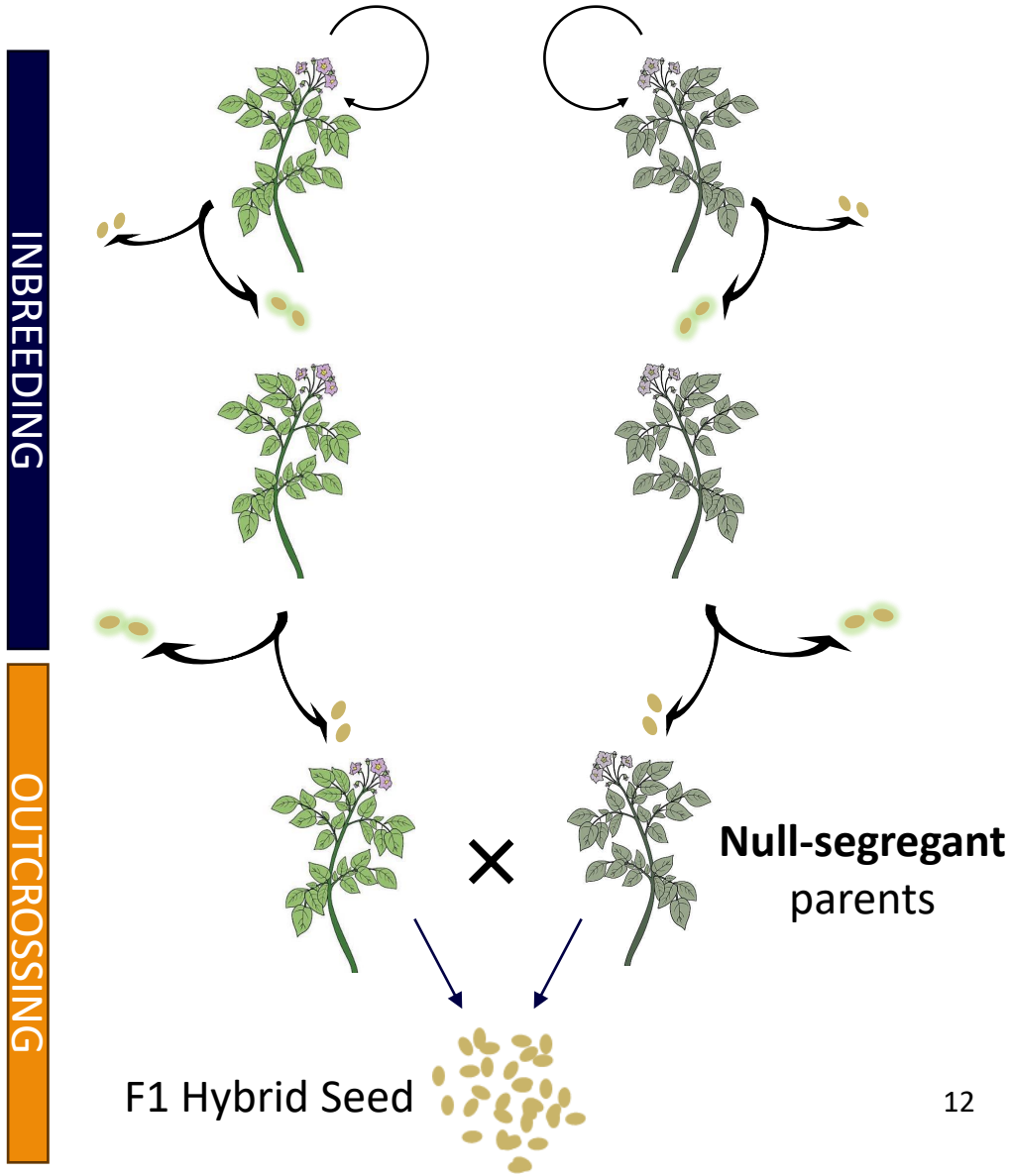
Wild-type (Aztec Gold)

CIAO+/- (Aztec Gold)



Combined Inbreeding And Outcrossing

- A transgene that can be used to create inbred lines from Self-Incompatible species
- The transgene is present in 50% of offspring, which can be identified by seed fluorescence
- Null-segregant (non-transgenic) offspring can be used to make F1 hybrid seed from both parents



Acknowledgements

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