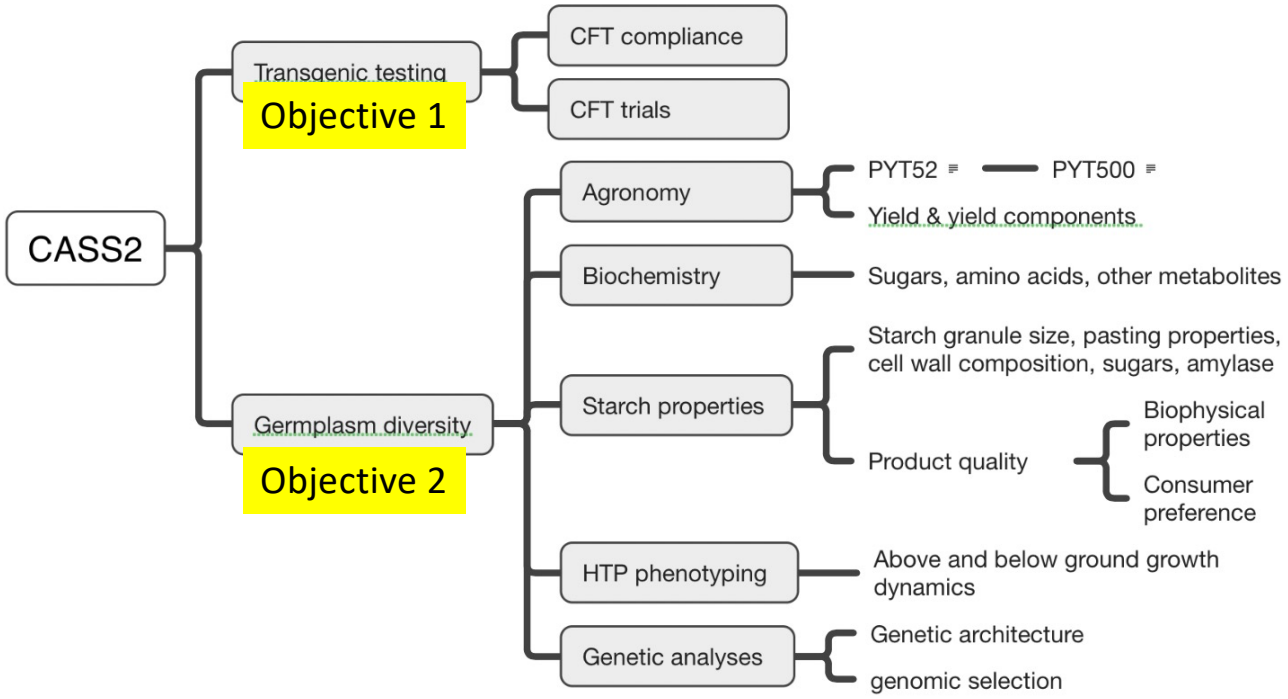
CASSII – IITA UPDATE

Highlights, latest results and brief outlook

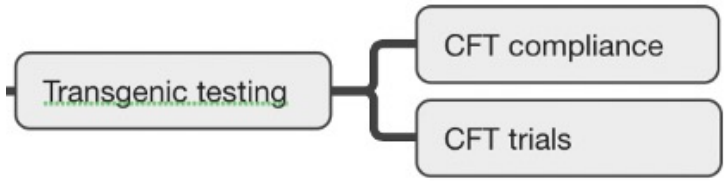
08 April 2024

Ismail Y. Rabbi

Activities conducted at IITA



Objective 1 (Confined Field Trials)



FEDERAL REPUBLIC OF NIGERIA

National Biosafety Management Agency NBMA

Permit Certificate

Issued to:

International Institute of Tropical Agriculture (IITA), Ibadan.

For Confined Field Trial of Genetically Modified Cassava for increased storage root yield (i.e. bigger root or more storage root per plant)

This permit is with effect from **14th October 2021 to 14th October 2023,** in line with the attached decision document with Code No: NBMA /CF /010.

Permit Code No: NBMA /CFT /010

Director General/ Chief Executive Officer

- CFT under permit number NBMA-CFT-010 just concluded
- Experiment planted both inside and outside screen-houses
- DOP – 27 March 2023
- DOH – 5-6 April 2024

Confined Field Trial – 2023-2024

Screenhouse planting results

- AAP1
- MITO-GS
- AAP1-MITOGS
- TMS60444
- Vector Control

Construct	SH1	SH2	SH3
aap1	**24**	**24**	**24**
aap1_4349	4	4	4
aap1_4353	4	4	4
aap1_4354	4	4	4
aap1_4355	4	4	4
aap1_4358	4	4	4
aap1_4359	4	4	4
mitoGS	**24**	**24**	**24**
mitoGS_4267	4	4	4
mitoGS_4268	4	4	4
mitoGS_4269	4	4	4
mitoGS_4272	4	4	4
mitoGS_4275	4	4	4
mitoGS_4277	4	4	4
mitoGS_aap1	**20**	**20**	**20**
mitoGS_aap1_4440	4	4	4
mitoGS_aap1_4448	4	4	4
mitoGS_aap1_4455	4	4	4
mitoGS_aap1_4459	4	4	4
mitoGS_aap1_4461	4	4	4
TMS60444	**4**	**4**	**4**
TMS60444	4	4	4
VC	**24**	**24**	**24**
Vector_control_4218	4	4	4
Vector_control_4220	4	4	4
Vector_control_4221	4	4	4
Vector_control_4234	4	4	4
Vector_control_4239	4	4	4
Vector_control_4243	4	4	4
N	**96**	**96**	**96**

The challenge of testing 60444 (root rot)

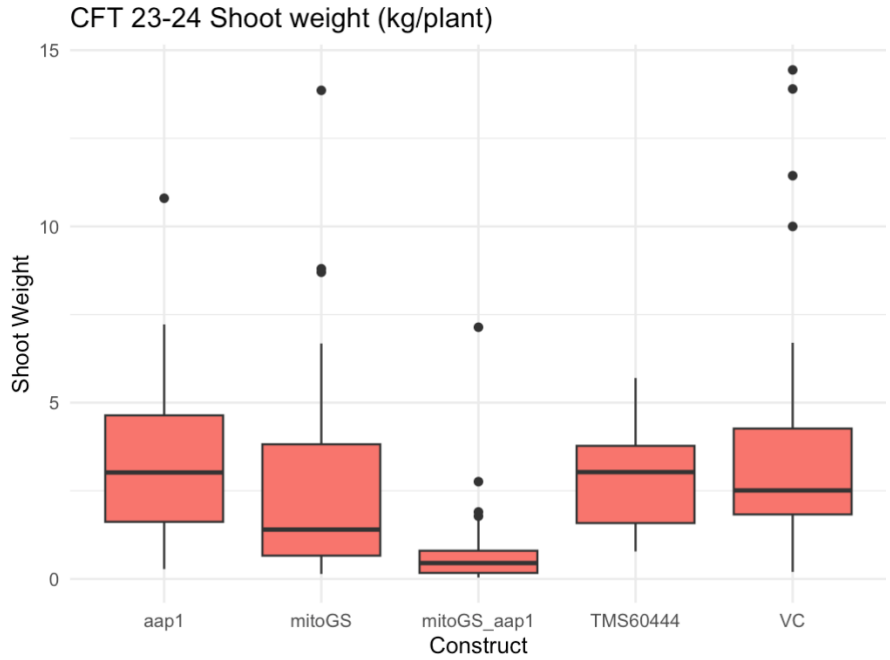
Construct	Stem	Roots
aap1_4349	4	
aap1_4353	4	3
aap1_4354	4	3
aap1_4355	SH1 4	1
aap1_4358	4	3
aap1_4359	4	3
mitoGS_4267	4	2
mitoGS_4268	3	1
mitoGS_4269	4	1
mitoGS_4272	4	1
mitoGS_4275	4	4
mitoGS_4277	3	
mitoGS_aap1_4440	4	
mitoGS_aap1_4448	3	
mitoGS_aap1_4455	3	
mitoGS_aap1_4459	1	
mitoGS_aap1_4461	2	
TMS60444	4	1
Vector_control_4218	4	3
Vector_control_4220	4	2
Vector_control_4221	4	3
Vector_control_4234	4	2
Vector_control_4239	4	2
Vector_control_4243	4	3

Construct	Stem	Roots
aap1_4349	4	2
aap1_4353	3	2
aap1_4354	4	4
aap1_4355	SH2 3	2
aap1_4358	4	2
aap1_4359	4	1
mitoGS_4267	3	2
mitoGS_4268	2	
mitoGS_4269	3	
mitoGS_4272	3	2
mitoGS_4275	3	2
mitoGS_4277	2	2
mitoGS_aap1_4440	1	
mitoGS_aap1_4448	1	1
mitoGS_aap1_4455	1	
mitoGS_aap1_4459	1	
mitoGS_aap1_4461	1	1
TMS60444	4	3
Vector_control_4218	4	1
Vector_control_4220	4	3
Vector_control_4221	2	2
Vector_control_4234	4	2
Vector_control_4239	3	2
Vector_control_4243	4	3

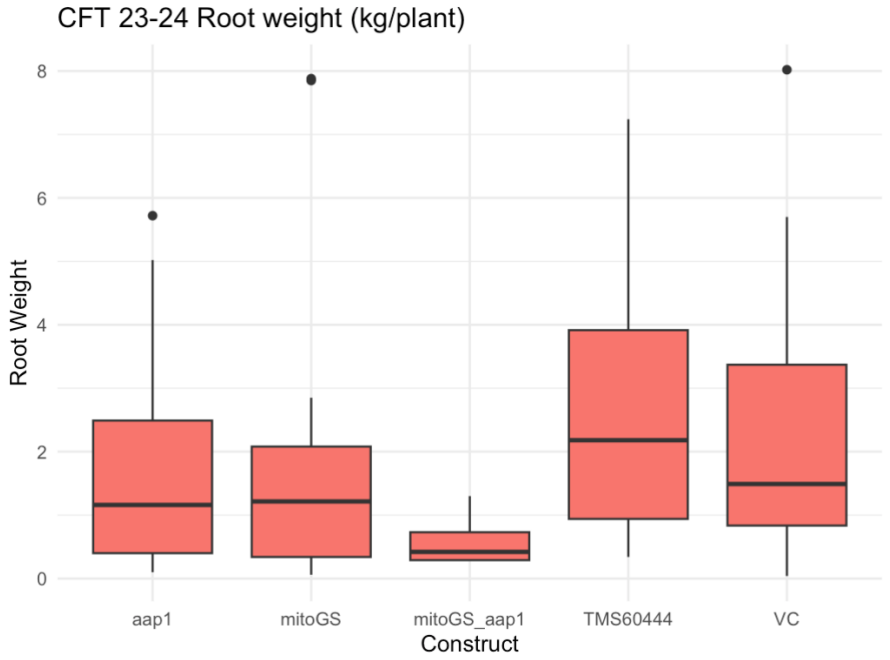
Construct	Stem	Roots
aap1_4349	4	3
aap1_4353	4	3
aap1_4354	4	4
aap1_4355	SH3 4	2
aap1_4358	4	2
aap1_4359	4	4
mitoGS_4267	4	3
mitoGS_4268	3	2
mitoGS_4269	4	1
mitoGS_4272	4	3
mitoGS_4275	4	3
mitoGS_4277	4	3
mitoGS_aap1_4440	4	1
mitoGS_aap1_4448	3	1
mitoGS_aap1_4455	4	1
mitoGS_aap1_4459	3	
mitoGS_aap1_4461	3	1
TMS60444	4	2
Vector_control_4218	4	2
Vector_control_4220	4	3
Vector_control_4221	3	2
Vector_control_4234	4	3
Vector_control_4239	4	3
Vector_control_4243	4	3

Screenhouse construct performance

Shoot weight

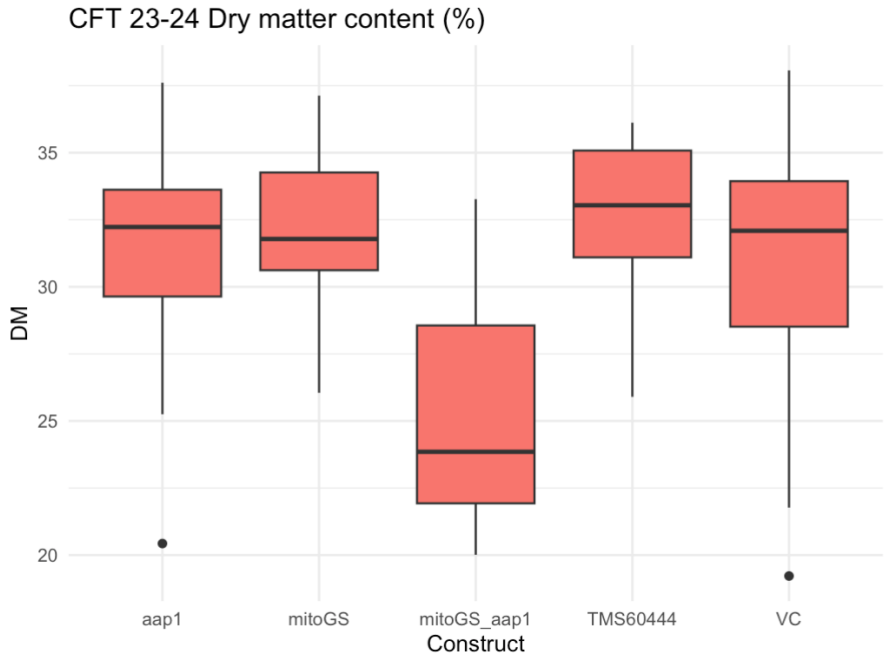


Root weight



Screenhouse construct performance

Dry matter content



Next steps

- Replanted the trial for maintenance and crosses if needed

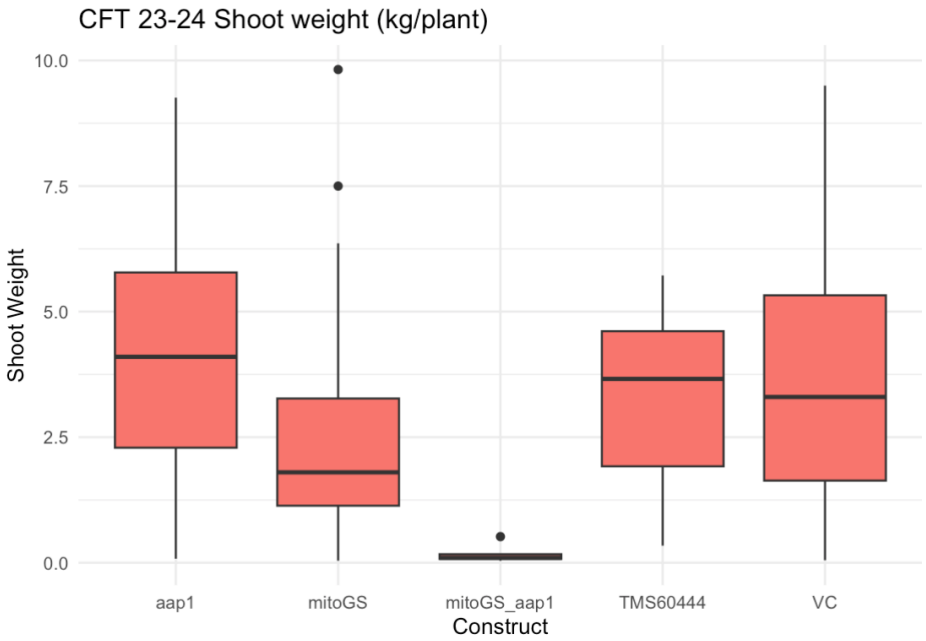
Open field planting results

- Aap1 and mitoGS did better in terms of establishment and survival compared with mitoGS_aap1 dual construct

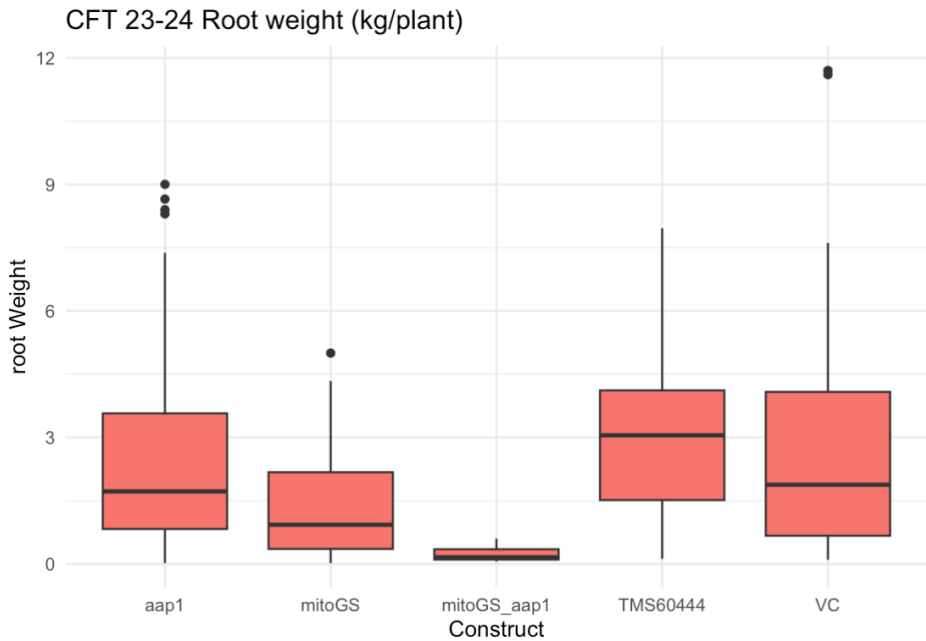
Construct	Plants
aap1	**86**
aap1_4353	18
aap1_4354	18
aap1_4355	14
aap1_4358	18
aap1_4359	18
mitoGS	**61**
mitoGS_4267	18
mitoGS_4269	18
mitoGS_4272	7
mitoGS_4275	14
mitoGS_4277	4
mitoGS_aap1	**17**
mitoGS_aap1_4435	9
mitoGS_aap1_4440	4
mitoGS_aap1_4461	4
TMS60444	**21**
TMS60444	21
VC	**85**
Vector_control_4218	17
Vector_control_4220	18
Vector_control_4234	18
Vector_control_4239	14
Vector_control_4243	18
Grand Total	**270**

Open field planting results

Shoot weight

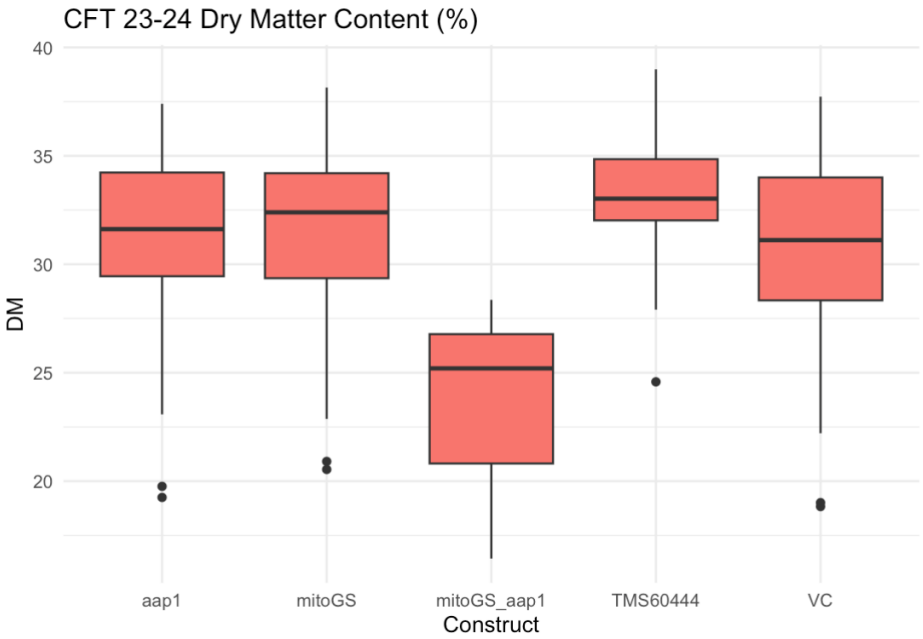


Root weight

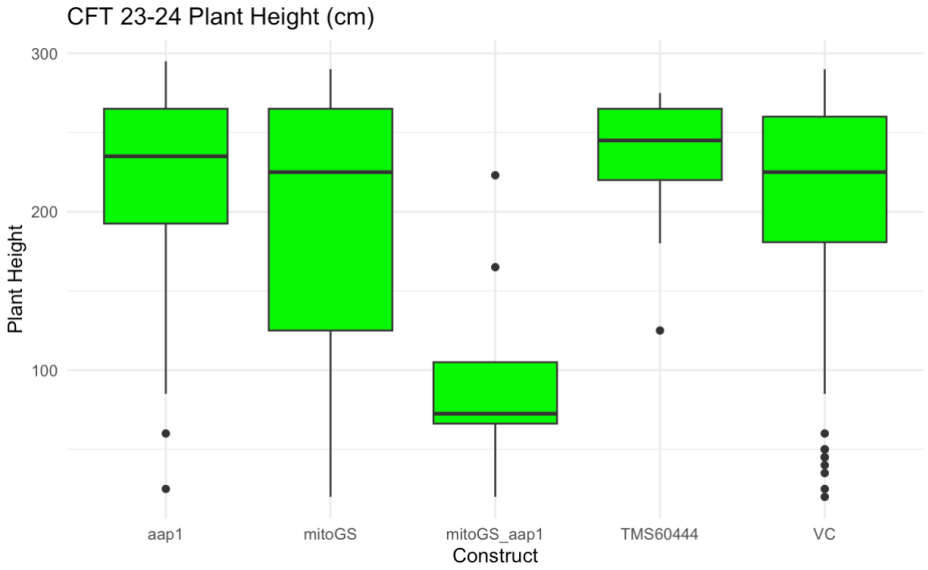


Open field planting results

Dry matter content



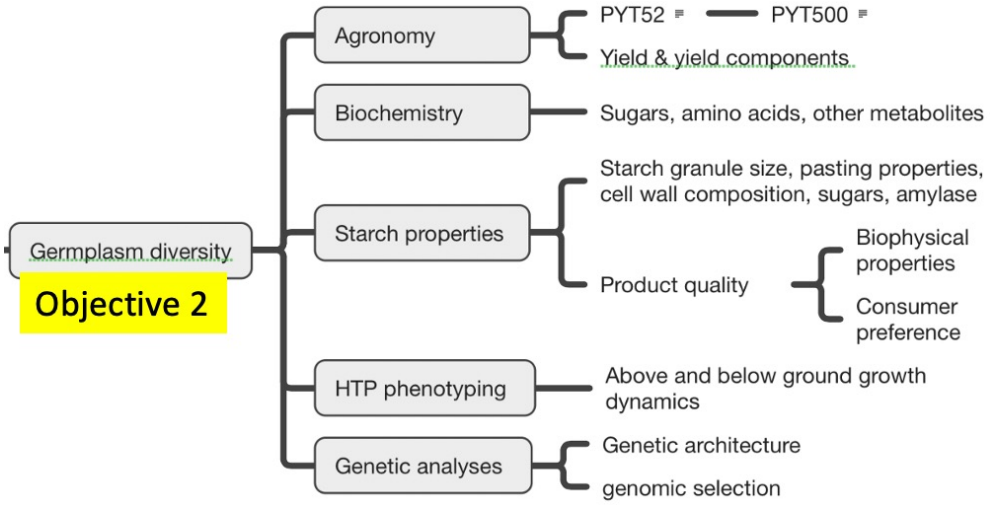
Plant height



Next steps

- Field trial replated as plots instead of individual plants
  - 2 rows, 5 plants / row, 2 replicates for available constructs
  - Additional planting to be carried out
- Expansion of the CFT field area to include adjascent field
- Permission requested from NBMA to make experimental crosses between GM and adapted varieties
  - Waiting for approval

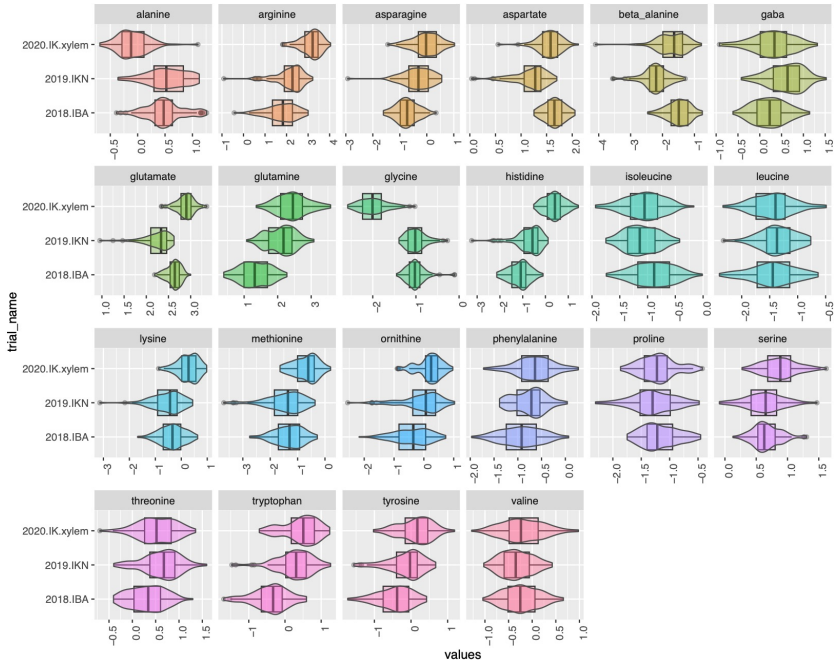
Objective 2: Multi-omics characterization of genetic variation in breeding germplasm



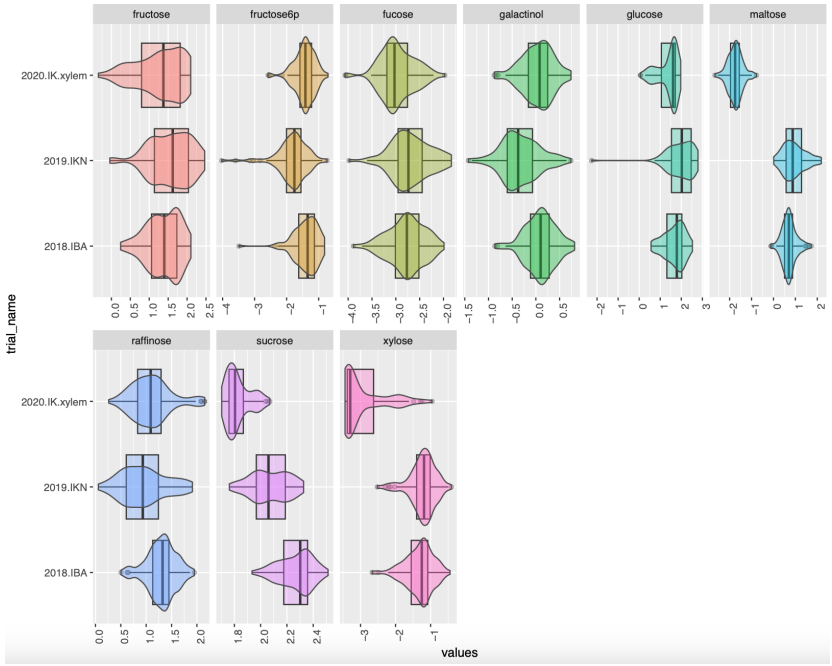
- 52 diverse clones
- 2 replicates
- 3 seasons
- 6 x 6 plots
- randomized complete block design

Most metabolites show same average and distribution across the three planting seasons

Amino acids

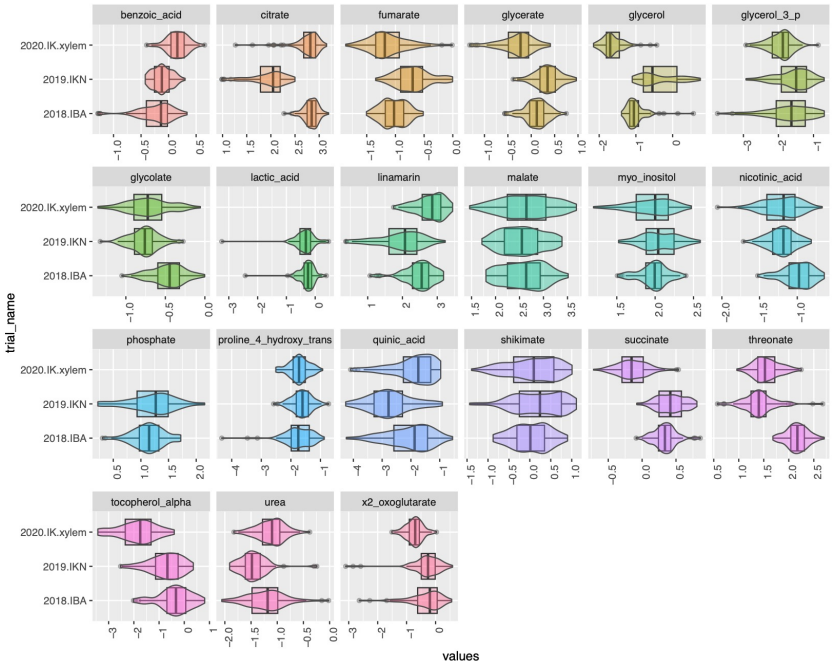


Sugars

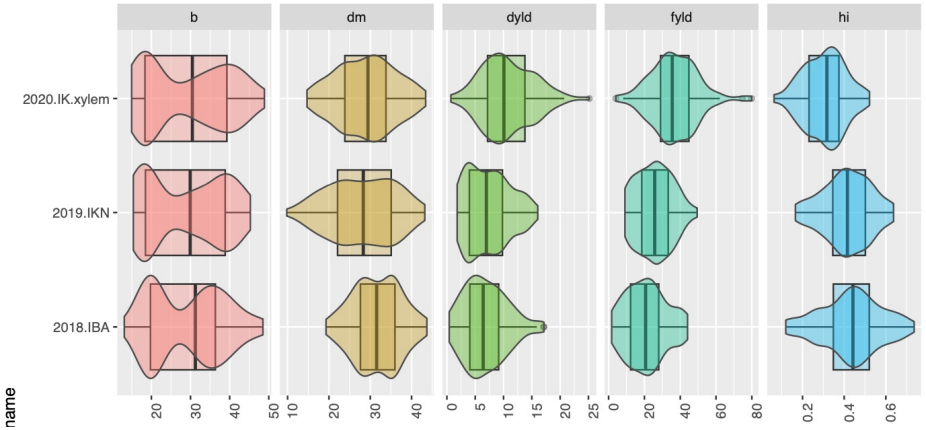


Variation of agronomic and yield traits

Other metabolites



b-carotene, dry matter, yield, and yield components

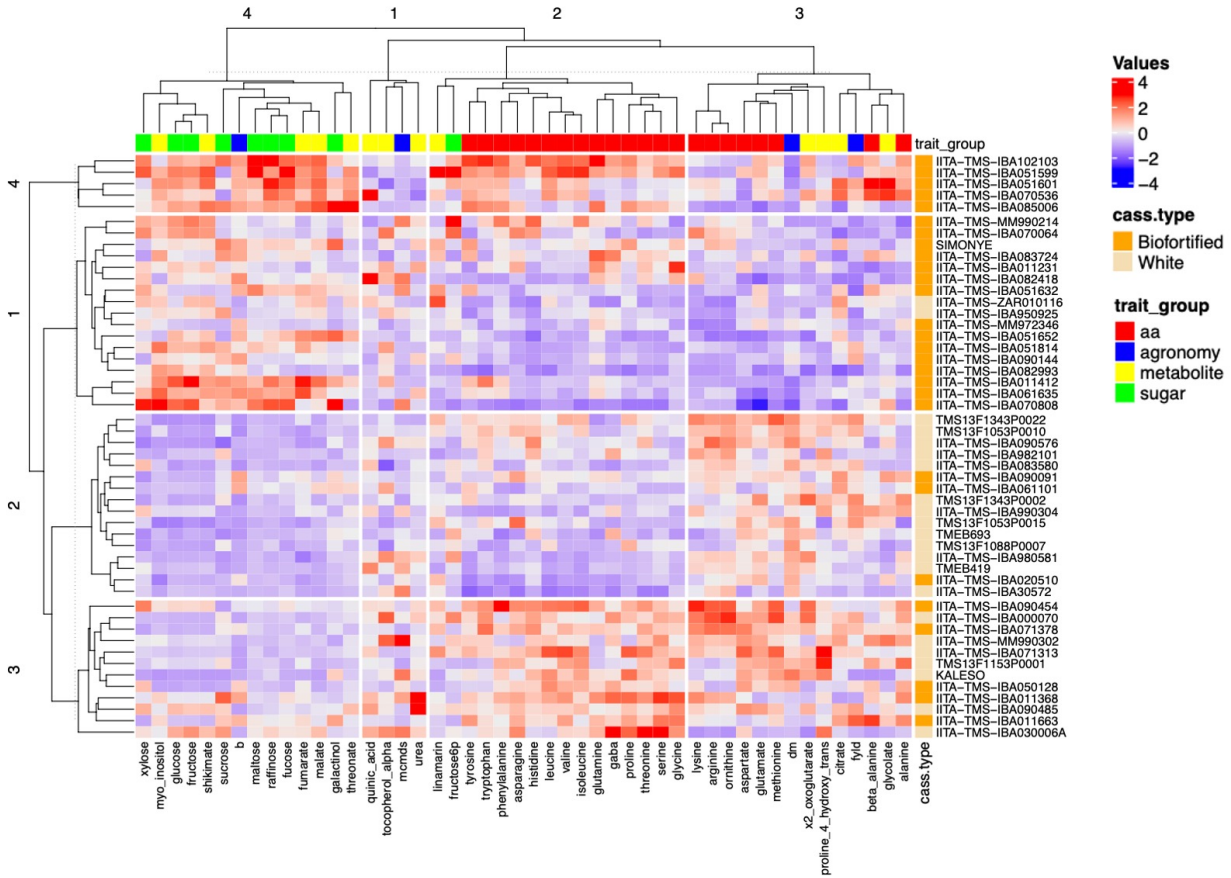


B = b-carotene
DM = dry matter

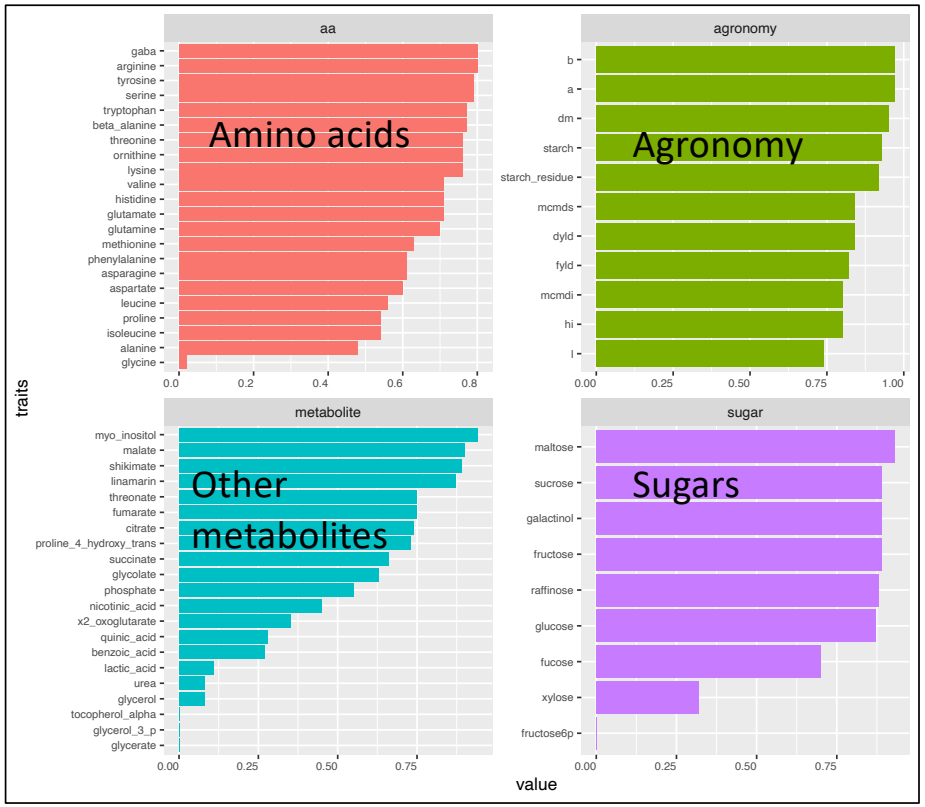
FYLD = fresh root yield
DYLD = dry root yield
HI = Harvest Index

Cluster plot of metabolite + agronomic data and accessions

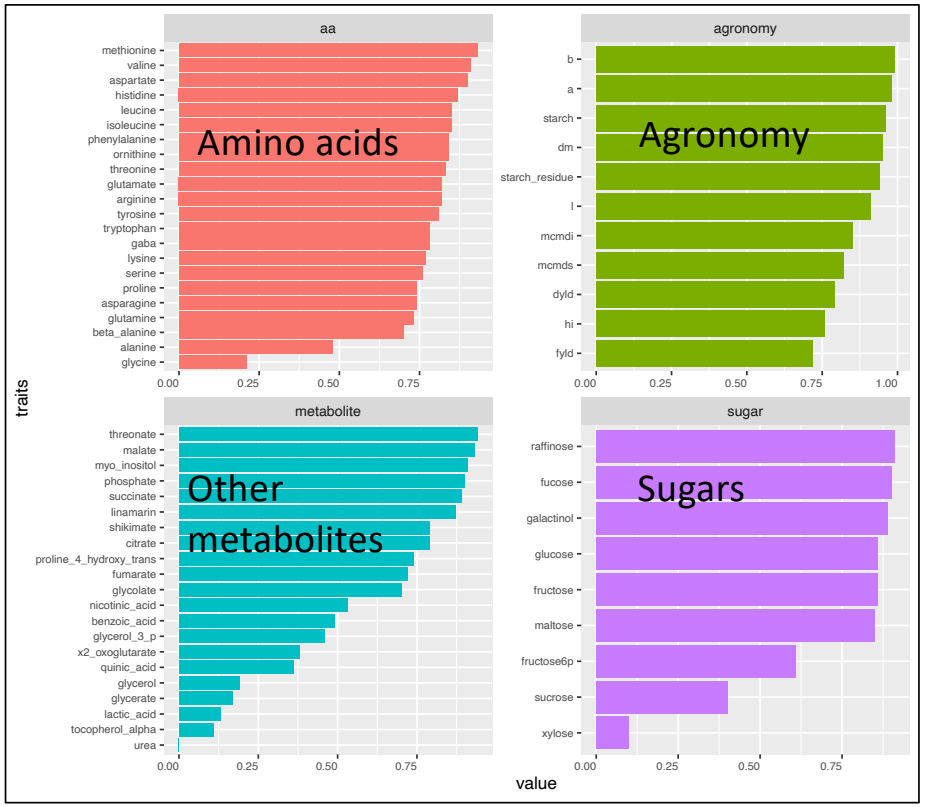
Germplasm collection clustered by dry matter and beta-carotenoids



Measured traits have high repeatability (broad-sense heritability) indicating strong genetic effect

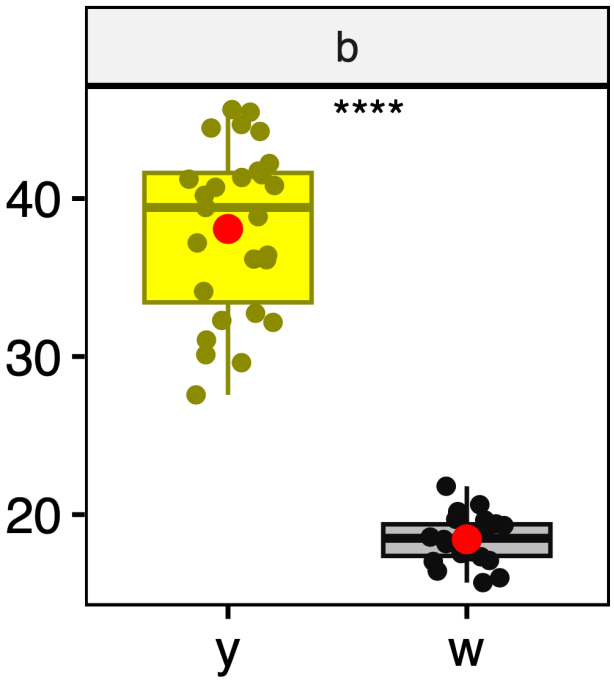


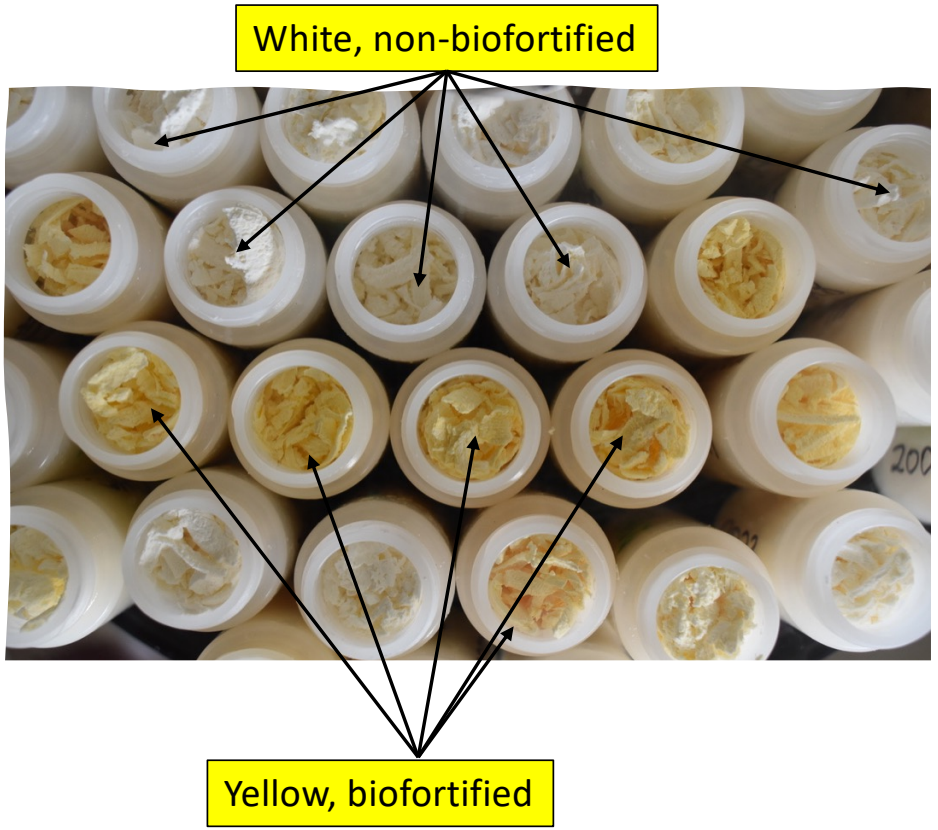
Season 1



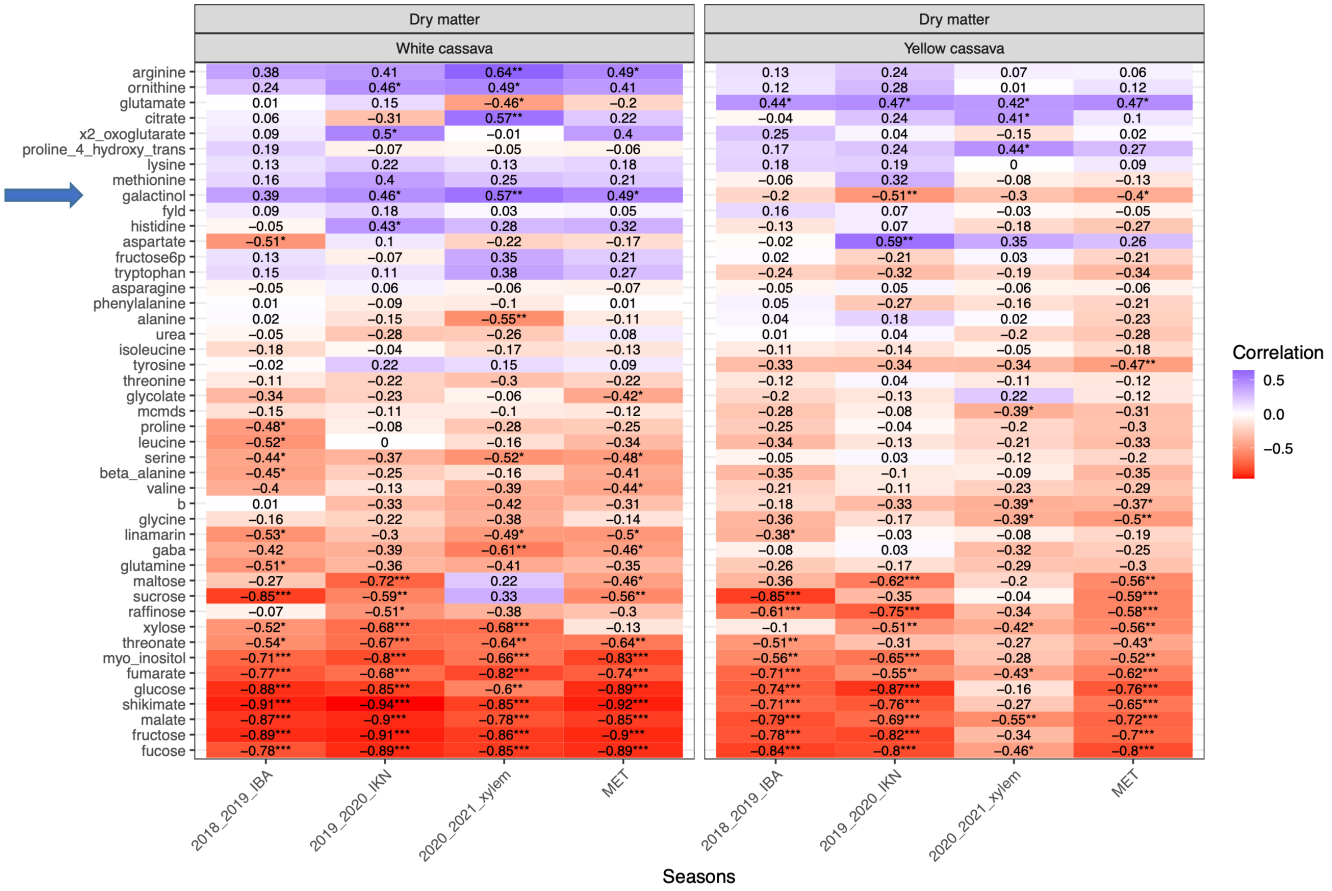
Season 2

Biochemical basis of the dry matter variation in biofortified vs non-biofortified germplasm





Which metabolites are correlated with dry matter in white vs yellow-root gerplasm?

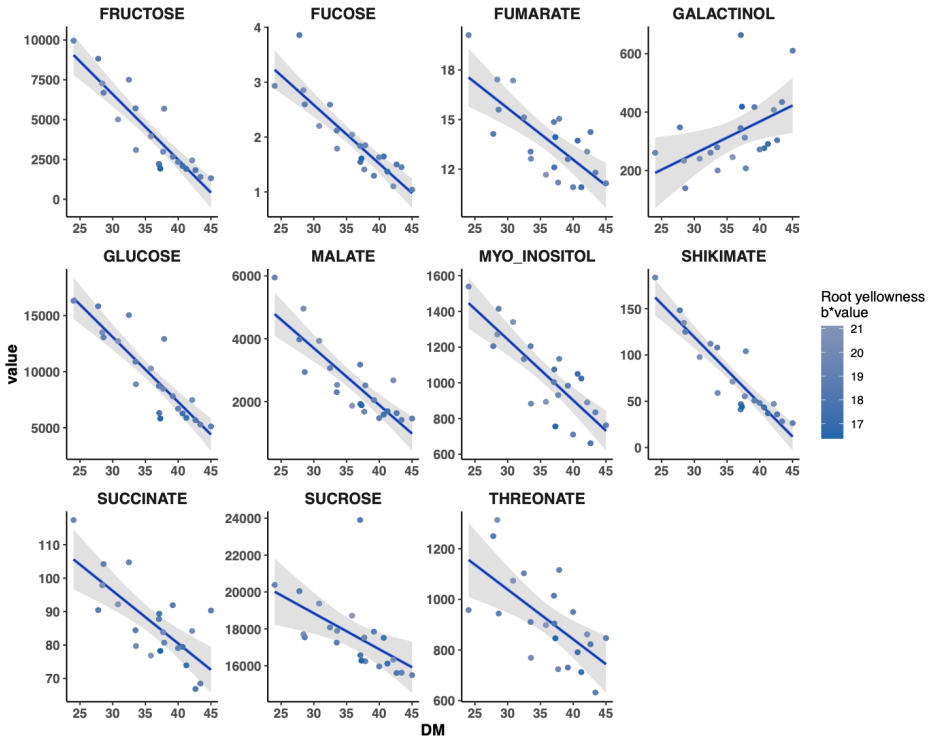


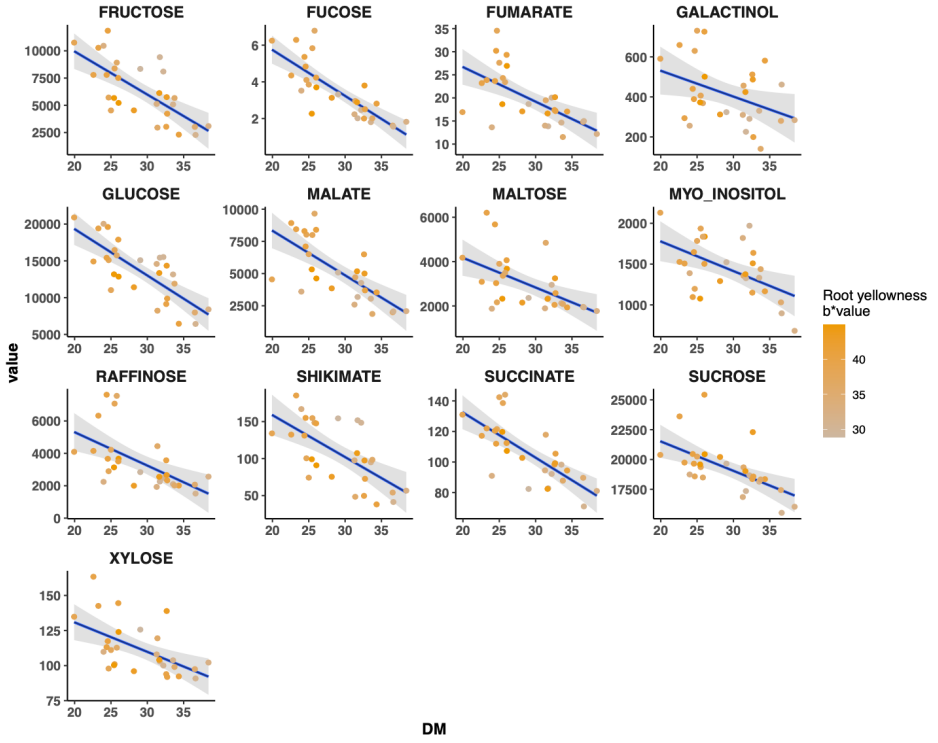
Magnitude and direction of correlation between individual metabolites and dry matter is consistent in the two subsets of germplasm

Few notable exceptions are: Galactinol, and a few amino acids

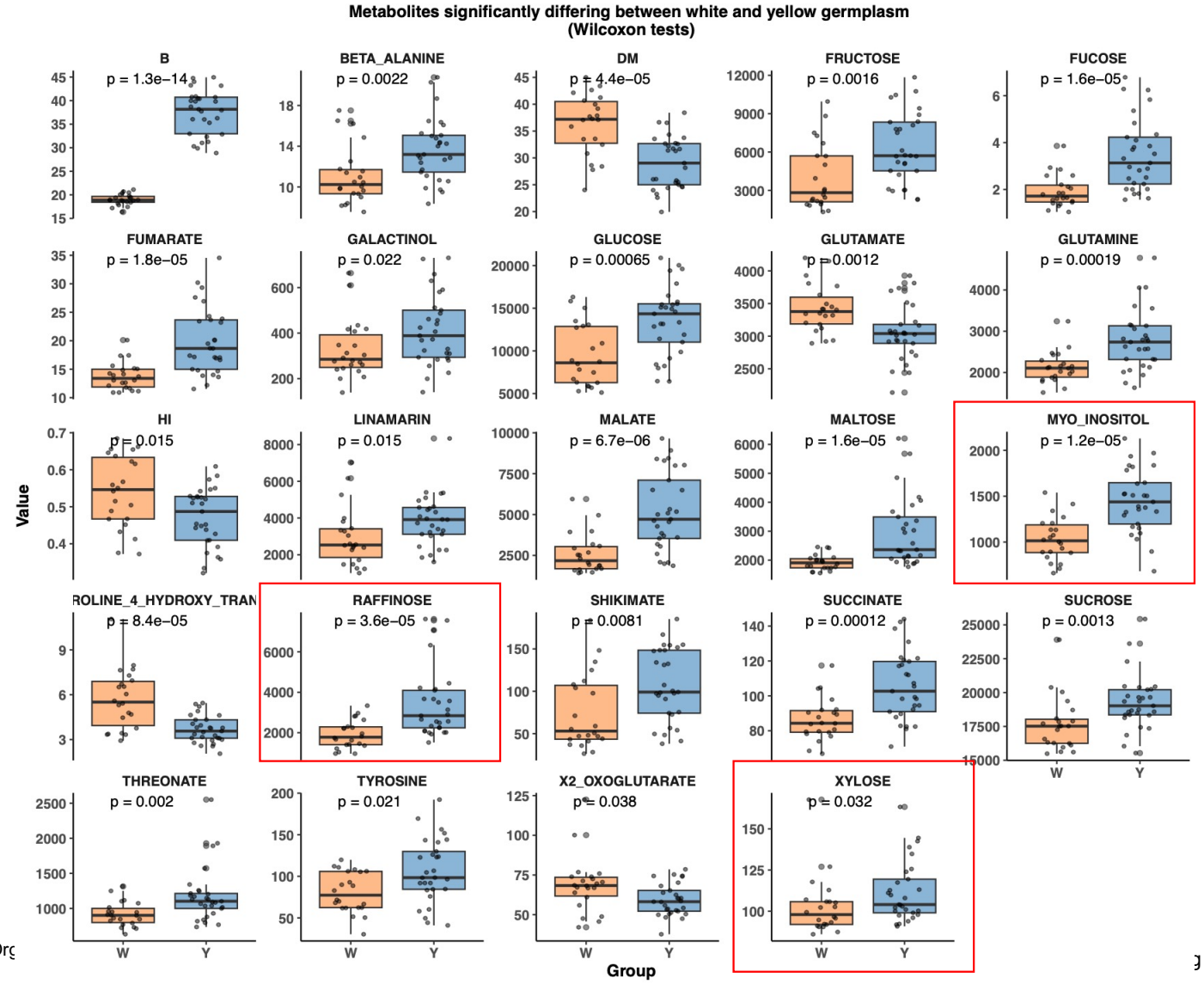
What role does ganactinol have in modulation of starch content ? Or how is presence of carotenoids affecting galactinol content and dry matter content?

Which metabolites are correlated with dry matter in white vs yellow-root gerplasm?





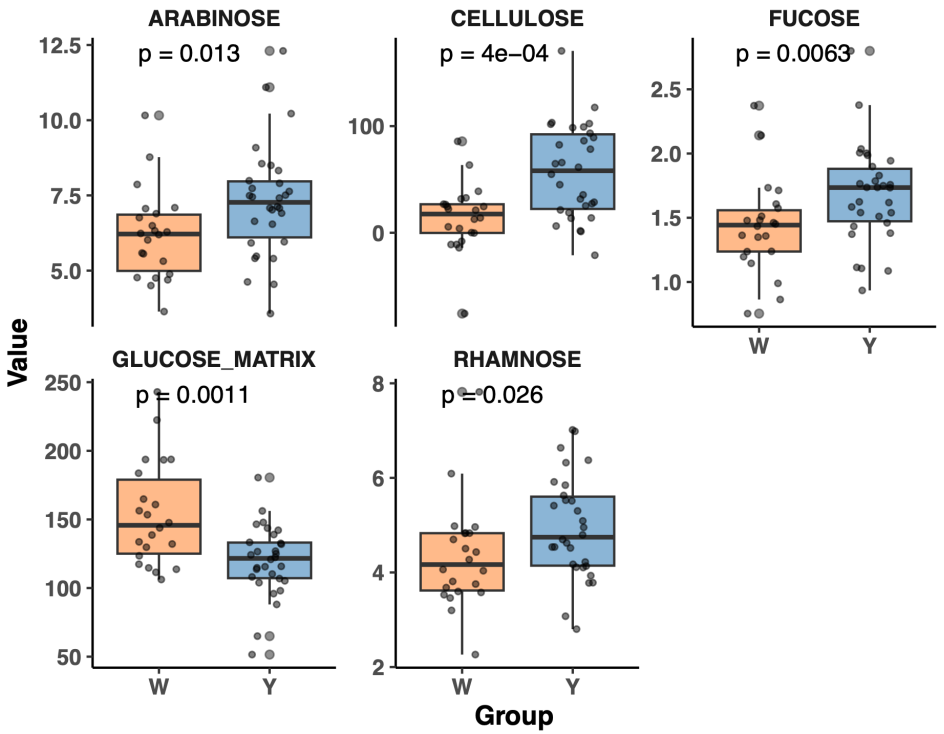
Which metabolites significantly differ between white and yellow germplasm ?



Cell wall component analysis on white and yellow-root genotypes

Cell wall component analysis supports the metabolite results

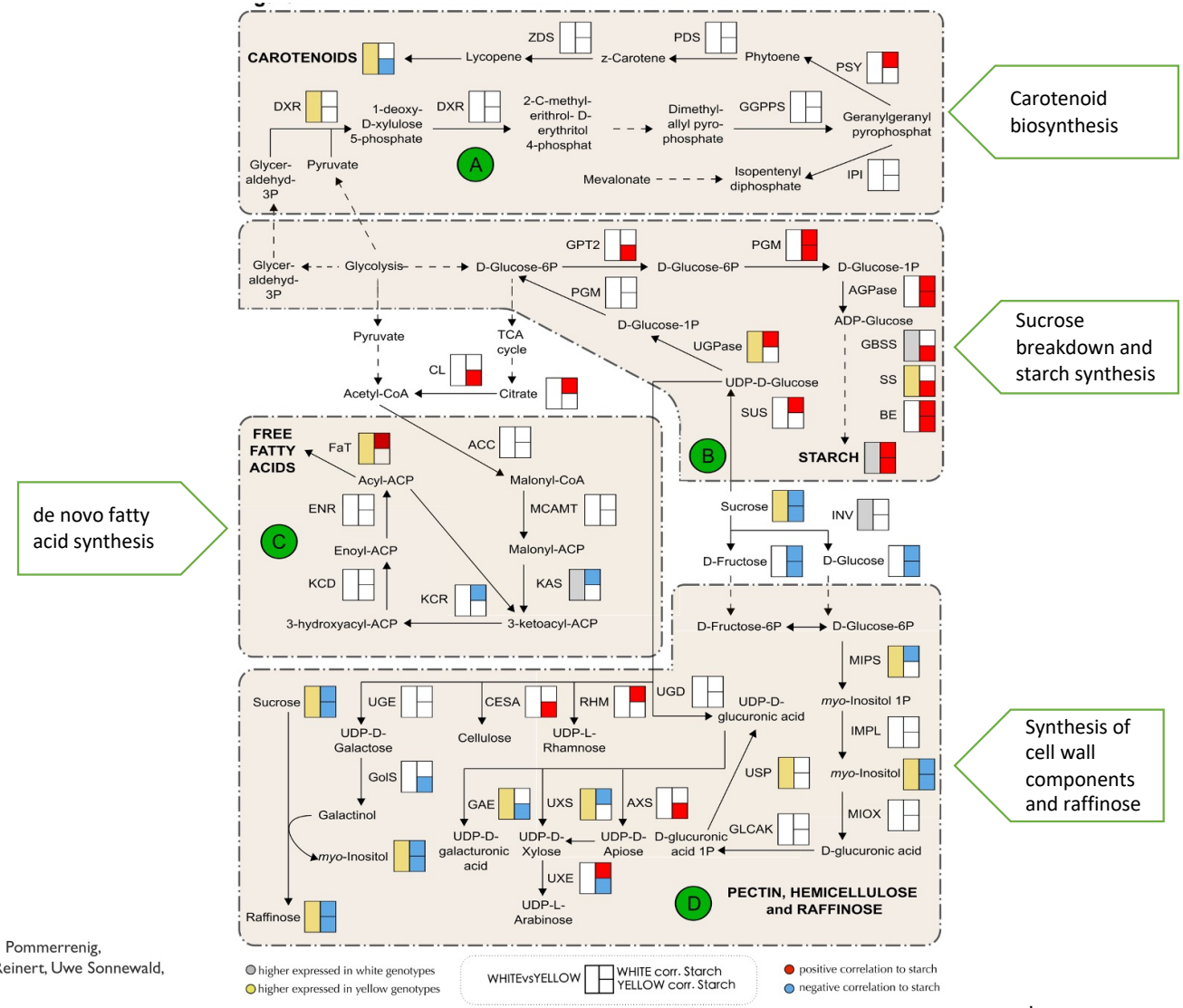
Data from ETH starch and flour analysis



Sam Zeeman, Simon Bull @ ETH - Zurich

Shift in carbon use in yellow-fleshed storage roots

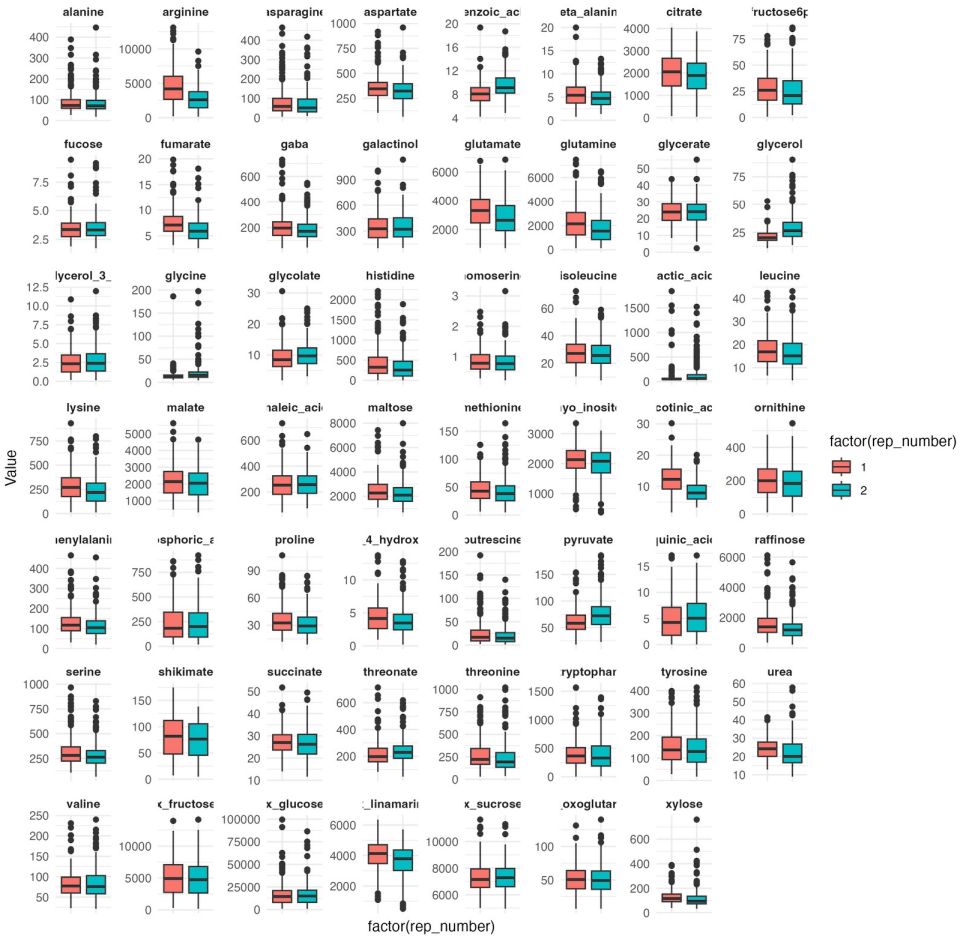
- Transcript and metabolite levels highlight significant higher abundance of genes and metabolites associated with myo-inositol, raffinose and cell wall biosynthesis in yellow genotypes
- Significant shift from **starch biosynthesis** to cell wall and raffinose biosynthesis via the myo-inositol pathway



Sindy Gutschker, David Ruescher, Ismail Y. Rabbi, Laise Rosado-Souza, Benjamin Pommerrenig, Anna M. van Doorn, Armin Schlereth, H. Ekkehard Neuhaus, Alisdair R. Fernie, Stephan Reinert, Uwe Sonnewald, Wolfgang Zierer

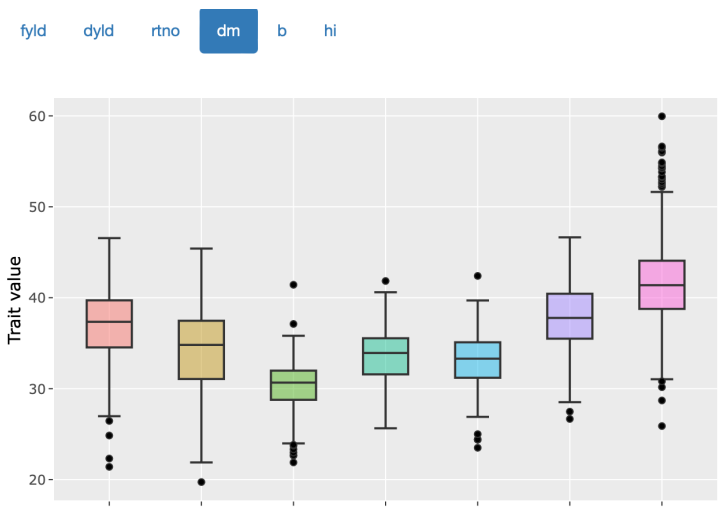
PYT500 population metabolite profiling

- Large population at Preliminary Yield Trial stage (n = 500)
- Genotyped at >50 K SNP markers
- Evaluated in 7 environments in Nigeria for agronomic traits
- 250 selected for metabolite profiling (55 metabolites)
-

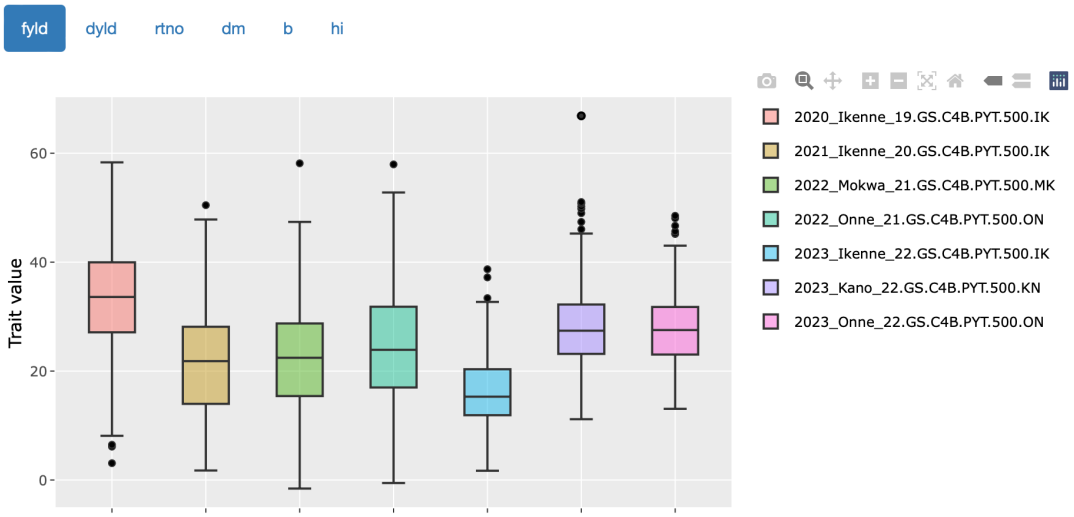


PYT500 phenotype variation

Dry matter content

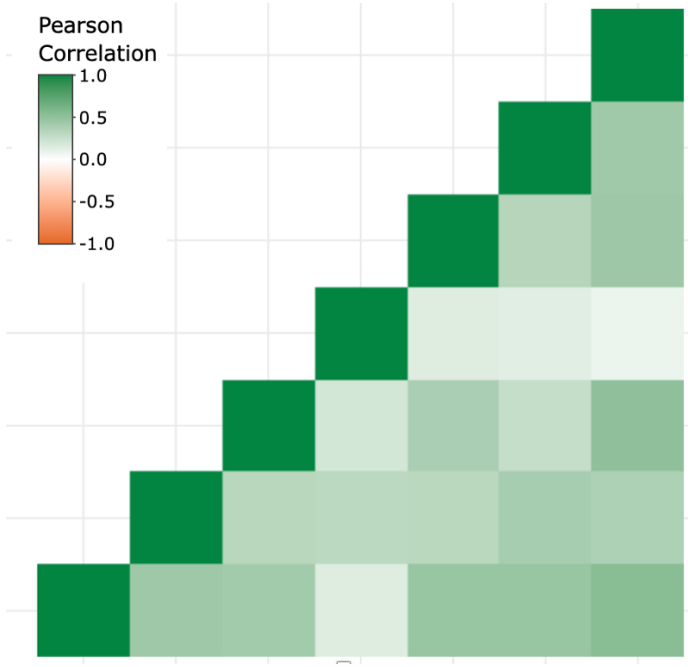


Fresh root yield

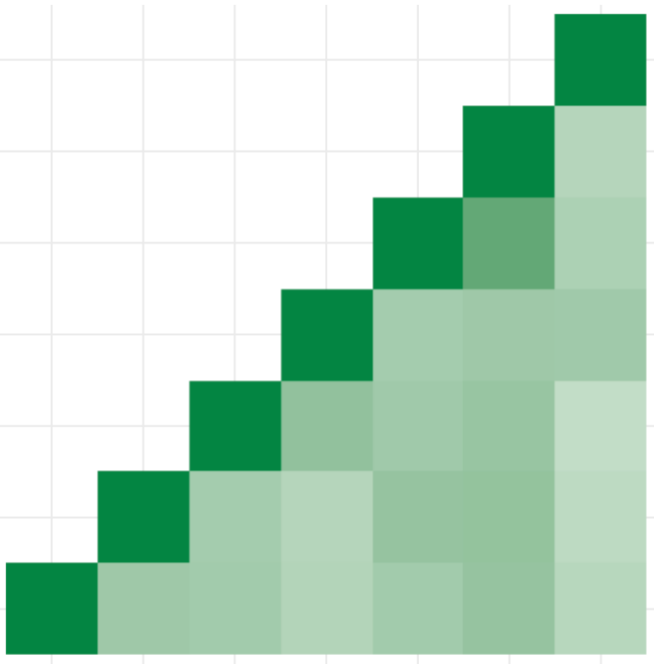


Correlation betweeb environments

Fresh yield



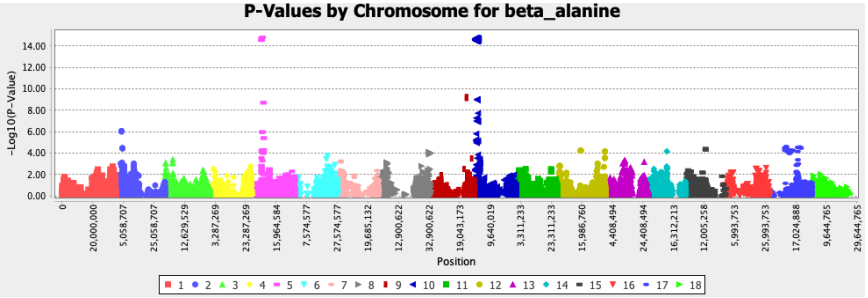
Dry matter (starch)

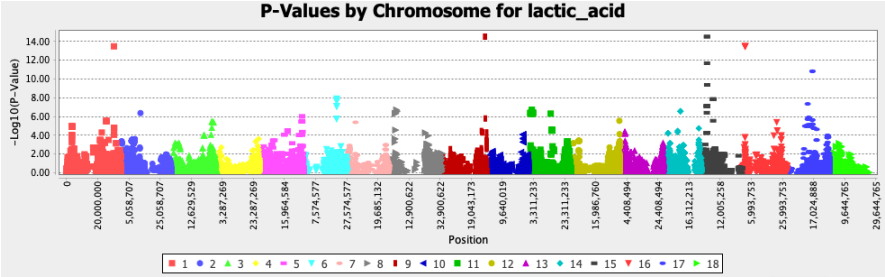


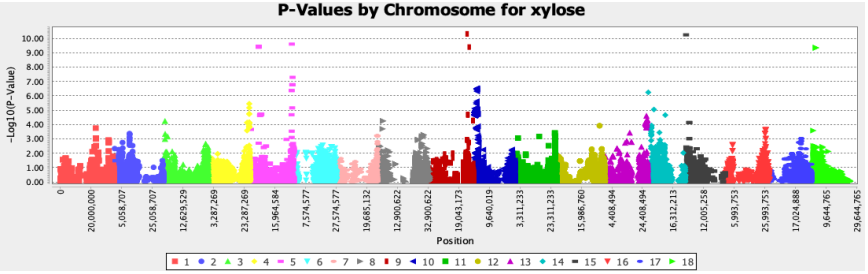
Genome-wide association (GWAS)

GWAS underway to identify major loci underlying variation in the studied metabolites

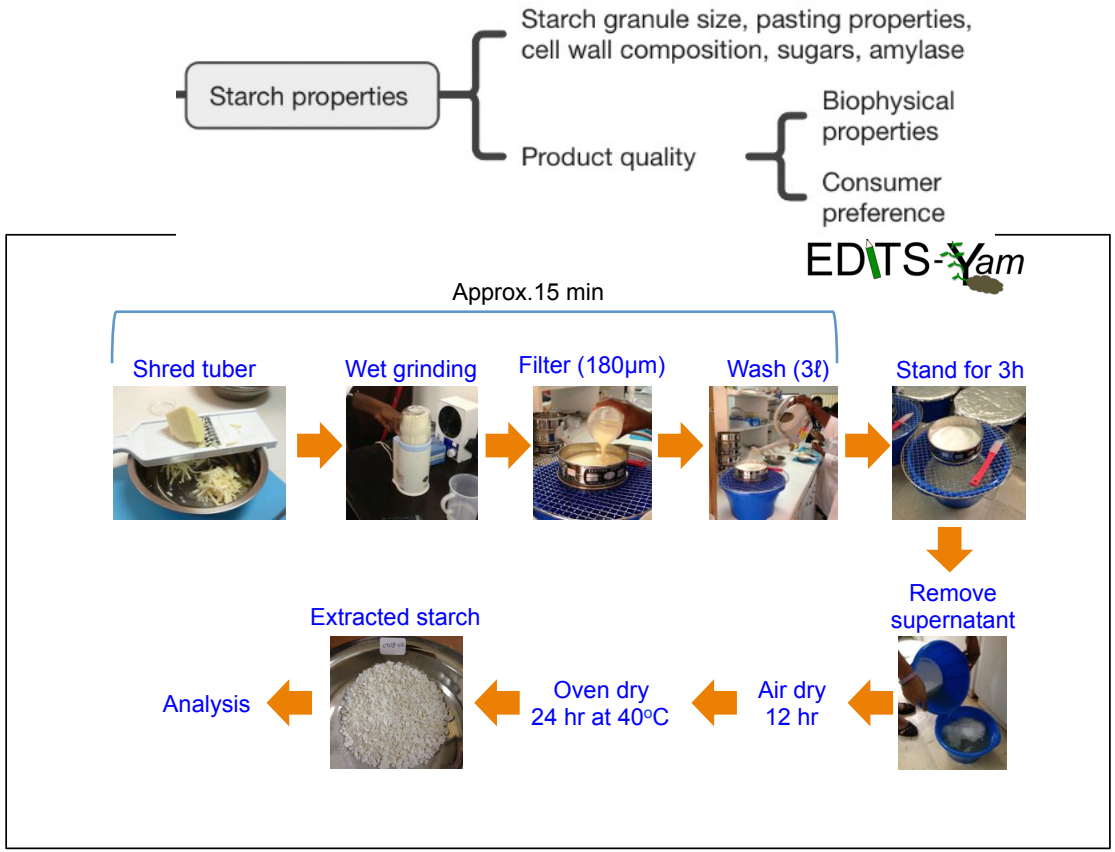
Preliminary results indicates major loci for a number of traits







Starch analysis (IITA-ETH collaboration)

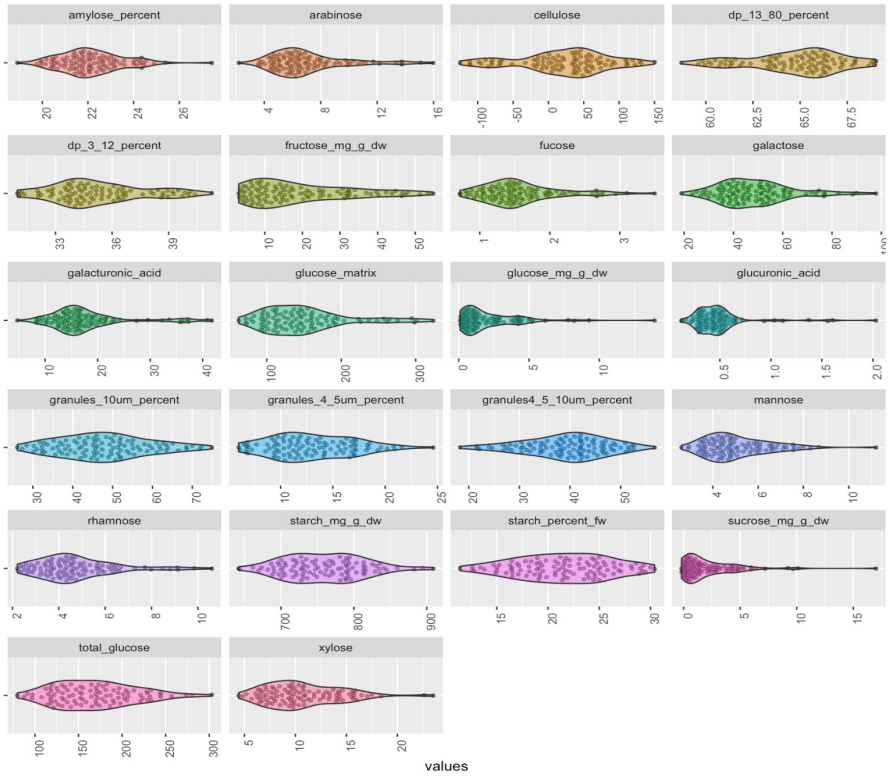


Granules<4.5um (%)	Amylose (%)
Granules4.5-10um (%)	dp 3-12 (%)
Granules>10um (%)	dp 13-80 (%)
	Fucose
starch (mg/gDW)	Rhamnose
Sucrose (mg/gDW)	Arabinose
Glucose (mg/gDW)	Galactose
Fructose (mg/gDW)	Cellulose
starch(%_FW)	Glucose matrix
Sucrose (%_FW)	Total Glucose
Glucose (%_FW)	Xylose
Fructose (%_FW)	Mannose
	Galacturonic acid
	Glucuronic acid
flour pasting temp	starch pasting temp
flour peak viscocity	starch peak viscocity
flour holding strength	starch holding strength
flour breakdown	starch breakdown
flour final viscosity	starch final viscosity
flour setback from peak	starch setback from peak
flour setback from trough	starch setback from trough

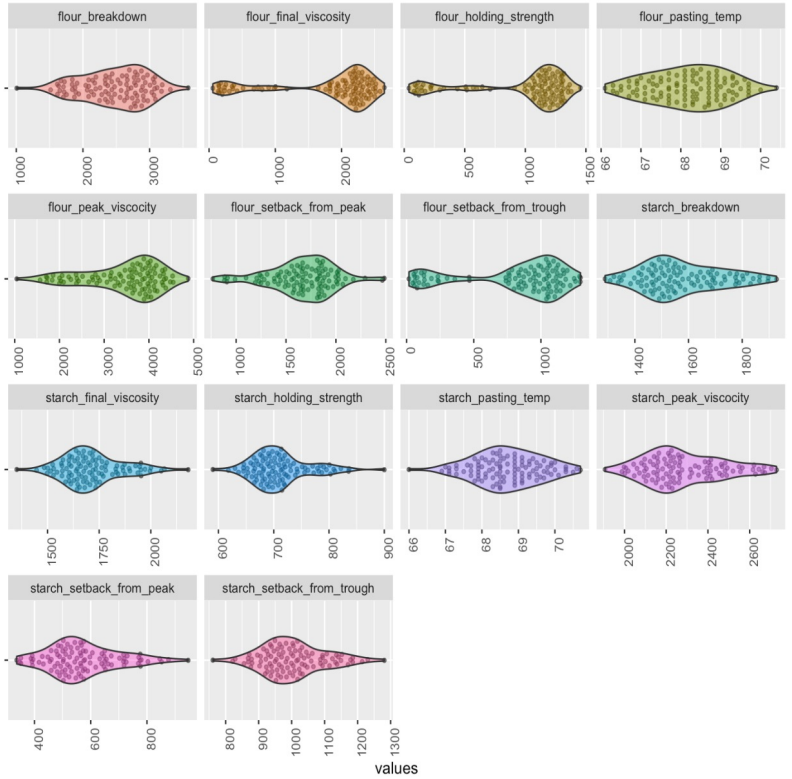
Analyses done at ETH (Laure, Simon)

Starch traits distribution in core collection

starch, sugars, cell wall



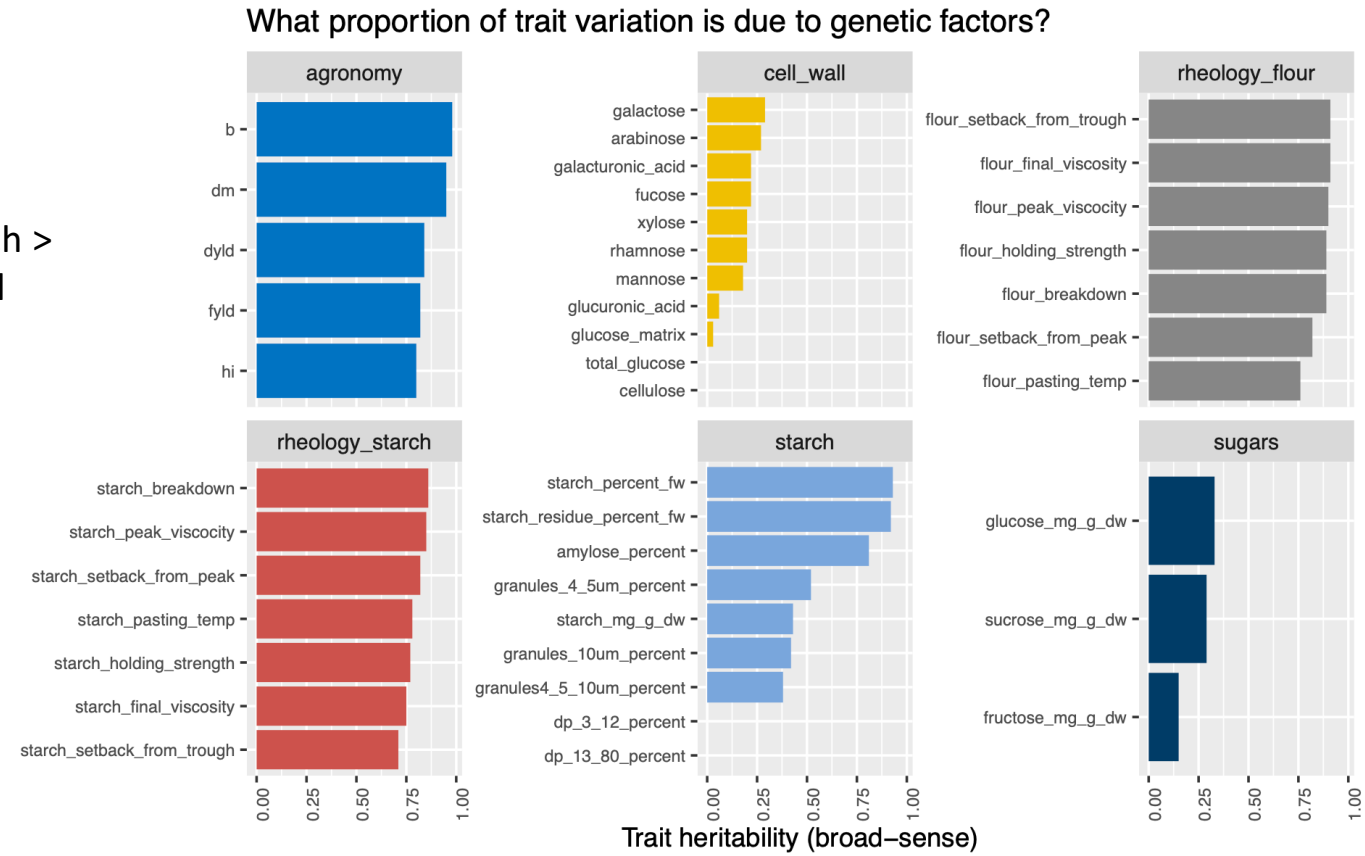
Rheology starch, rheology



Measured starch traits have high repeatability indicating strong genetic effect

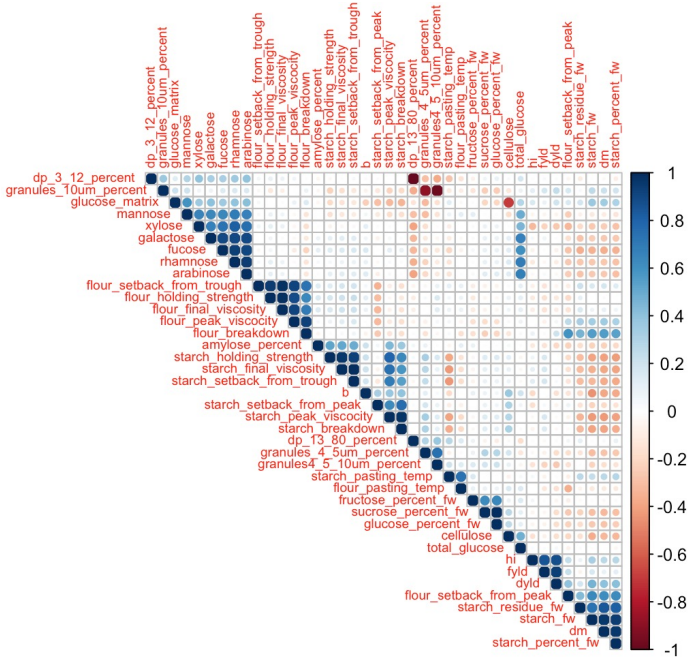
H2

Rheology > Starch > Sugars > cell wall

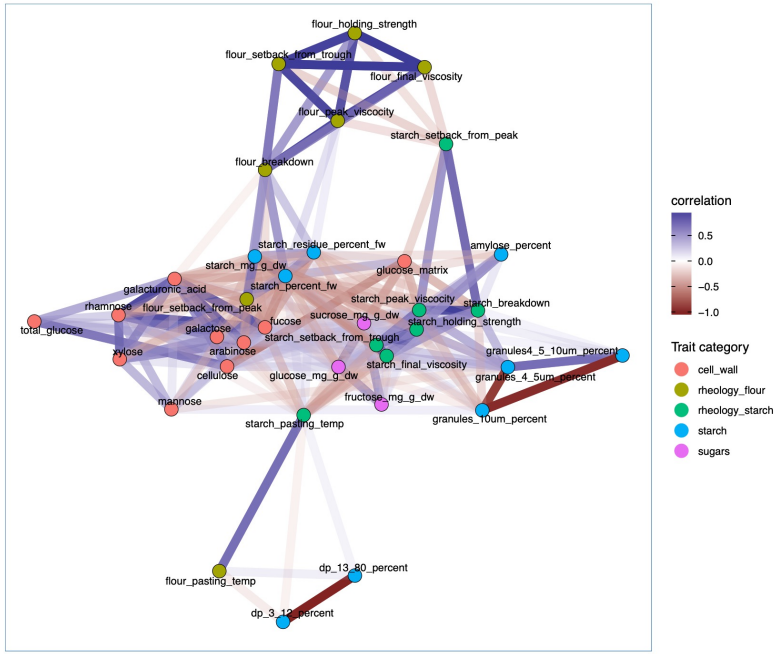


Relationship between measured properties of starch and flour

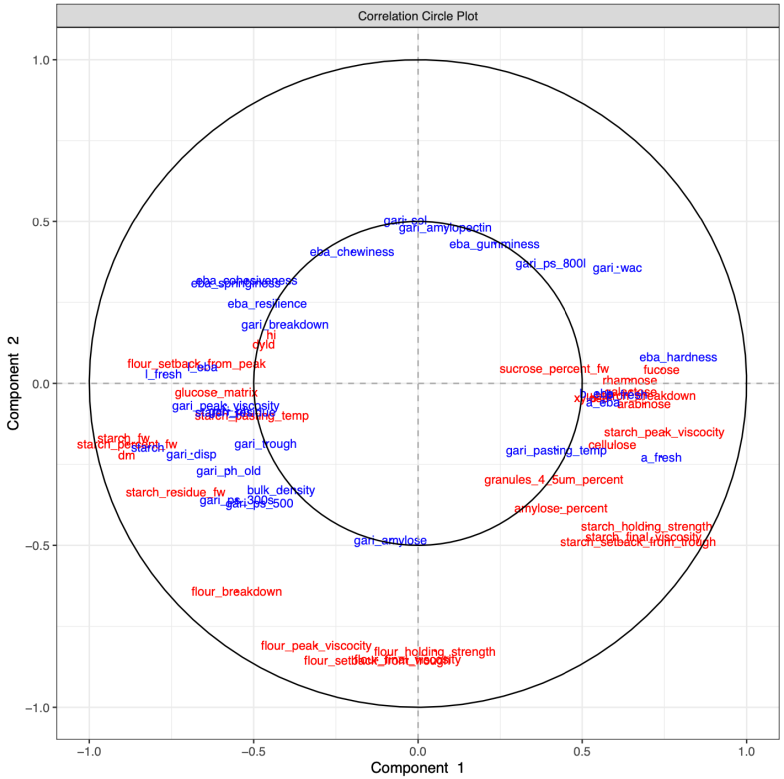
Global relationship between measured traits

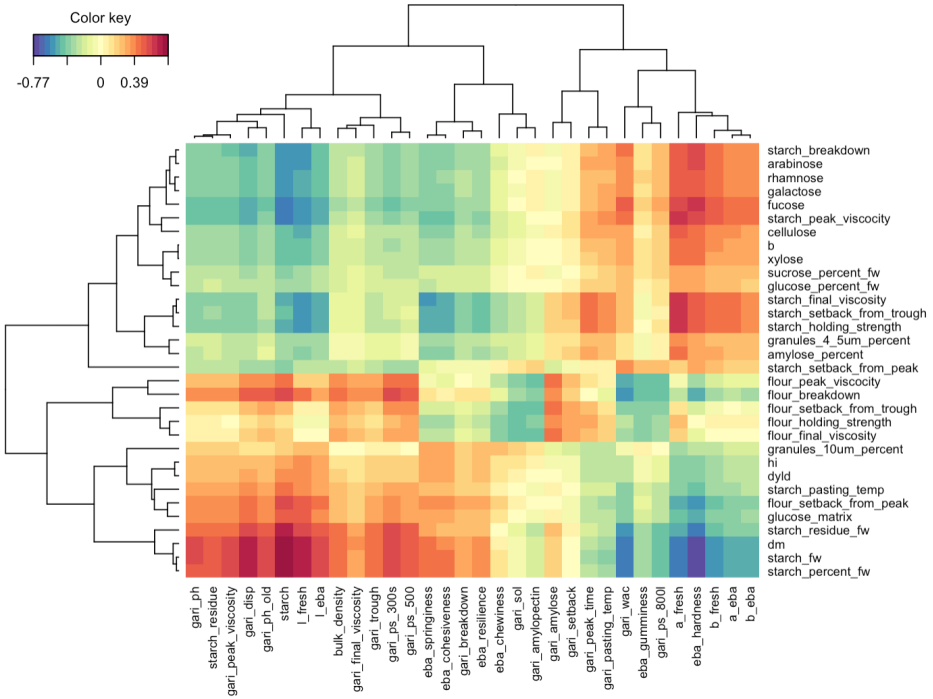


Flour and starch have different rheology properties



Relationship between starch and product qualities





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Thanks to all the partners who have contributed to the germplasm characterization!