Breeding Process Framework: A Powerful Tool for Enhancing Desirable Traits in Potato

David S. Douches

Natalie Kaiser, Joe Coombs, Paul Collins, Kelly Zarka, Kate Shaw, Chen Zhang, Felix Enciso, Swathi Nadakuduti, Dan Zarka, Thilani Jayakody, Matt Zuehlke, Jaime Willbur, Ray Hammerschmidt, Robin Buell

Dept. of Plant, Soil, and Microbial Sciences

Michigan State University

East Lansing, MI 48824

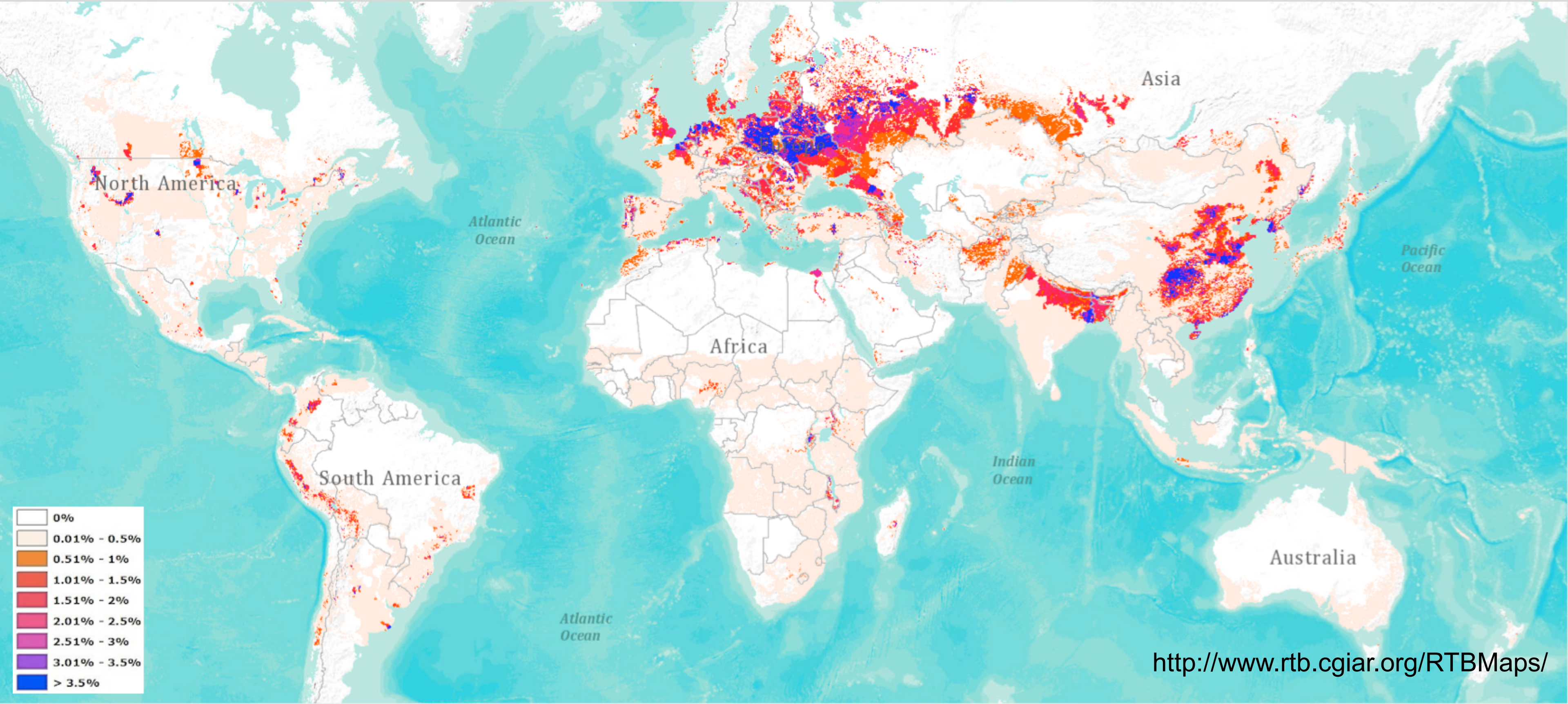
The Potato

(*Solanum tuberosum* L.)

- Fourth in world production
- No. 1 Vegetable in the world
- Most popular US vegetable

The potato produces more nutritious food, more quickly, on less land, and in harsher climates than any other major crop.

Potato is the most significant non-grain food crop in the world

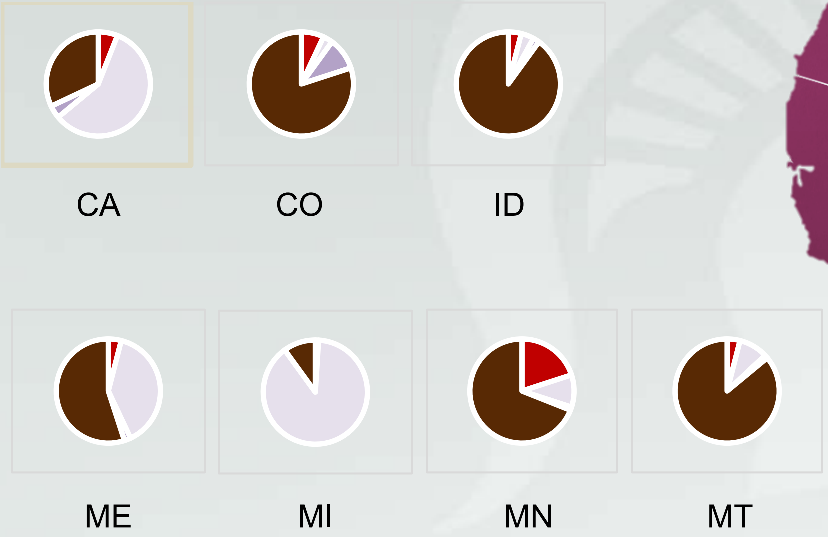


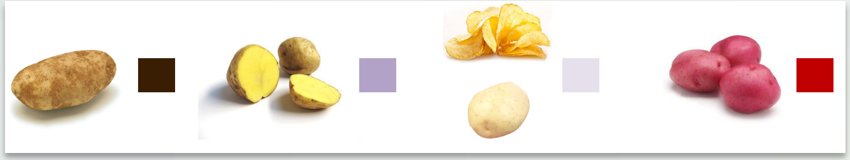
http://www.rtb.cgiar.org/RTBMaps/

US Potato Production

- $4 B Annual crop value on 1 M acres
- 5th in world production
- Horticultural crop
  - High cost of production: over $3,000/acre (managing insects, diseases, nutrients and water)

US Potato Production





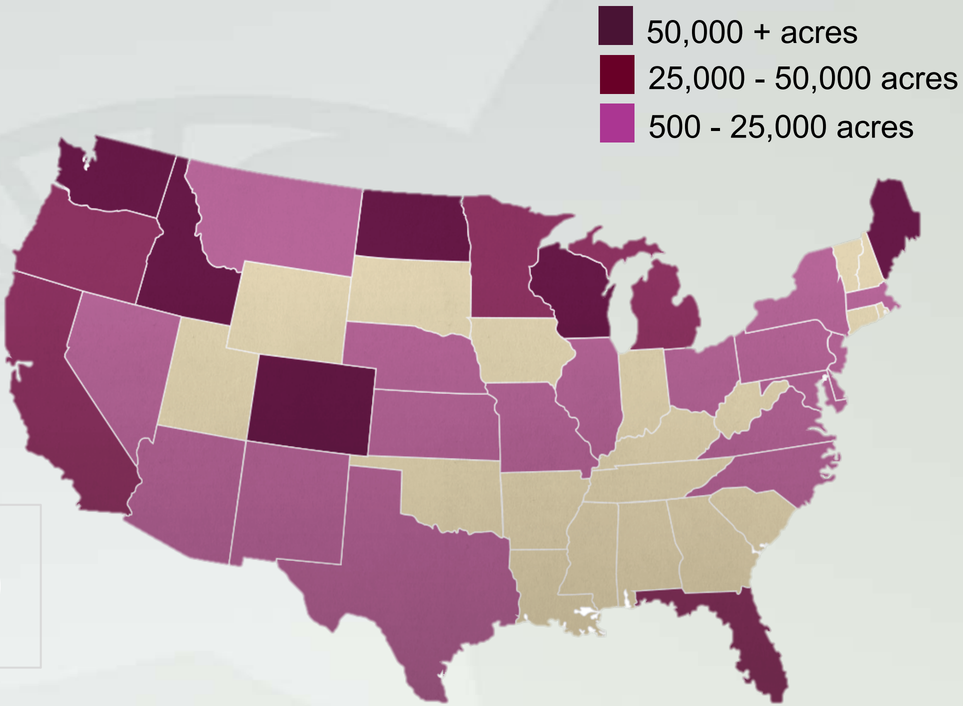
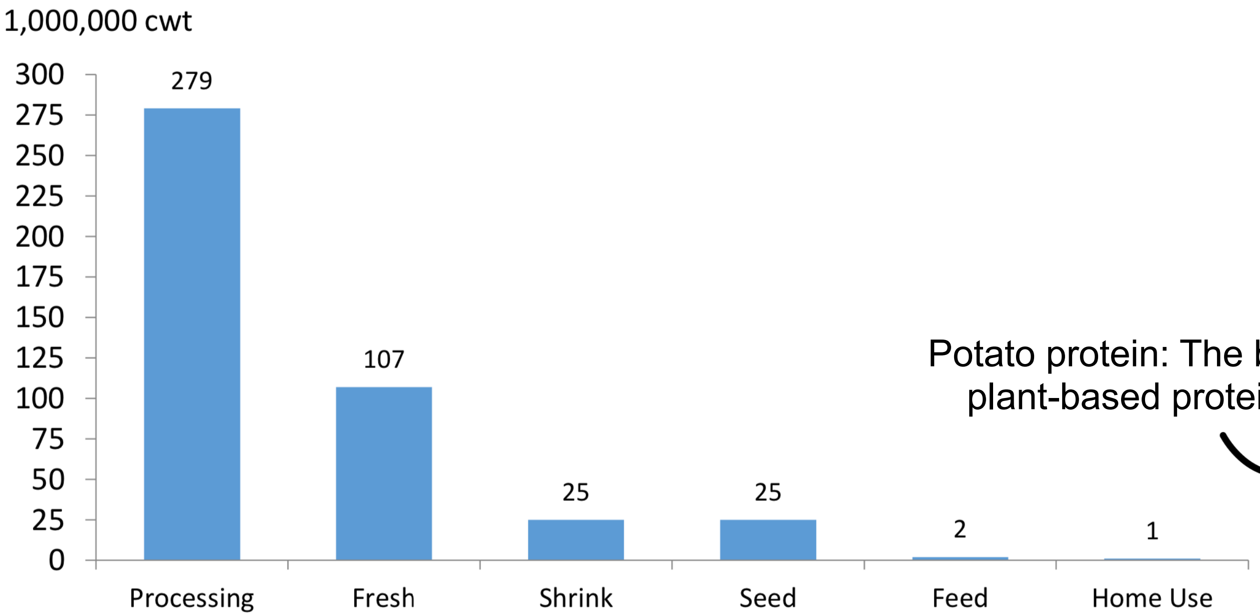
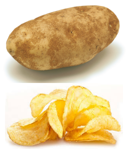


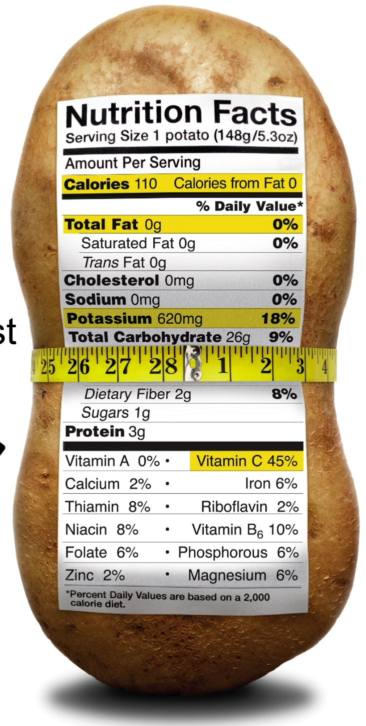
Photo credit: Potatoes USA
Data: USDA, NASS 2017

Potato consumption in the US





Potato protein: The best plant-based protein



USDA, NASS, 2017

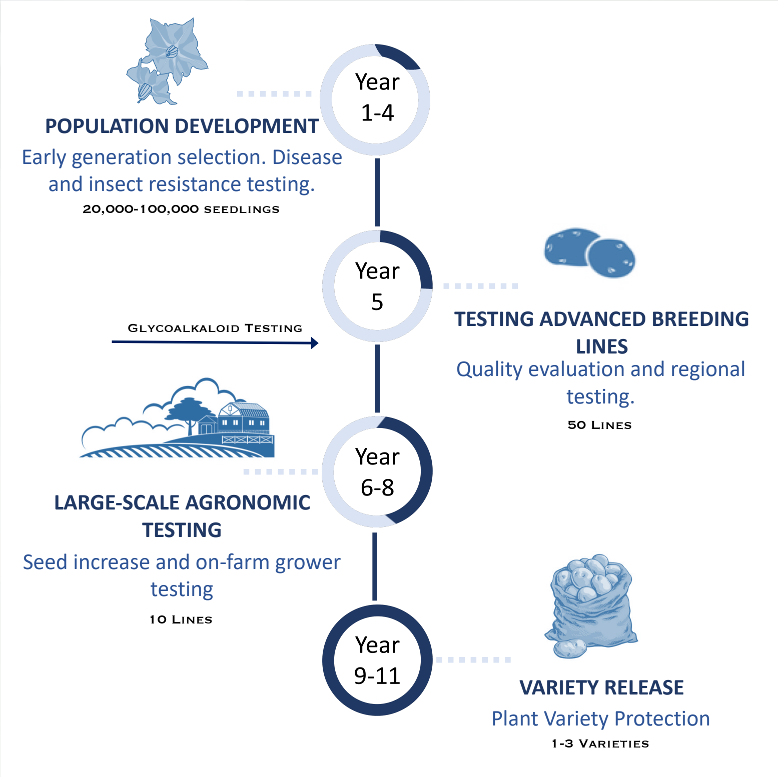
US Potato Breeding is Public

- 9 University breeding programs
- 4 USDA/ARS breeding/research
- 2 Private breeding programs

⊗ Public Breeding Programs

Potato Breeding: A challenging 8-12 Year Process

- Tetraploid (4x) and heterozygous
- Prevents the fixation of desirable alleles in variety development
- Each cross segregates for all desirable and market limiting traits

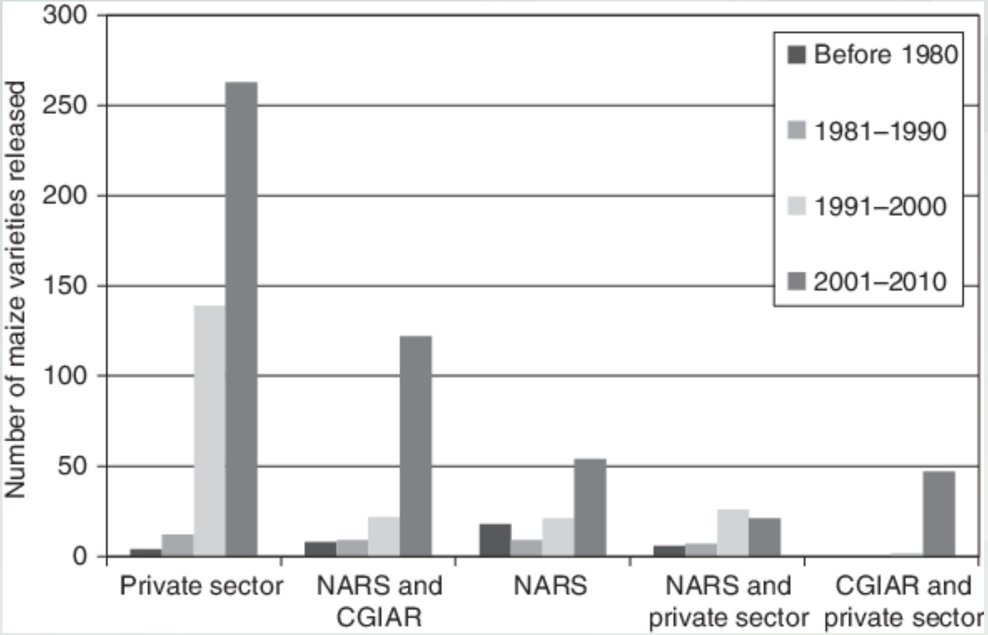


Kaiser et al., 2020 submitted

Potato Breeding Progress has been slow

- For 100 years, potato yield increase mostly due to improvement of production and management techniques
- Every cross mixes all the important traits for potato agronomic performance and market quality

Variety change: Top 20 US potato varieties vs maize
Variety adoption is slow



De Groote, H., et al. "11 Assessing the Effectiveness of Maize and Wheat Improvement from the Perspectives of Varietal Output and Adoption in East and Southern Africa." *Crop Improvement, Adoption, and Impact of Improved Varieties in Food Crops in Sub-Saharan Africa* (2015): 206.

Only 2 of the top 20 varieties were released after 2000.

Russet Burbank is over 100 years old!

Russet Burbank - 1876

Norkotah Russet - 1986

Ranger Russet - 1990

Milestones in Potato Breeding and Genetics: Integrating Conventional Breeding, Biotechnology and Genomics

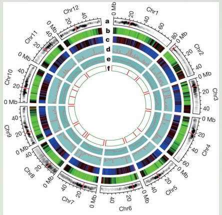
Since 1850s
Conventional Tetraploid (4x) Breeding
-Vegetatively propagated

1995
Genetic Engineering

2005
Diploid Self-Compatibility

2010
Diploid (2x) Breeding
-True seed production

2011
Potato Genome Published
-Genomic tools



2015
Gene Editing

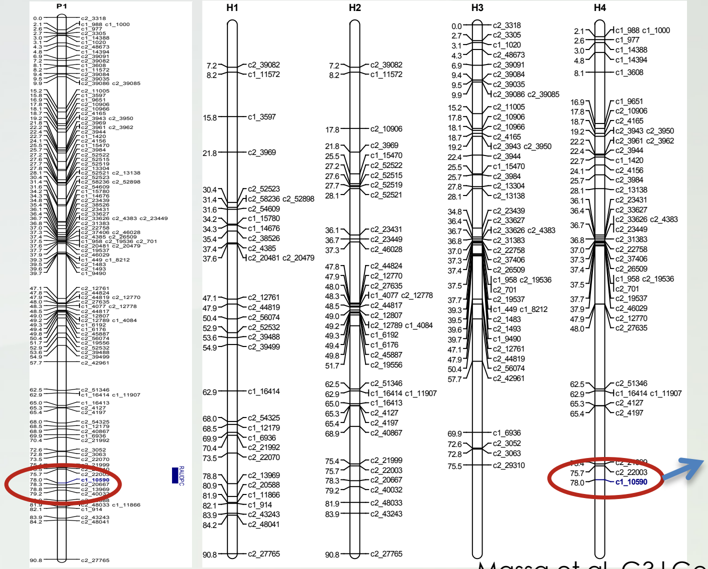
Opportunities for Potato in the Genomics Era

- Genome wide markers
  - SNP array technology and other platforms
- Sequencing technologies
  - Assembled genome(s)/annotation
  - RNA-seq
- Genetic engineering
  - Silencing genes
  - New genes for important traits
  - Targeted gene editing

Marker Assisted Breeding – SNP example

- Tetraploid mapping using SNPs to find markers associated with trait
  - Need for 2-4,000 markers to have density for QTL mapping
  - Use of potato browser to develop markers for breeders and annotate genes
- Now have breeder selection tools for many disease resistance and some quality traits

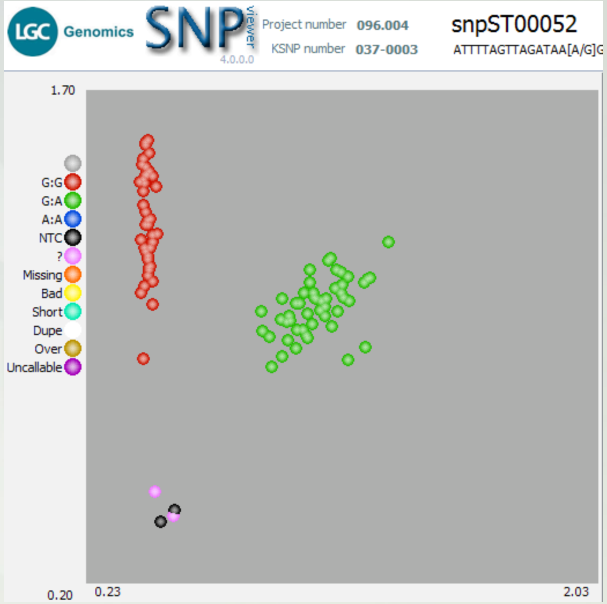
Late blight resistance



Massa et al. G3|Genes|Genomes|Genetics 2015

Marker Assisted Breeding

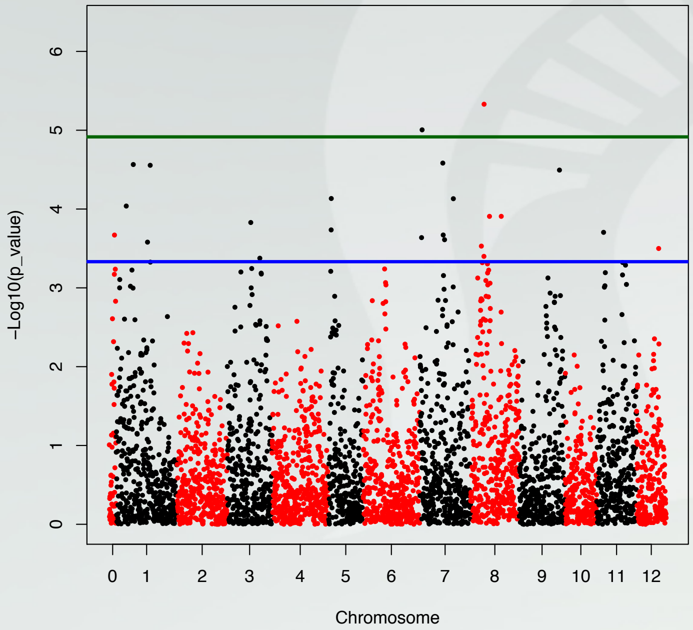
- 10 KASP (SNP-based) markers for economically important traits/sample for potato
- High Throughput Genotyping Services (HTPG) is a shared KASP genotyping platform serving CGIAR centers and their partner programs
- HTPG was established to provide low-cost and world class genotyping services to breeding programs working on CGIAR mandate crops, including potato



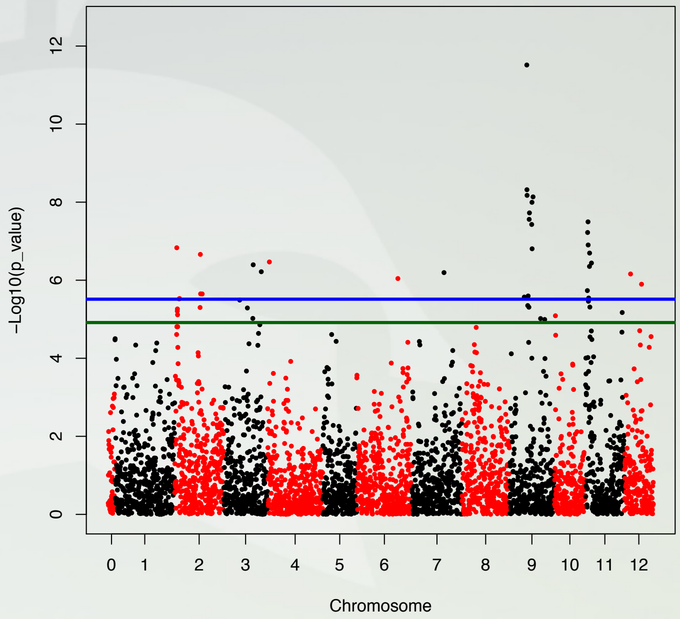
Genome wide association studies (GWAS)

- understanding contributions to resistance traits in breeding population

Late Blight



Scab

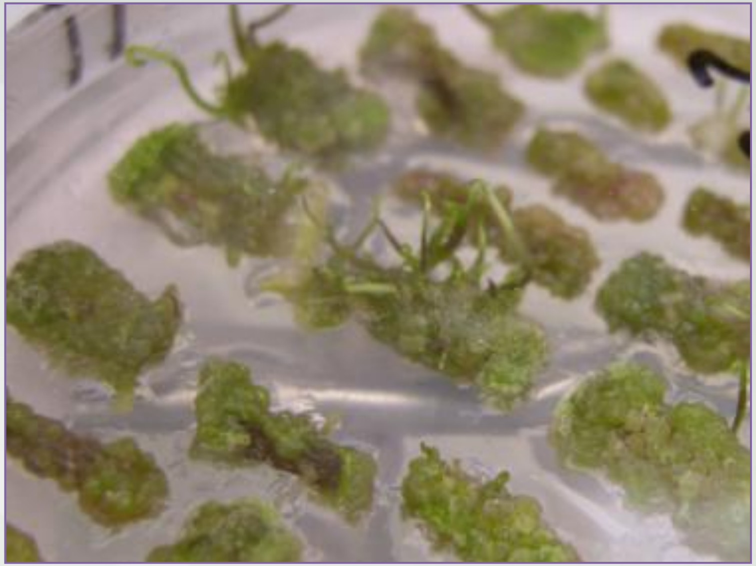


Identified SNPs associated with different R-gene hotspots.

Enciso, et al., 2018

GM Approach: Key Economic Traits of Interest

- Silencing of vacuolar acid invertase (*VInv*) to reduce conversion of sucrose to reducing sugars in potato tubers
- Water use efficiency in potato
- Enhancing Late Blight (*P. infestans*) Resistance of Potato Breeding Lines with three R-genes from *Solanum* species



VInv silencing for improving storability for processing

- RNAi invertase gene silencing events with Kalkaska variety
- Simplot Plant Sciences has released three *VInv* silenced potato varieties in the US in 2016 that are in the commercial market

Kal.91.03

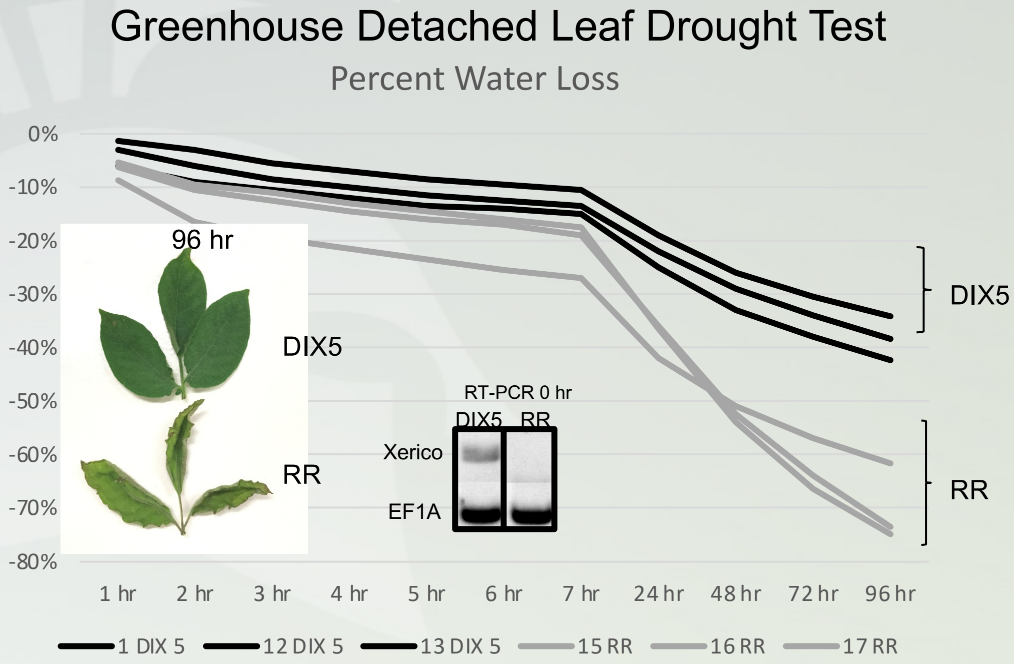
Kalkaska

Chipped directly after 3 months at 40F

XERICO Drought Tolerance Technology for Potato

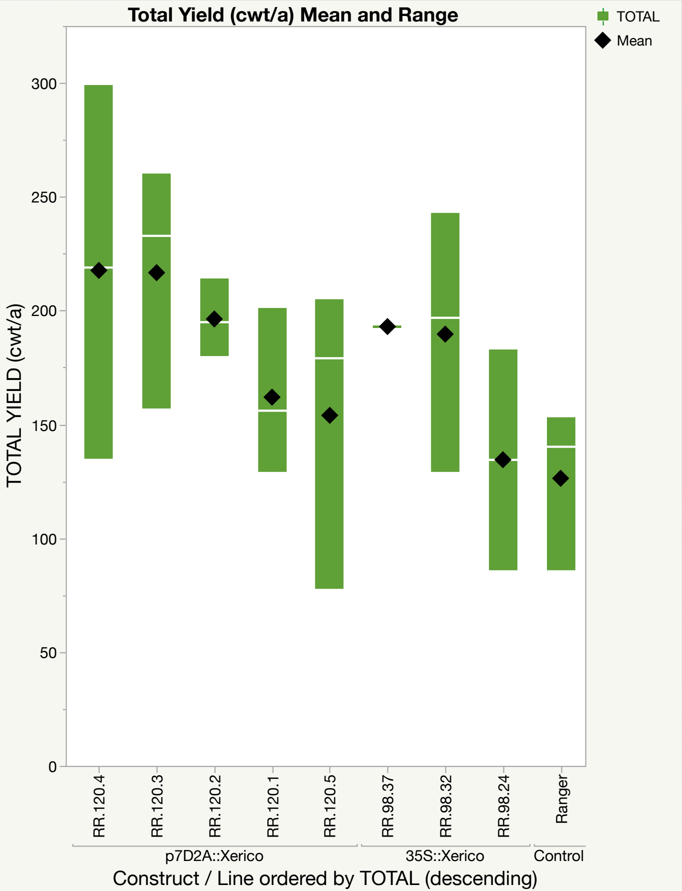
A trait for climate change

- Transformations using the XERICO gene coupled with drought inducible promoter in a commercially important potato variety
- Greenhouse studies to verify function
- Field trials to assess agronomic traits

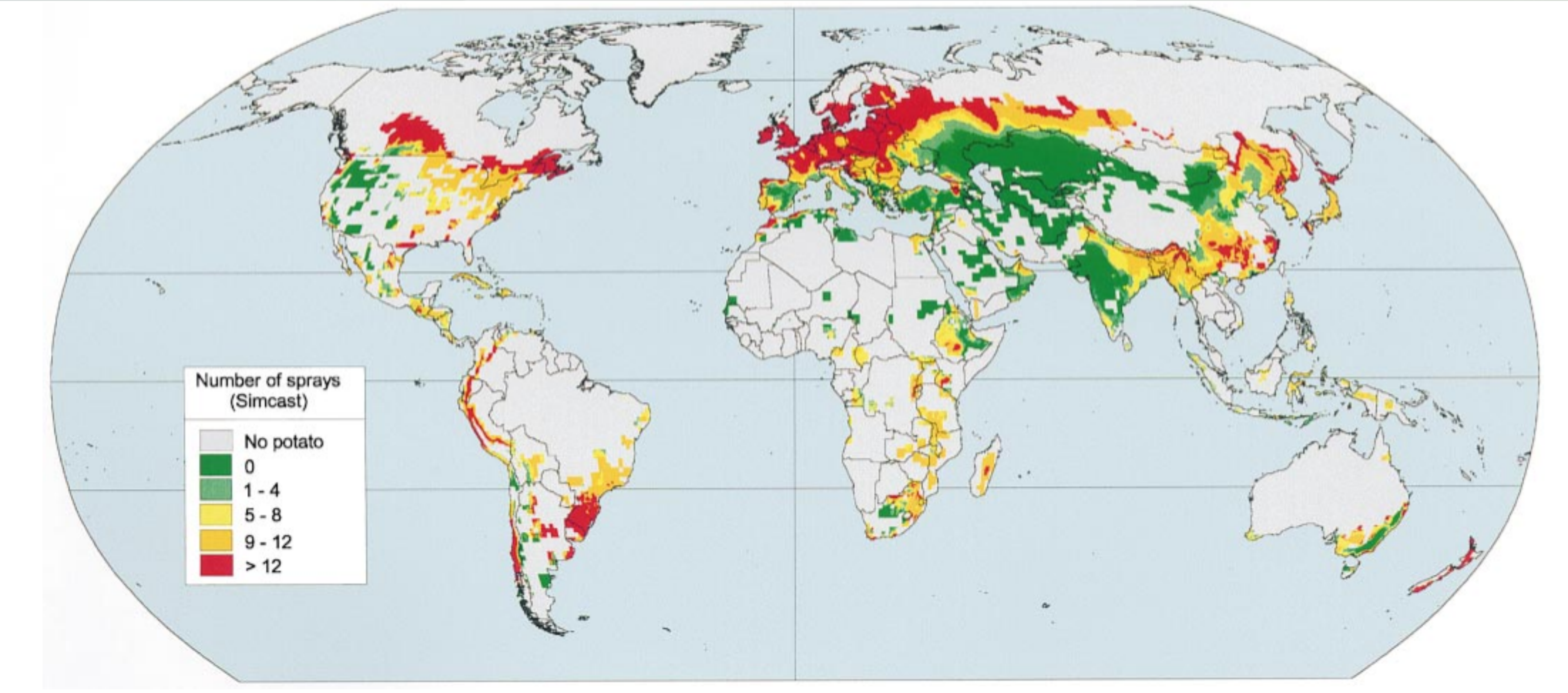


2019 XERICO Field Trials at MSU

- Test trait in potato *in situ*
- No yield penalty
- Increase in starch content



Late Blight - Global Severity



Plant Pathology (2000), v49

Effectiveness of 3 R-gene Late Blight Resistance

- Proof of Concept
- MSU 3 R-gene Events
- Field Trial Test 2017
- Inoculated with US23
- Border rows
  - Atlantic (non-transgenic)
- Center 4 rows include:
  - Single-gene event
  - 3 R-gene events

USAID Feed the Future Biotechnology Potato Project

FEED THE FUTURE
The U.S. Government's Global Hunger & Food Security Initiative

- GOAL:
  - Bring durable biotech late blight resistant potato varieties to smallholder farmers in Bangladesh and Indonesia.
- VISION:
  - Project leverages partnerships between private and public sector biotechnology stakeholders

USAID FROM THE AMERICAN PEOPLE | UNIVERSITY OF MINNESOTA | University of Idaho | Simplot PLANT SCIENCES | MICHIGAN STATE UNIVERSITY

USAID Target Countries: Bangladesh and Indonesia

- Potatoes feed the hungry
  - Bangladesh is 7th in production
  - Small holder farmers
  - High cost of inputs
  - Crop damage by insect pests and disease (particularly late blight)
  - Reduction of yield in field and damage in storage

USAID FROM THE AMERICAN PEOPLE | UNIVERSITY OF MINNESOTA | University of Idaho | Simplot PLANT SCIENCES | MICHIGAN STATE UNIVERSITY

Durable Late Blight Resistance: Stacking multiple genes

3 R-gene potatoes creates a more durable and high level resistance to late blight

3 R-Gene Late Blight Resistant Potatoes for South East Asia

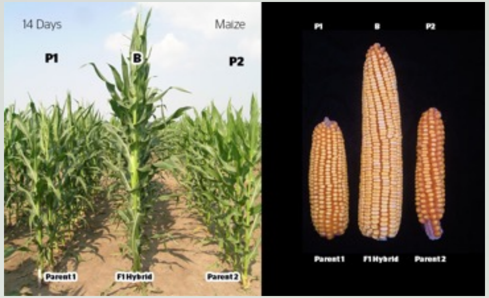
- Indonesian and Bangladesh potato varieties
- MSU received plantlets of 20 selected events of Granola and Diamant from Simplot
- Molecular characterization and confined field trials at MSU
- MSU hydroponic greenhouse minituber production for distributing certified seed
- Field trials to be conducted in 2020 in Bangladesh and Indonesia

"We will be guided by the science-based information, not by the nonscientific whispering of a section of people. As human beings, it is our moral obligation that all people in our country should get food and not go to bed on an empty stomach. Biotechnology can play an important role in this effect."

- *Matia Chowdhury*
  Minister of Agriculture
  Bangladesh

Revolutionizing Potato Breeding – *Diploid* Hybrid Potatoes (Potato 2.0)

Comparable with Hybrid Maize Breeding



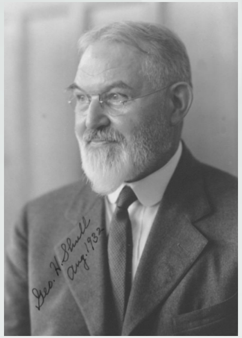
Iowa State University

1877

WJ Beal (Michigan Agricultural College) crosses two open-pollinated maize cultivars to exploit hybrid vigor

1908

George H. Shull crosses two inbred maize cultivars to better exploit hybrid vigor. He coins the term heterosis



1926

Henry A. Wallace founds Pioneer Hi-Bred to commercialize hybrid maize

1962

By 1962, 95% of maize acreage in the US is hybrid maize

Key Points of Potato 2.0

Diploid Hybrid Potato

- Varieties will be diploid (2x) F1 hybrids
  - Breeders can “fix” weaknesses in a F1 hybrid
- Breeders can access powerful breeding strategies not currently feasible for tetraploid potato
- The breeding process is more efficient and effective
- More feasible to study and exploit traits for breeding
- Able to access genomic tools and gene editing more effectively

Potato 2.0: 2x F1 Hybrid Varieties

- Advantages:
  - Simple genetic system for breeding
  - 100 wild species: Sources of economic-related traits
  - Can create inbreds
- Past Disadvantages:
  - Gametophytic self-incompatible (SI) system
  - Germplasm unadapted to US
- Key messages:
  - **Self-compatibility trait from *Solanum chacoense***
  - Breeding will take us from “interesting tubers” to market classes

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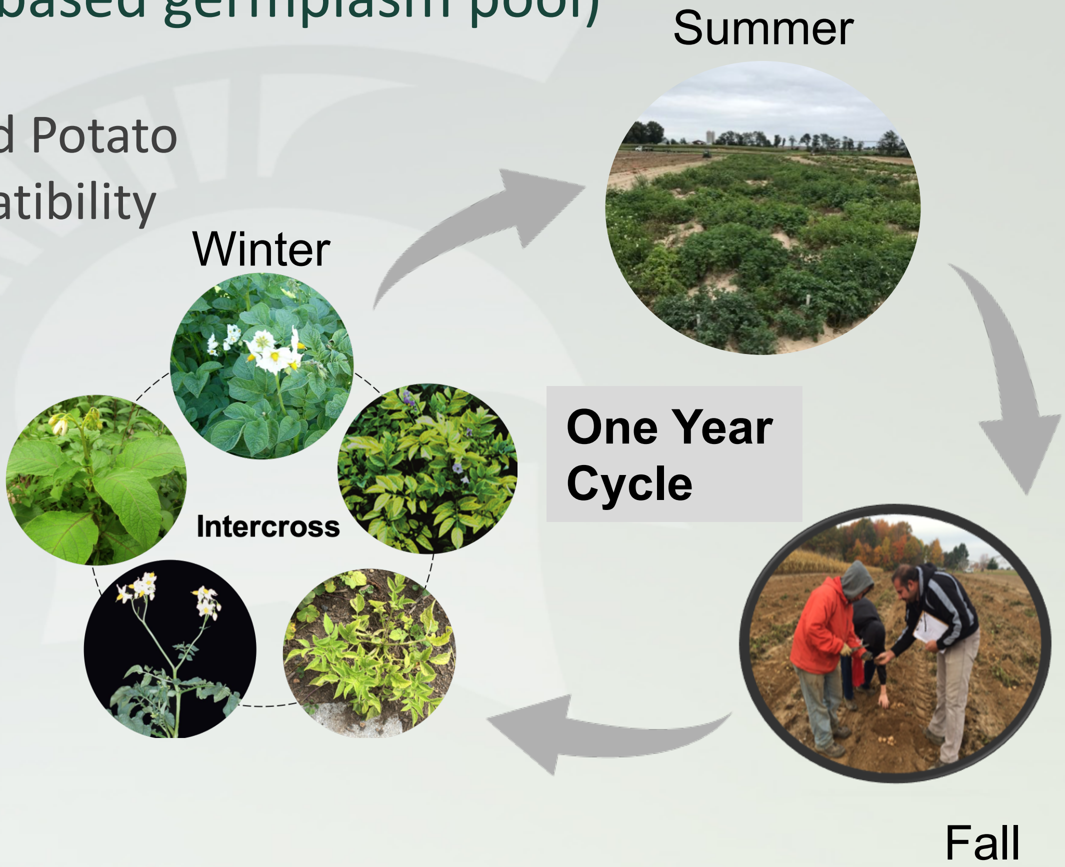
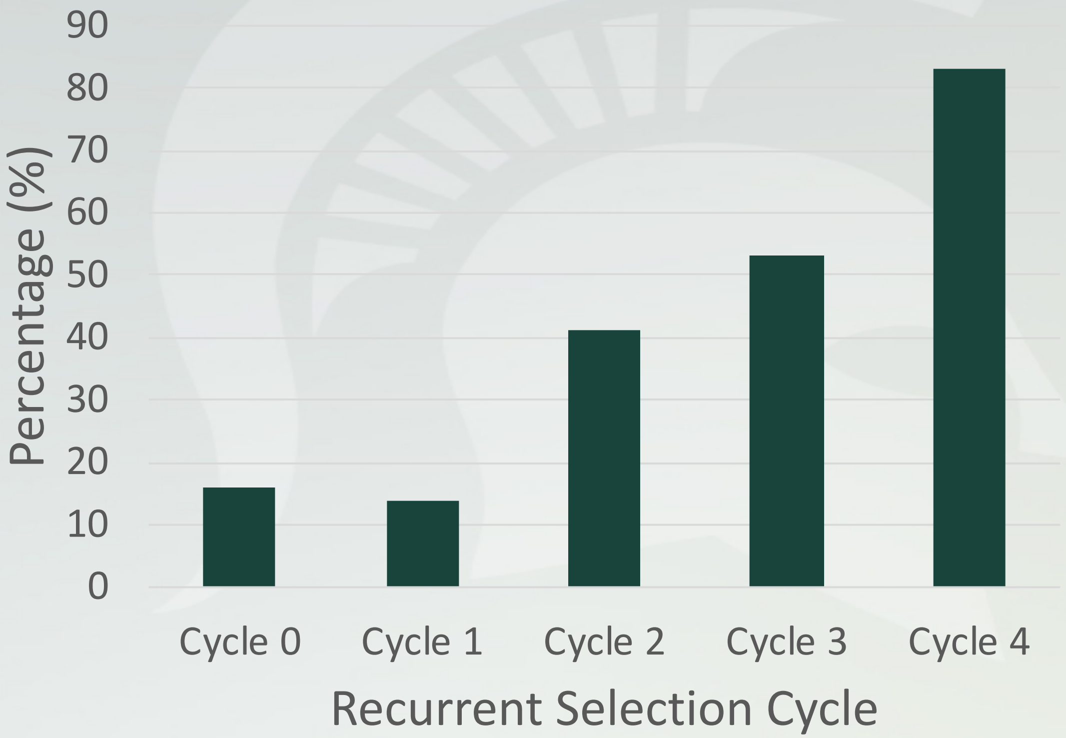
MSU Diploid Potato Breeding: Recurrent Selection Breeding Strategy (species-based germplasm pool)

Overall Goal: Develop Diploid Potato Germplasm with Self-compatibility

M6 – self compatibility donor



Improvement of Self-compatibility through Recurrent Selection



Self-compatibility Chi-square p=0.0025

Selections from Five Cycles of Recurrent Selection

Cycle 0
MSBB938-01

Cycle 1
MSCC822-03

Cycle 2
MSDD829-09

Cycle 3
MSEE737-05

Cycle 4
MSFF608-08

Atlantic
4x cultivar

Germplasm Base: Dihaploids from Cultivated Tetraploid Potato (adapted commercial varieties and breeding lines)

Tetraploid
(2n=4x=48)

↓

Dihaploid
(2n=2x=24)

↓

Male sterile
and/or
Female sterile

4x Potato Cultivar X Haploid Inducer

U Maine, H. Tan

Dihaploids Extracted from Cultivated Potato at MSU

(Past 5 Years)

- **Dihaploidization** is a critical genetic sieve to remove deleterious alleles carried in tetraploid parents
- Dihaploids from 20 tetraploid varieties and breeding lines
- Over 200 dihaploid selections in the breeding pool

ATL.M.198

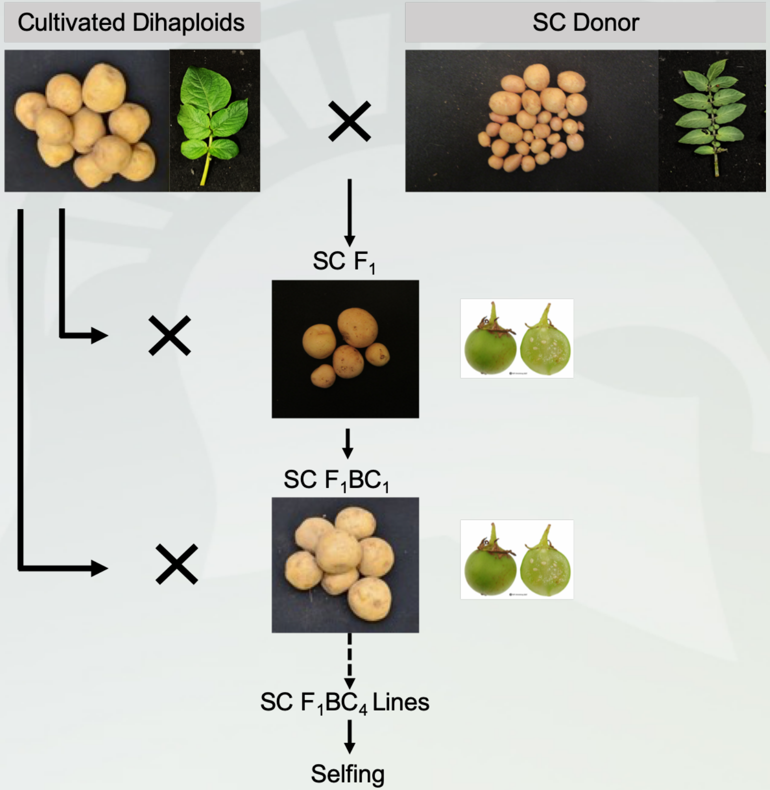
ATL.M.188

ATL.M.120

- Key traits:
  - Chip-processing
  - Specific gravity
  - Scab resistance
  - PVY resistance
  - PLRV resistance
  - PVX resistance
  - Late blight resistance
  - Golden nematode resistance

Atlantic 4x control

Backcross Breeding to Introgress Self-compatibility (SC) into *Solanum tuberosum* Dihaploids (cultivated germplasm pool)



Selections from F1, BC1, and BC2 Generations

F_1 | BC_1 | BC_2

MSCC864-17

MSEE872-03

MSFF696-01/SC

MSCC864-19

MSEE823-05

MSFF774-04

1980s Version of Diploid Potatoes

International Potato Center

21st Century Version of Diploid Potatoes

Cycle 0 | Cycle 2 | Cycle 4 | S1 | S2

Recurrent Selection

BC1 | BC1 | BC2 | BC2

Backcross

Next Steps: Assessing Heterosis and Inbreeding in the Field for F1 Hybrids

- Crossing between germplasm pools
- 10,000 diploid plants in 2019 field season
- Elite diploid selections with yield and quality

Key Points of Potato 2.0

- Varieties will be F1 hybrids
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- The breeding process is more efficient and effective
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Diploid Breeding for Insect Resistance

Breaking through decades of stasis

1980

Host plant resistance to the Colorado potato beetle identified in the wild species *S. chacoense*

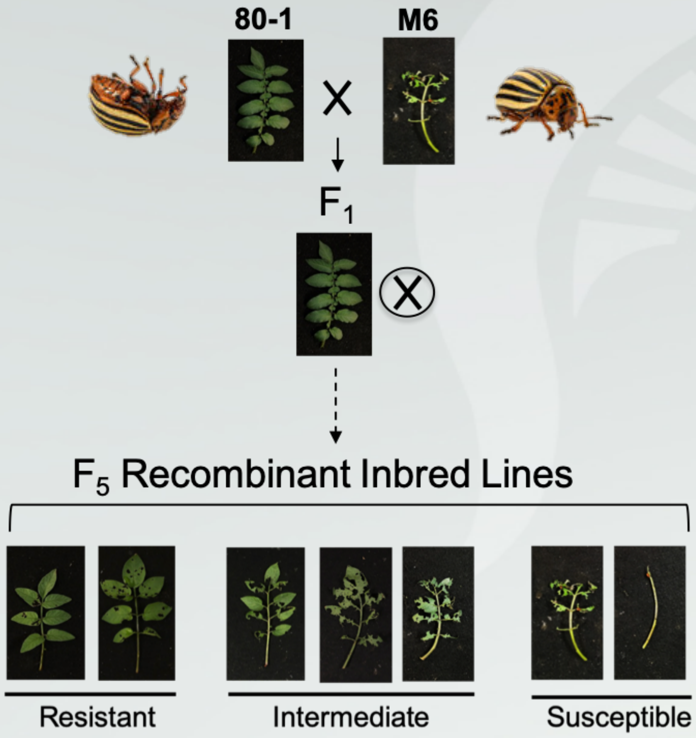
Complex genetics of the trait
Diploid self-incompatibility
Sparse DNA markers

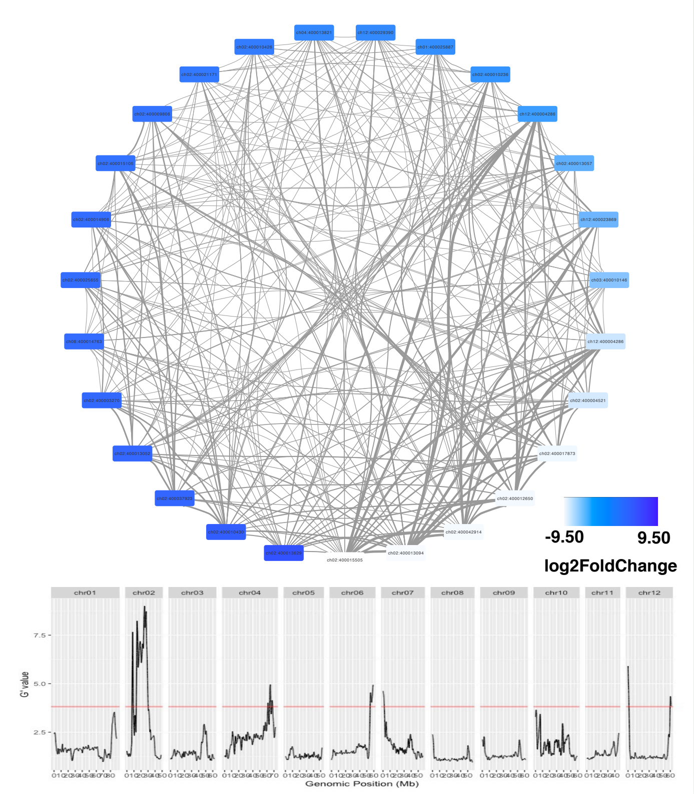
Present

No commercial resistant varieties available

Diploid Breeding for Insect Resistance

Exploiting self compatibility and next generation sequencing





(Kaiser et al., 2020)

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Gene editing – A powerful tool in potato improvement

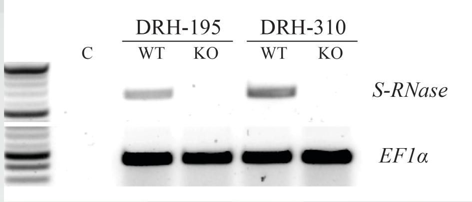
- Ability to knock-out and knock-in genes is valuable crop improvement tool
  - Fast changing technology
- Self compatible inbred diploid potatoes may be the best germplasm to exploit gene editing tools for breeding

Self compatibility was achieved in diploid potato lines and edits were transmitted to the progeny

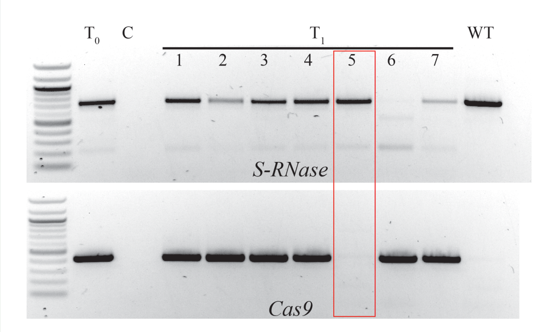
Self compatible diploid potato fruit

Fruit set in T_0 lines

S-RNase expression after 24 hours post pollination



S-RNase and *Cas9* screening in T_1 progeny



Potato Summary

- Potato is most important US vegetable and a world food security crop
- 4x breeding has improved via:
  - Access species germplasm from gene bank to introgress economically important traits
  - Diploid reference genome(s)
  - SNP array technology – marker-assisted breeding
  - Transformation technology – improving market preferred varieties
    - Storage trait, biotic stress, abiotic stress

Potato Summary

- Breeders can access powerful breeding strategies not currently feasible for tetraploid potato
  - Two self-compatible germplasm pools (RS and BC) were generated that are genetically distinct and could be the first step of producing inbred potato lines at the diploid level
  - Two germplasm pools have been selected for agronomic traits
- In the future varieties will be F1 hybrids
  - We have initiated inbred line development and heterosis testing

Potato Summary

- The breeding process is more efficient and effective
  - Breeding cycles for recurrent selection, backcrossing and inbreeding are accelerated (1-3 years vs. 5-8 years)
- Breeders can fix or introduce traits in a F1 hybrid
  - Backcross breeding can introduce traits into inbred line
- More feasible to study and exploit traits for breeding
  - We are introgressing insect resistance via marker-assisted breeding
- Able to access genomic tools and gene editing more effectively
  - Gene editing can be used effectively and efficiently without USDA regulation in many instances

What is next: Potato 2.0 (USDA/SCRI 2019)

- Improve self-compatibility and fertility
  - Combination of breeding and gene editing
- Broaden genetic base
  - Russets, reds, yellows, chippers
- Develop key parental inbreds for market classes
  - Select for tuber traits and maintain fertility
- Research and test seed production systems

Acknowledgements

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Azamat Sardarbekov	Kate Shaw	Dan Zarka

National Science Foundation

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Project GREEEN

USDA/NIFA
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