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Update on the GT4SP Genomic **TOOLS** for Sweetpotato Improvement Project

Developing next generation breeding tools for SSA sweetpotato breeders

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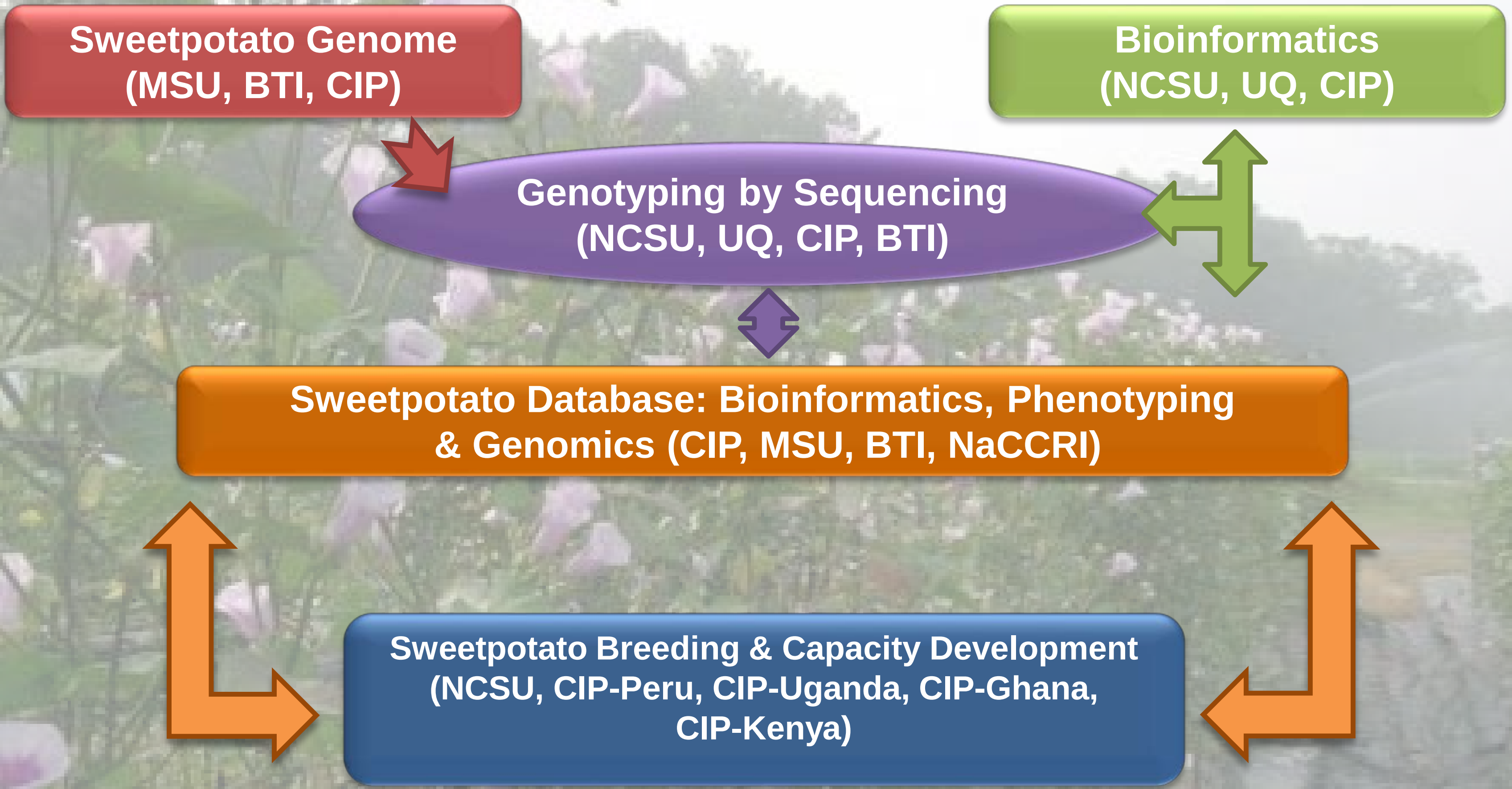
College of Agricultural and Life Sciences
North Carolina State University

SPHI 10th Annual Meeting
Kigali, Rwanda
Aug. 23 - 25, 2019

GT4SP
GENOMIC TOOLS
FOR SWEETPOTATO
IMPROVEMENT

The Genomic Tools for Sweetpotato Improvement Project
GT4SP



Sweetpotato Genome
(MSU, BTI, CIP)

Bioinformatics
(NCSU, UQ, CIP)

Genotyping by Sequencing
(NCSU, UQ, CIP, BTI)

Sweetpotato Database: Bioinformatics, Phenotyping
& Genomics (CIP, MSU, BTI, NaCCRI)

Sweetpotato Breeding & Capacity Development
(NCSU, CIP-Peru, CIP-Uganda, CIP-Ghana,
CIP-Kenya)

An ambitious project to sequence sweetpotato and develop modern breeding tools for a food crop that sustains million of people in SSA.

Collaborators: Boyce Thompson Institute at Cornell, Michigan State University, University of Queensland, Australia; The International Potato Center, Peru; BioSciences East and Central Africa, Kenya; National Crops Resources Research Institute, Uganda; Crops Research Institute, Ghana

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Marcelo Mollinari (Postdoc)
Dahlia Nielson (Bioinformatics)
Lina Quesada (coPI) Pathology

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Shan Wu, Postdoc
Alex Ogbonna, Database Development
Bryan Ellerbrock, Database Development

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International Potato Center
CIP
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biosciences eastern and central africa

CIP Lima
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CIP SSA
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Mercy Kitavi, Molecular Breeding, Capacity Development

NaCRRI
NATIONAL CROPS RESOURCES RESEARCH INSTITUTE

Benard Yada, (coPI) Breeder
Milton Otema Anyanga, Entomologist

THE UNIVERSITY OF QUEENSLAND AUSTRALIA | IMB Institute for Molecular Bioscience

Lachlan Coin (coPI)
Chenxi Zhou, PhD Student, China

CSIR COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH GHANA

Marian Quain, Biotechnology, TC

GT4SP – Major Accomplishments

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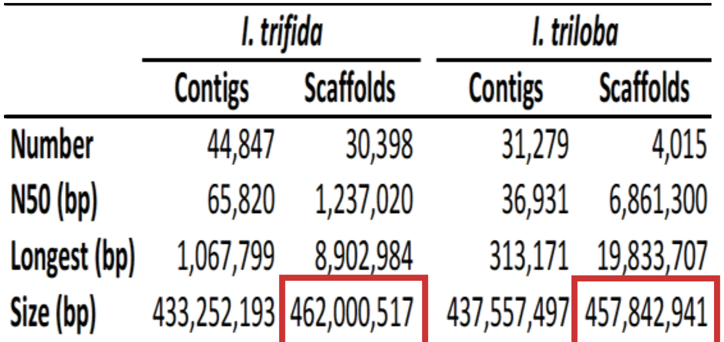
Sweetpotato Reference Genome(s)

Cultivated sweetpotato is a highly heterozygous allo-auto-hexaploid (2n=6x=90) with a large genome (~1.6 Gb; Arumuganthan and Earle, 1991).

The origin of cultivated sweetpotato

Hypothesis I: Derived from the *I. trifida* autopolyploid complex (ranging from diploid to hexaploid) (Kobayashi, 1984).

Hypothesis II: Generated by natural hybridization between *I. trifida* and *I. triloba* (Austin, 1988).

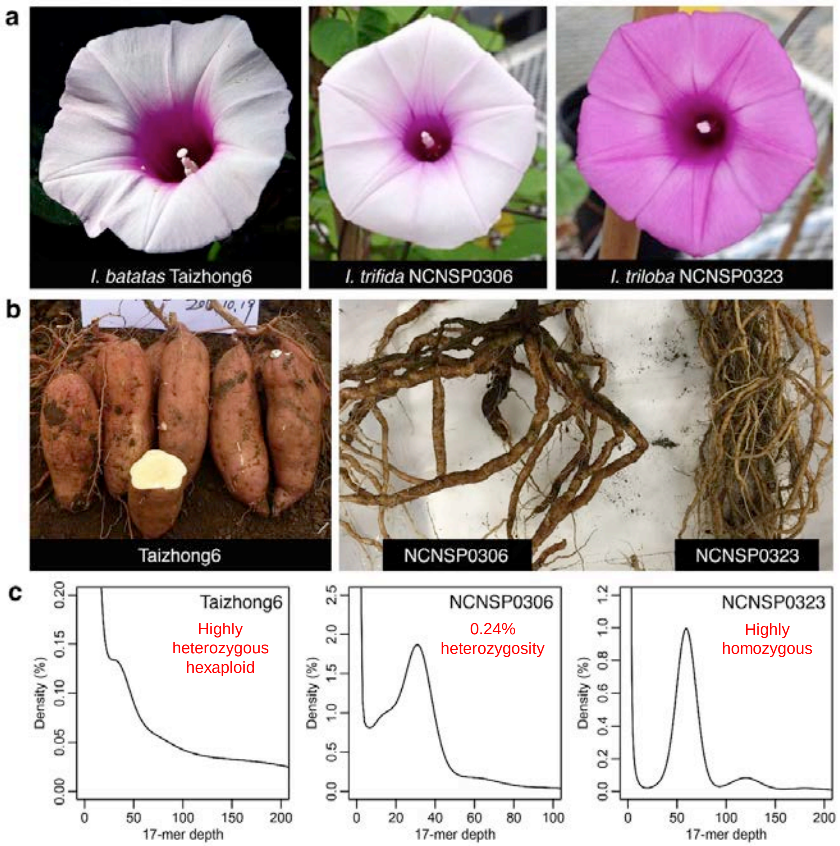


	I. trifida		*I. triloba*	
	Contigs	Scaffolds	Contigs	Scaffolds
Number	44,847	30,398	31,279	4,015
N50 (bp)	65,820	1,237,020	36,931	6,861,300
Longest (bp)	1,067,799	8,902,984	313,171	19,833,707
Size (bp)	433,252,193	462,000,517	437,557,497	457,842,941

87.7 %

92.3 %

of the estimated genome sizes (526.5 Mb and 495.9 Mb for *I. trifida* and *I. triloba*, respectively.)



Highly heterozygous hexaploid

0.24% heterozygosity

Highly homozygous

"Taizhong6" recently sequenced by Yang et al. 2017.

Fei Lab

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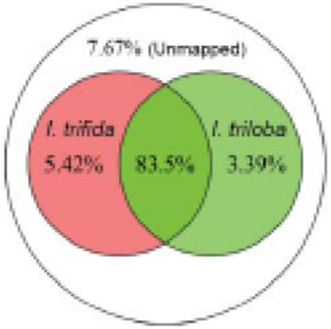
Comparative analysis of the hexaploid cultivated sweetpotato genome with genome assemblies of two wild relatives.

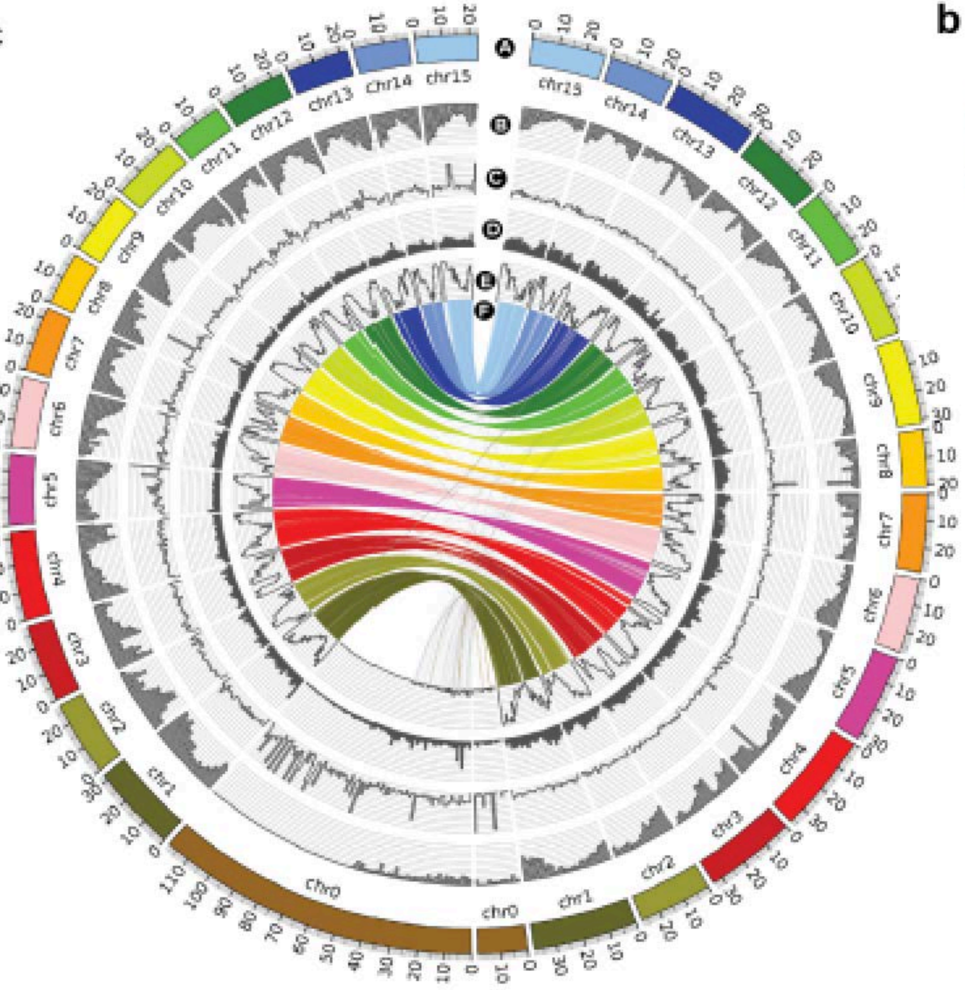
Wu et.al. Nature Communications. (2018) 9:4580 | DOI: 10.1038/s41467-018-06983-8

a

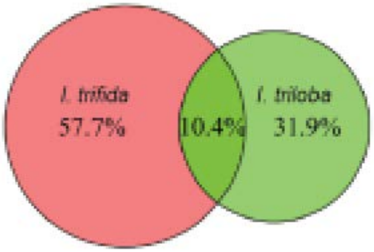


a, Percentages of mapped and unmapped 10x Genomics linked reads of hexaploid ‘Tanzania’ to the *I. trifida* and *I. triloba* genome assemblies.

c



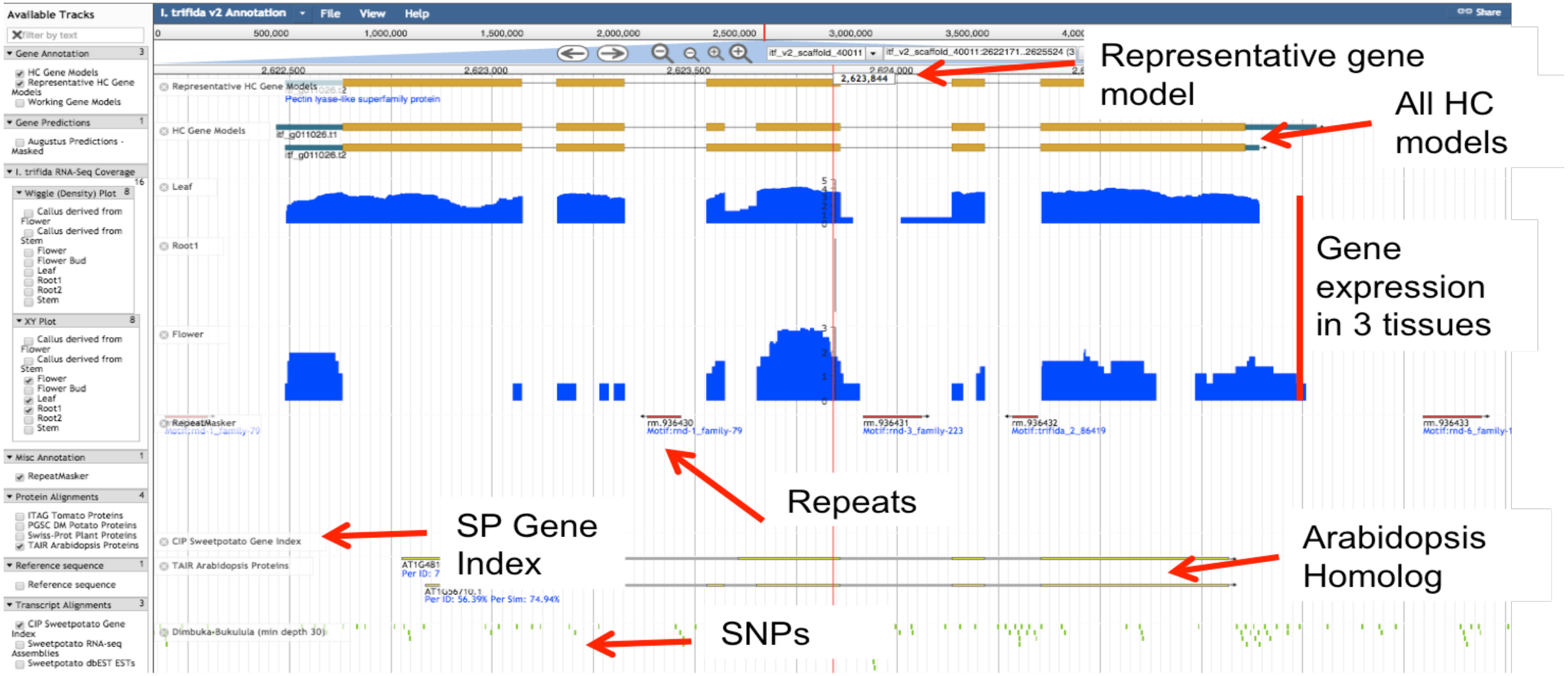
b



b, Percentages of 10x Genomics reads with better alignments when mapped to one genome assembly compared to the other.

c, Comparison of the hexaploid sweetpotato molecules (center) with the two diploid assemblies: Trifida (left)/Triloba (right)

Jbrowse Genome Browser

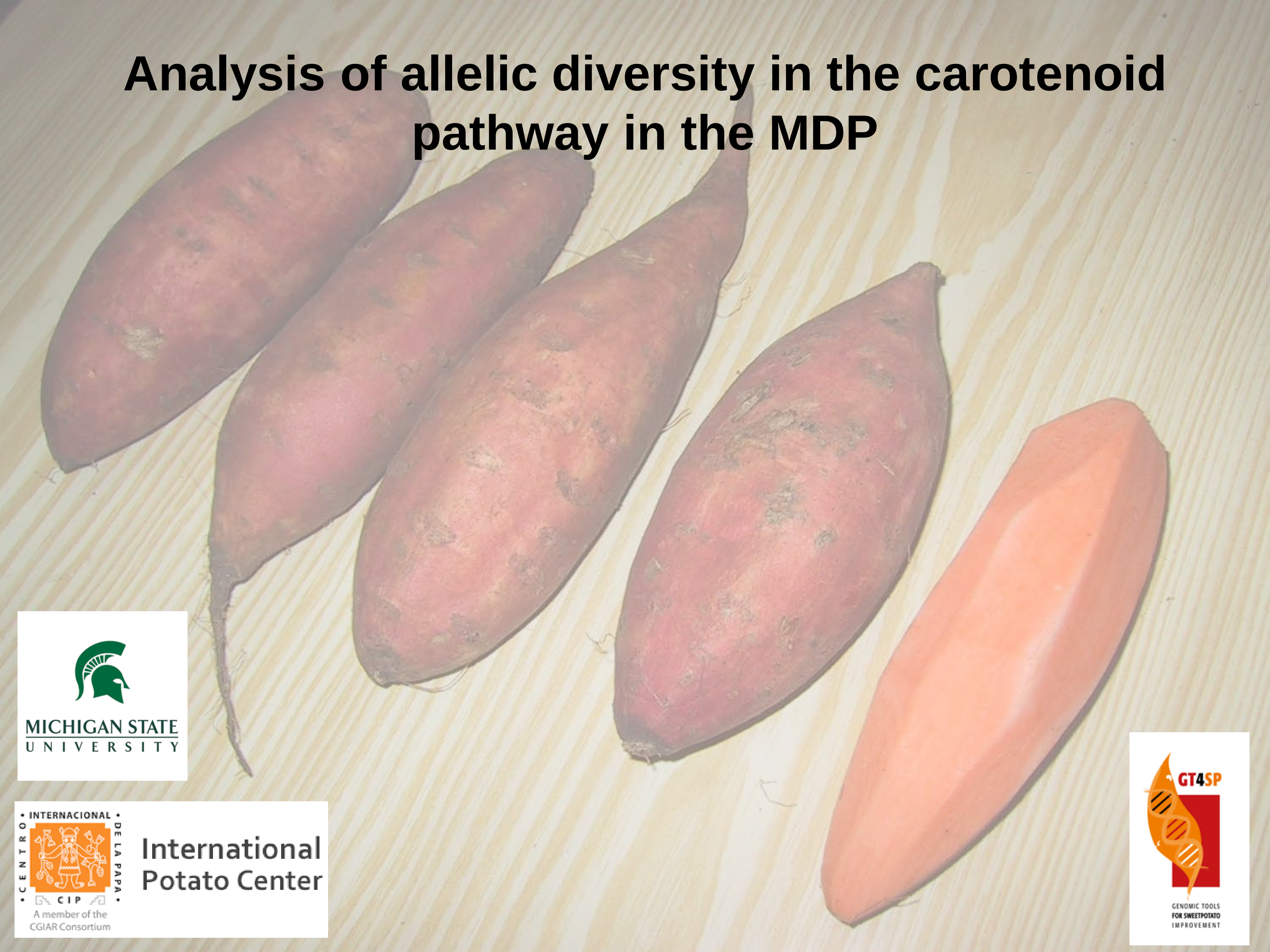


Tracks available on the Jbrowse:

- Genome Annotation (Loci, Gene Models)
- Gene Predictions (Augustus, SNAP, FGENESH)
- MAKER Transcript Evidence
- MAKER Protein Evidence
- CIP Sweetpotato Gene Index Alignments
- RepeatMasker Repeats
- RNA-Seq Coverage – Wiggle
- RNA-Seq Coverage – XY
- I. trifida 0431-1 SNPs

MICHIGAN STATE UNIVERSITY Dr. Robin Buell http://sweetpotato.plantbiology.msu.edu/

Analysis of allelic diversity in the carotenoid pathway in the MDP



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The Mwanga Diversity Panel (MDP)

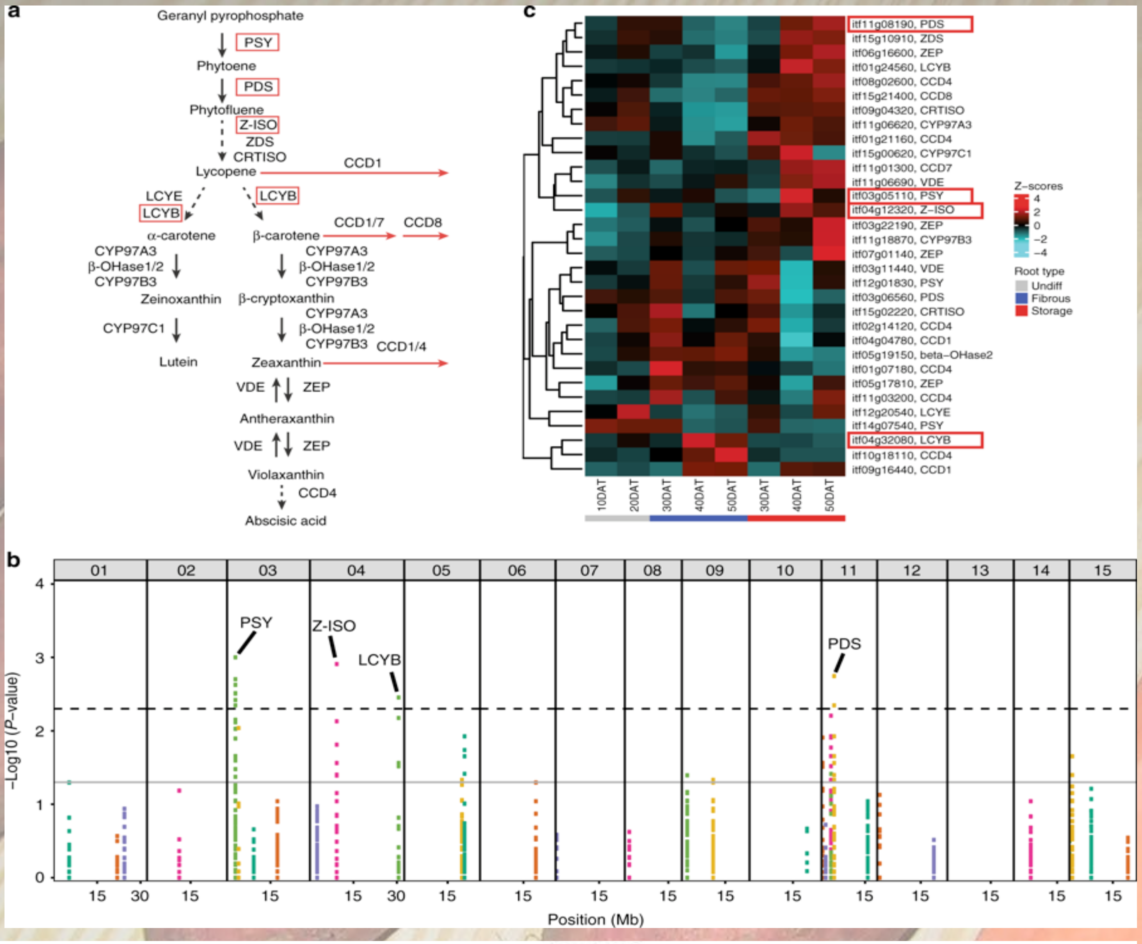
- 16 clones selected to make an 8x8 diallel population to exploit heterosis.

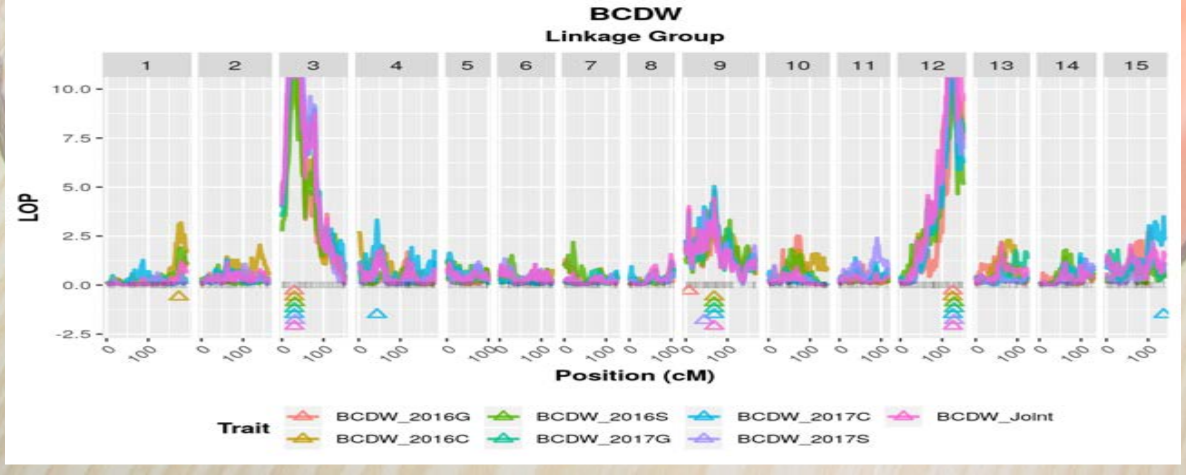
Population A

Name	Origin	Flesh color
Ejumula	Uganda	Orange
Kabode	Uganda	Orange
Kakamega	Kenya	Orange
NASPOT 5/58	Uganda	Orange
NASPOT 7	Uganda	Orange
Dimbuka-Bukulula	Uganda	Cream
NASPOT 1	Uganda	Cream
NK259L	Uganda	White

Population B

Name	Origin	Flesh color
Huarmeyano	CIP/Peru	Orange
NASPOT 5	Uganda	Orange
Resisto	USA(Kenya)	Orange
Magabali	Uganda	Cream
Mugande	Uganda	White
NASPOT 11	Uganda	Cream
New Kawogo	Uganda	Cream
Wagabolige	Uganda	Cream


a
Geranyl pyrophosphate
PSY
Phytoene
PDS
Phytofluene
Z-ISO
ZDS
CRTISO
Lycopene
CCD1
LCYE
LCYB
LCYB
α-carotene
β-carotene
CCD1/7
CCD8
CYP97A3
β-OHase1/2
CYP97B3
CYP97A3
β-OHase1/2
CYP97B3
Zeinoxanthin
β-cryptoxanthin
CYP97A3
β-OHase1/2
CYP97B3
CYP97C1
CCD1/4
Lutein
Zeaxanthin
VDE
ZEP
Antheraxanthin
VDE
ZEP
Violaxanthin
CCD4
Abscisic acid
c
itf11g08190, PDS
itf15g10910, ZDS
itf06g16600, ZEP
itf01g24560, LCYB
itf08g02600, CCD4
itf15g21400, CCD8
itf09g04320, CRTISO
itf11g06620, CYP97A3
itf01g21160, CCD4
itf15g00620, CYP97C1
itf11g01300, CCD7
itf11g06690, VDE
itf03g05110, PSY
itf04g12320, Z–ISO
itf03g22190, ZEP
itf11g18870, CYP97B3
itf07g01140, ZEP
itf03g11440, VDE
itf12g01830, PSY
itf03g06560, PDS
itf15g02220, CRTISO
itf02g14120, CCD4
itf04g04780, CCD1
itf05g19150, beta–OHase2
itf01g07180, CCD4
itf05g17810, ZEP
itf11g03200, CCD4
itf12g20540, LCYE
itf14g07540, PSY
itf04g32080, LCYB
itf10g18110, CCD4
itf09g16440, CCD1
10DAT
20DAT
30DAT
40DAT
50DAT
30DAT
40DAT
50DAT
Z–scores
4
2
0
–2
–4
Root type
Undiff
Fibrous
Storage
b
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15
PSY
Z-ISO
LCYB
PDS
4
3
2
1
0
–Log10 (P–value)
15 30 15 15 15 30 15 15 15 15 15 15 15 15 15 15
Position (Mb)


BCDW
Linkage Group
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
10.0
7.5
5.0
2.5
0.0
-2.5
LOP
0 100
Position (cM)
Trait
BCDW_2016G
BCDW_2016C
BCDW_2016S
BCDW_2017G
BCDW_2017C
BCDW_2017S
BCDW_Joint

GT4SP Mapping/Breeding Populations

- M9 x M19 – Diploid *I. trifida*
  - 212 clones
- Beauregard x Tanzania (BT)
  - 316 clones
- Tanzania x Beauregard (TB)
  - 247 clones
- New Kawogo x Beauregard (NKB),
  - 287 clones
- Mwanga Diversity Population,
  - 8 x 8 mating design,
  - 1,920 clones
- Multi-location, multi-year phenotyping in Peru, USA, Ghana and Uganda

Multisite Trials for Genetic Studies

Ica, Peru

Kumasi, Ghana

San Ramon, Peru

Pucallpa, Peru

Tamale, Ghana

BT

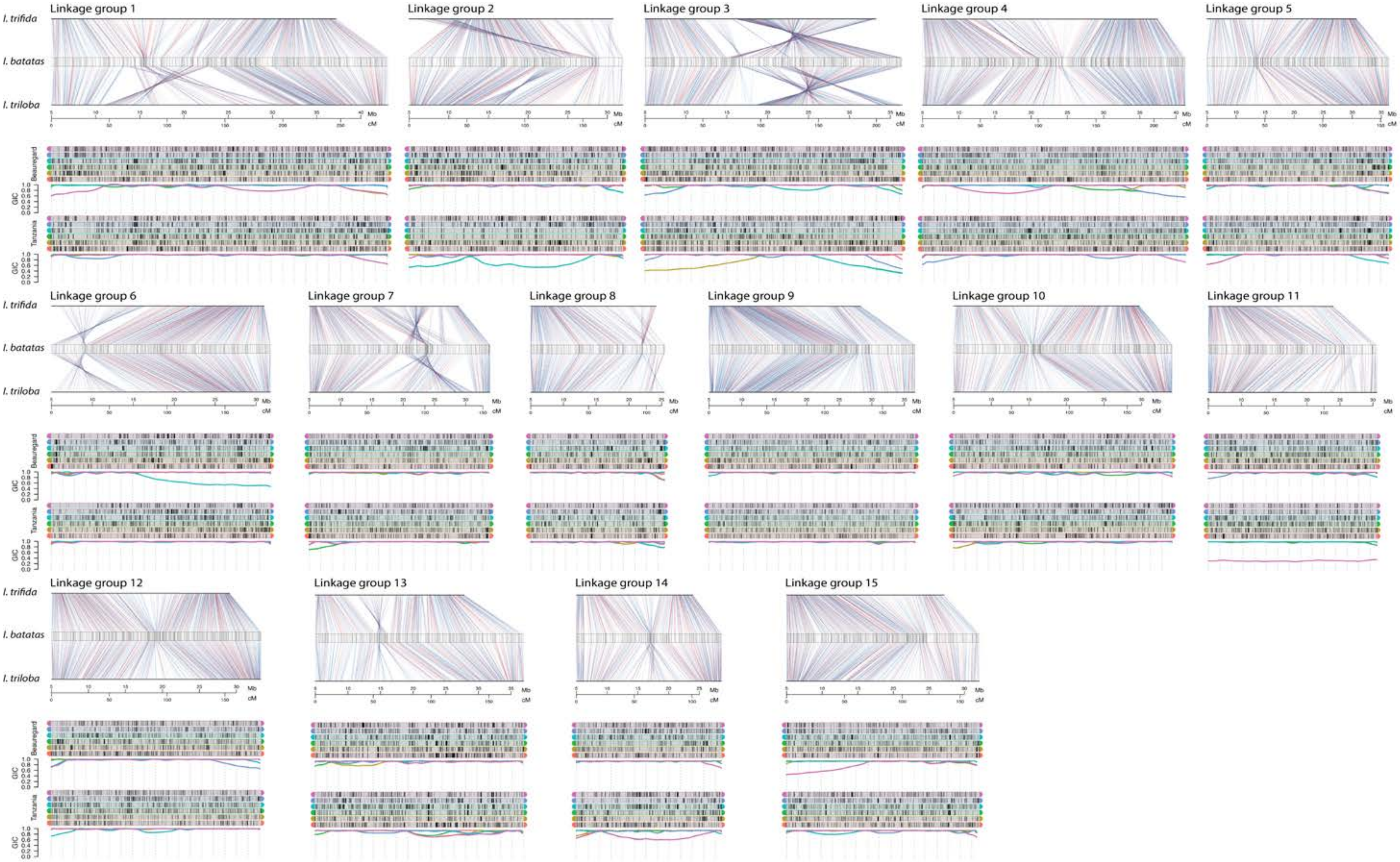
Beauregard

x

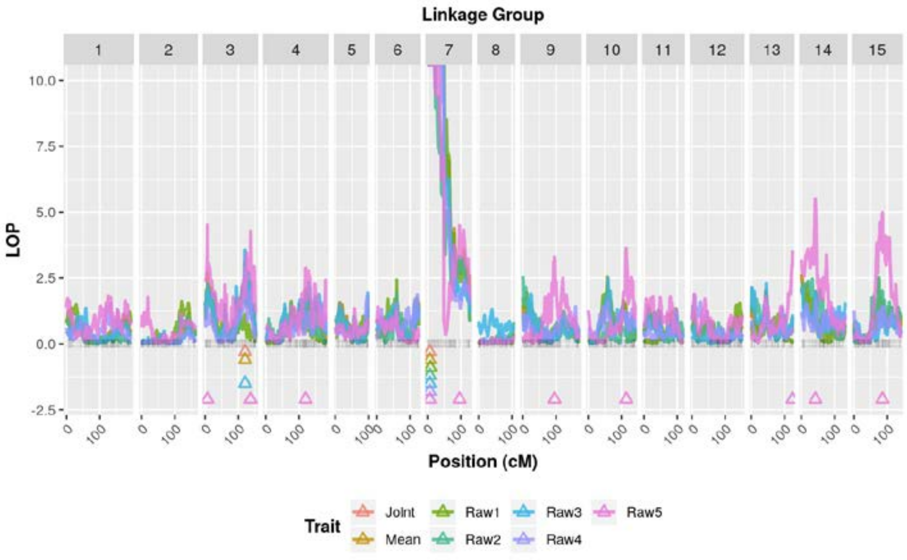
Tanzania

Reference Genome-Based Phasing and Linkage Mapping

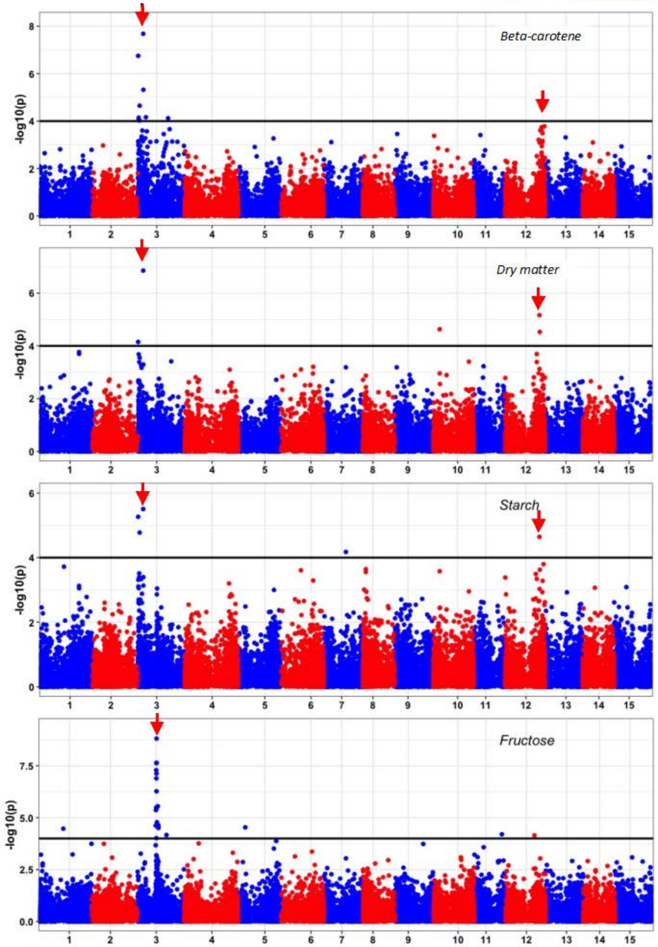
Drs. Zhao-Bang Zeng, Marcelo Molinari, Guilherme Da Silva Pereira



Major QTL and MAS



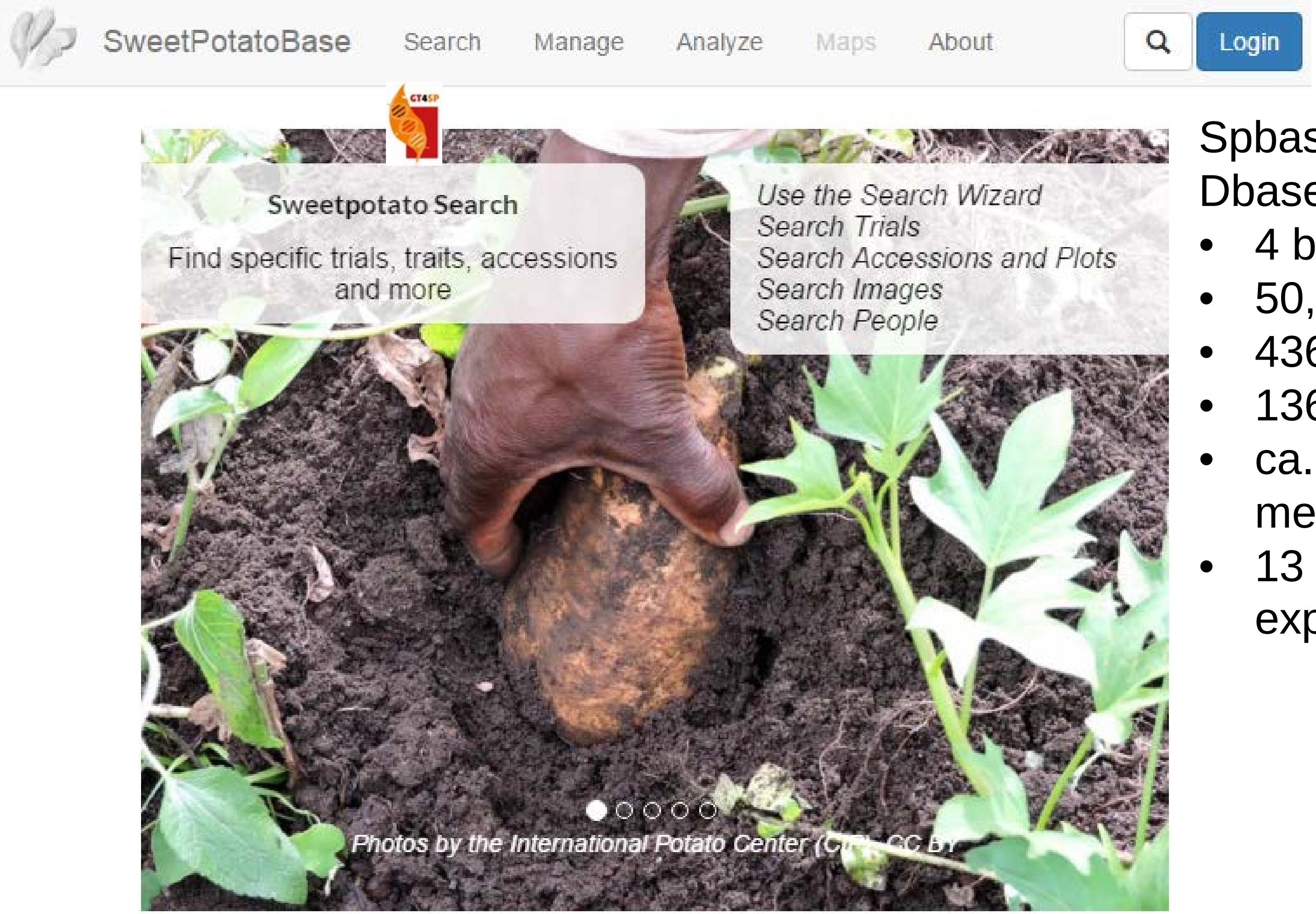
Root Knot Nematode: M. incognita



Quality traits: DMC, Beta-carotene, Starch, Sugars

Dr. Lukas Mueller

Managing Phenotypic Data SweetPotatoBase and FieldBook App.



Sweetpotato Search

Find specific trials, traits, accessions and more

Use the Search Wizard
Search Trials
Search Accessions and Plots
Search Images
Search People

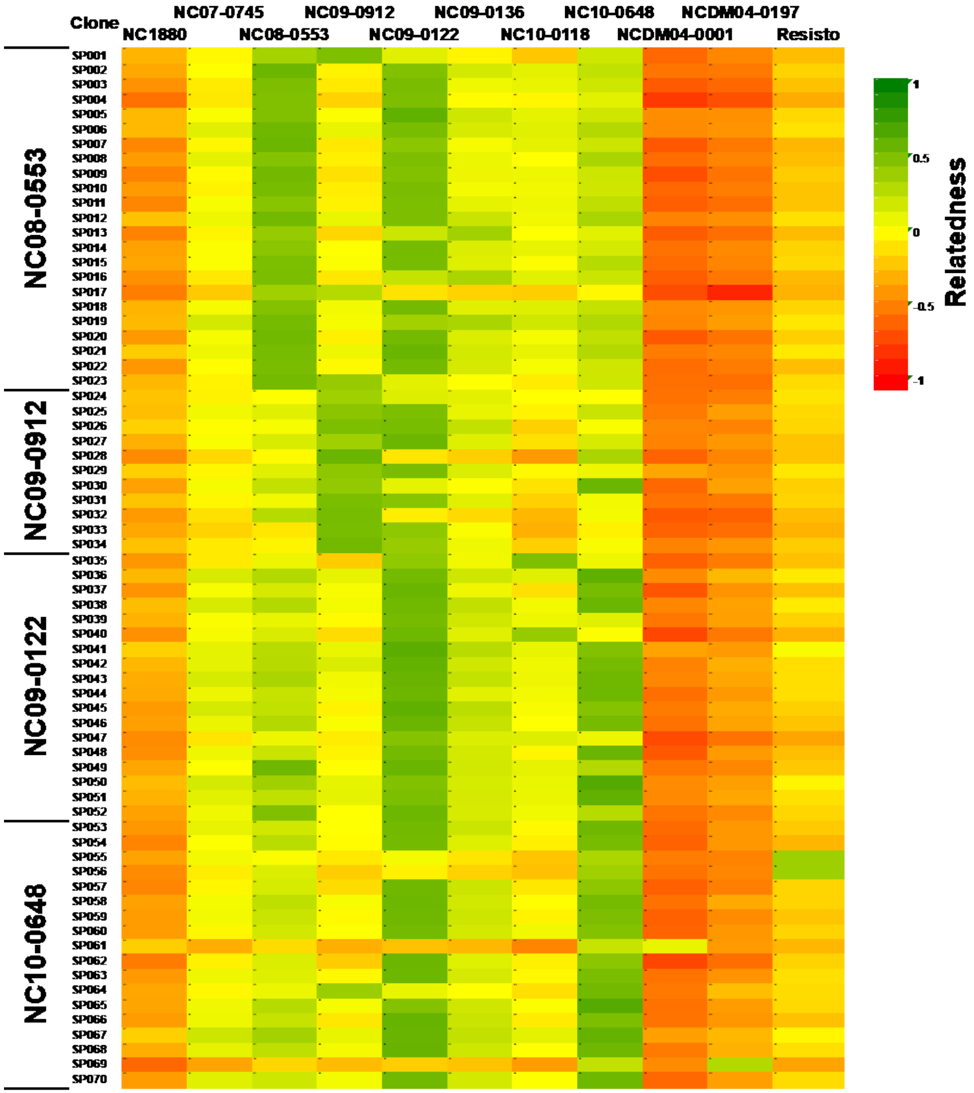
Photos by the International Potato Center (CIP), CC BY

Spbase…from empty Dbase to …:

- 4 breeding platforms;
- 50,000 accessions;
- 436 field trials;
- 136,702 plots;
- ca. 1 million trait measurements;
- 13 new programs exploring utility.

SweetpotatoBase

Prediction of parentage from seedling selections obtained from a polycross nursery

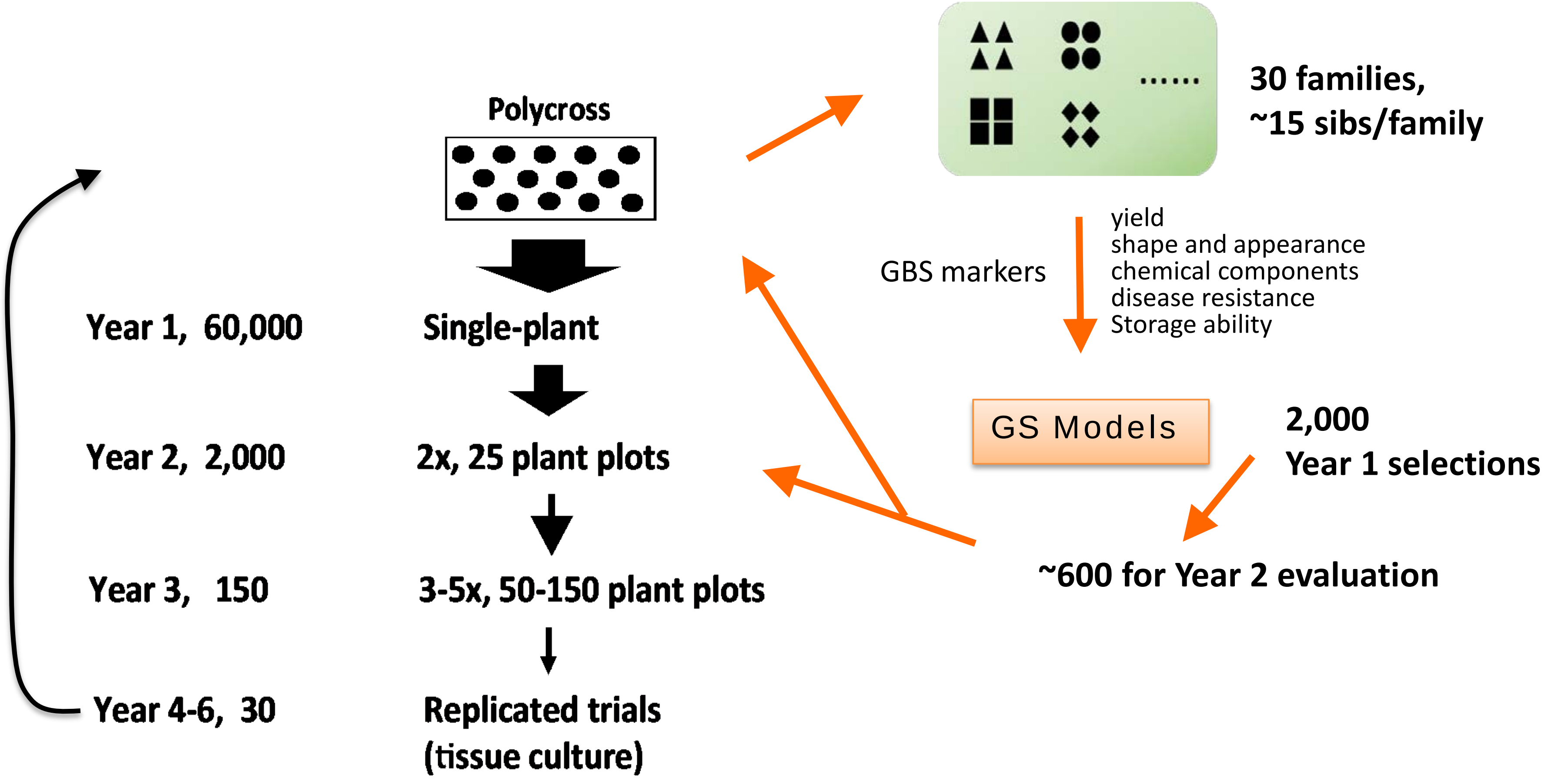


Key Points

- Can predict parentage with high degree of confidence.
- Polycross nurseries - mostly a collection of paired crosses
- Nearest neighbors important
- Pedigree information critical for future Genomic Selection in SP

Xiaofei Zhang and Ken Pecota, NCSU

GS-based Breeding Scheme



Polycross

Year 1, 60,000 — Single-plant

Year 2, 2,000 — 2x, 25 plant plots

Year 3, 150 — 3-5x, 50-150 plant plots

Year 4-6, 30 — Replicated trials (tissue culture)

Year 6-8, 1-2 — Variety Release

30 families,
~15 sibs/family

GBS markers

yield
shape and appearance
chemical components
disease resistance
Storage ability

GS Models

2,000
Year 1 selections

~600 for Year 2 evaluation

$$\Delta G = R_t = i\, r\, \delta_A / L$$

LESSONS LEARNED

- Genomics = new possibilities, new insights
- Breeding programs have to change to adopt.
- Still expensive – US$15/genotype x 1,000 = $15K
- Accurate, high quality phenotyping essential
- Analytical pipelines are complex, not everyone can use; therefore, research matrix-based teamwork is critical.
- Communication and dedicated people are critical.
- New tools/methods bring new ideas to the table
- SweetGAINS - Sweetpotato Genetic Advances and Innovative Seed Systems ….stay tuned

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SASHA Sweetpotato Action for Security and Health in Africa

Together
We’ve come a long way!

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1st Annual SASHA-GT4SP Joint Meeting, Mukono, Uganda, 2014

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