Phenotyping and QTL Analysis for Storage Root Chemistry Traits of Sweetpotato

Victor Amankwaah and Team

BSc. Agriculture, MSc. Plant Breeding (KNUST, Ghana)

17th Sweetpotato SpeedBreeders' and Genomics Community of Practice Meeting

June 7th, 2018

ACKNOWLEDGEMENTS

GRADUATE COMMITTEE

DR. CRAIG YENCHO (CHAIR)
DR. VAN-DEN TRUONG
DR. ZHAO-BANG ZENG
DR. BODE OLUKOLU
DR. EDWARD CAREY (TECHNICAL ADVISOR)

BILL & MELINDA GATES *foundation*

NC STATE UNIVERSITY

COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH · GHANA · CSIR

GT4SP GENOMIC TOOLS FOR SWEETPOTATO IMPROVEMENT

CIP INTERNATIONAL POTATO CENTER
A CGIAR RESEARCH CENTER

MEMBERS OF THE NCSU SP BREEDING PROGRAM

Outline

- TIMELINES
- RATIONALE AND SIGNIFICANCE
- TB NIRS WORK AND ANALYSIS
- TB AMYLASE ASSAY WORK AND ANALYSIS
- COOKED SUGAR EXTRACTION AND ANALYSIS
- CALIBRATION OF COOKED SUGARS AND AMYLASE ACTIVITY USING NIRS
- QTL MAPPING
- SUMMARY

Timelines

Fall 2015

Spring 2016

Fall 2016

Spring 2017

- 2.5 years of intensive CLASSES
- 2016 field establishment of TB and BT in Ghana trial for NIRS and amylase assay
- Dry matter processing and NIRS analysis

- 2017 TB field establishment
- Amylase assay for 2016 trial
- Engaged in linkage map construction of TB construction of
- Passed preliminary exams

Fall 2017

- Harvesting and dry matter processing and NIRS
- Amylase assay for 2017 trial
- Sugar extraction of baked samples
- Development of calibration curve for alpha and beta amylase and maltose

Spring 2018

Fall 2018

- Phenotypic data analysis of BT storage root quality data from Ghana
- QTL mapping of nutritional quality traits from TB and BT
- Thesis preparation and defense

Rational and Significance

- All types of sweetpotato contain minerals and vitamins
- Only the orange ones have large amounts of beta-carotene.
- OFSP can be used effectively to combat vitamin A deficiency (VAD) among vulnerable populations.

- SASHA project has been involved in repositioning sweetpotato in food economies of West Africa by developing essential capacities and products

- GT4SP can contribute to the initial efforts of SASHA project in West Africa of improving livelihoods and quality of sweetpotato that meet consumer preference through improved breeding

Rationale and Significance

- Commercial benefits of sweetpotato may not materialize due to the limited availability of consumer-preferred varieties influenced by desired quality traits

- Critical need for the development of new varieties that are optimized for current and future market needs relates to finding genetic factors and understanding molecular and chemical basis underlying simple and complex traits in sweetpotato

PhD Objectives

1. Evaluate nutritional quality traits of green, cured and storage samples of TB
2. Evaluate nutritional quality traits of BT mapping population
3. Evaluate α-and β-amylases activities in TB mapping population
4. Evaluate baked samples for sugar contents and correlate maltose with enzyme activity in the TB mapping population
5. Develop NIRS calibration curve for alpha and beta amylase activity
6. Develop NIRS calibration curve for cooked sugars
7. Construct a linkage map using SNP markers in the TB mapping populations
8. Identify QTLs for nutritional quality traits
9. Identify QTLs for enzyme activity underlying agronomic traits in each of the populations

Evaluation of consumer related nutritional quality traits in BT and TB mapping population

- The sugars contained in sweetpotato are an important component of its eating quality and they have been directly associated with its characteristic flavor.

- Consumers in WA prefer varieties which have
  - ✓ low sugars (non-sweet types)
  - ✓ high dry matter content
  - ✓ appreciable levels of β-carotene

- Consumers in USA prefer varieties which have
  - ✓ high sugars (sweet types)
  - ✓ low dry matter content
  - ✓ appreciable levels of β-carotene

Challenges of breeding for quality traits

- Strong negative relationship between sugar and dry matter contents
- A strong negative correlation between dry matter content and β-carotene content
- Inadequate locally adapted consumer preferred OFSP varieties
- Difference in sugar profiles of genotypes when it is green compared to after they have been processed.

How can Victor and team contribute in solving these challenges??

Materials and methods

BT AND TB MAPPING POPULATIONS

- TB developed at NCSU
- 2 parents, 248 progenies

- BT developed at CIP
- 2 parents, 315 progenies

PARENTAL CLONES

BEAUREGARD TANZANIA

COMPARISON BETWEEN PARENTAL CLONES

Parental clone	Origin	Flesh color	β-carotene content	Dry matter	Maturity period (days)
Beauregard	USA	Orange	High	Low (~ 20%)	100-110
Tanzania	East Africa	Cream	Low	High (~ 30%)	140

2016 Field season-Clinton (TB)

- 5 plants per plot
- 1 replication
- Planted June 2016
- Harvested November 2016
- Repeated in 2017

2017 Field season-Nyankpala (BT)

- 16 plants per plot
- 2 reps
- Planted Dec 2016
- Harvested May 2017
- Repeated in 2018

TB Dry matter processing

1. Washing of Samples

2. Peeling

3. Slicing

4.Food processing

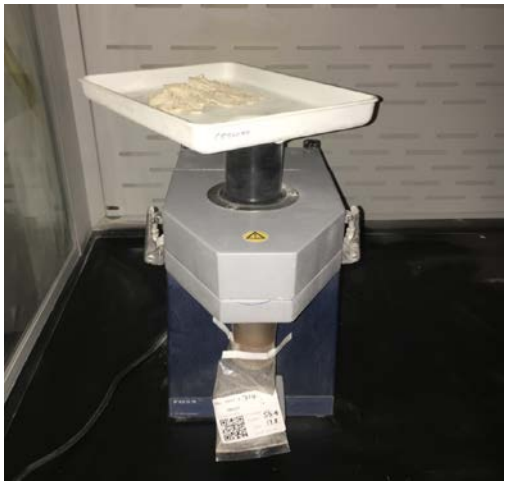
5. Weighing of samples

6. Samples ready for freeze drying

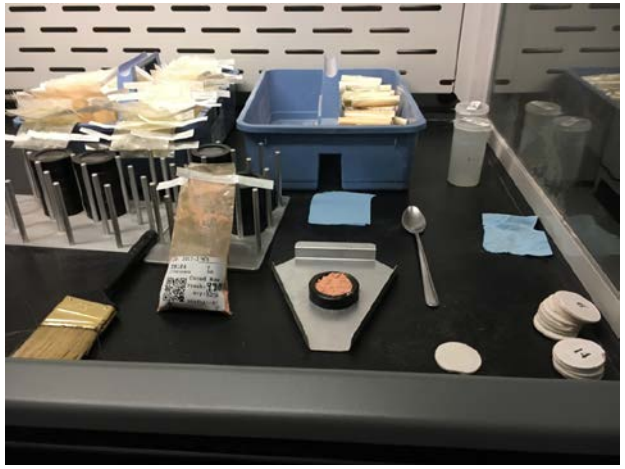
High throughput NIRS phenotyping

- Simple and many traits evaluated simultaneously.

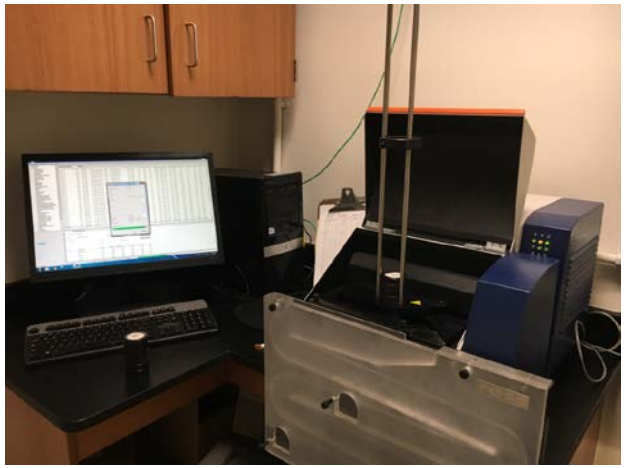
1. Milling of freeze dried samples



2.Preparation of samples for scanning



3. Scanning at 400-2500 nm



Quality traits evaluated and data analysis

- Quality traits evaluated
  - ✓ Dry matter content
  - ✓ Starch content
  - ✓ Total sugars (fructose, glucose and sucrose)
  - ✓ Beta carotene content

- Samples were dry matter processed at different post harvest stages:
  - ✓ Immediately after harvest (green)- BT and TB
  - ✓ After curing for 1 week (Curing at ~29 C) and 85% RH for one week)-TB
  - ✓ 11 weeks in storage (Curing at ~14 C)-TB

- Data collected from NIRS were averaged over the two-year period

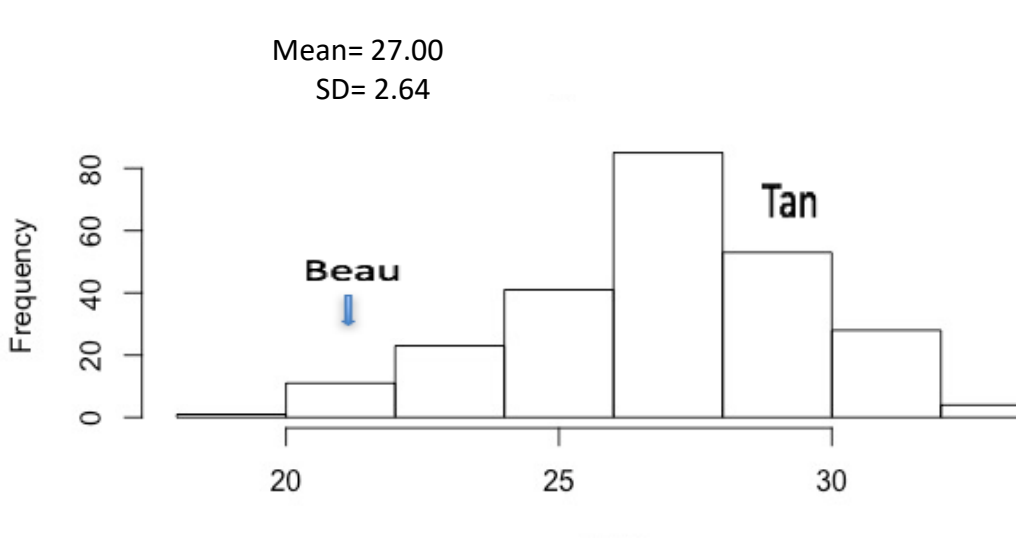
- Boxplots and histogram were drawn using R version 3.4.1

- Simple correlations between traits were done using the corrplot package in R

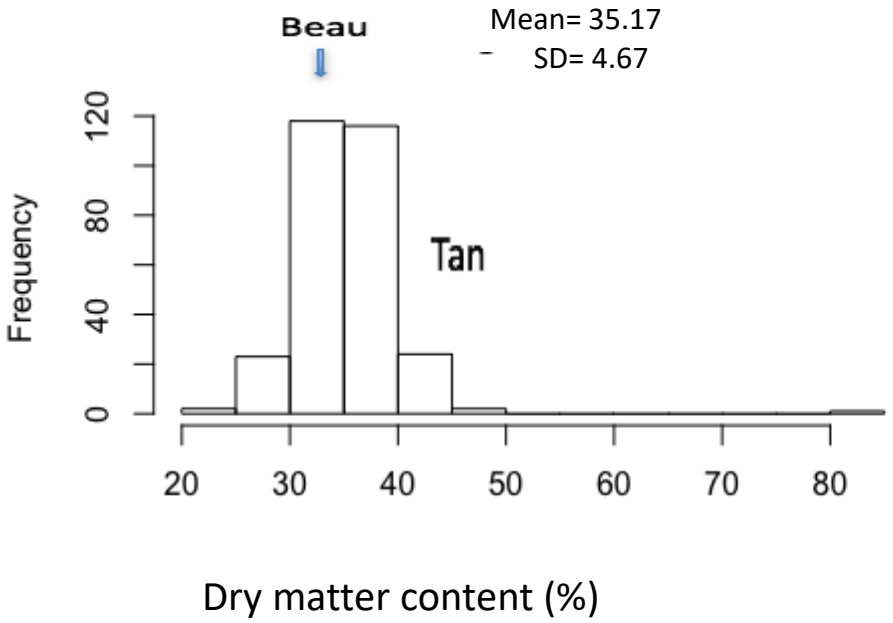
Results (Evaluation of Quality traits)

Histogram of dry matter content of BT AND TB Green Samples

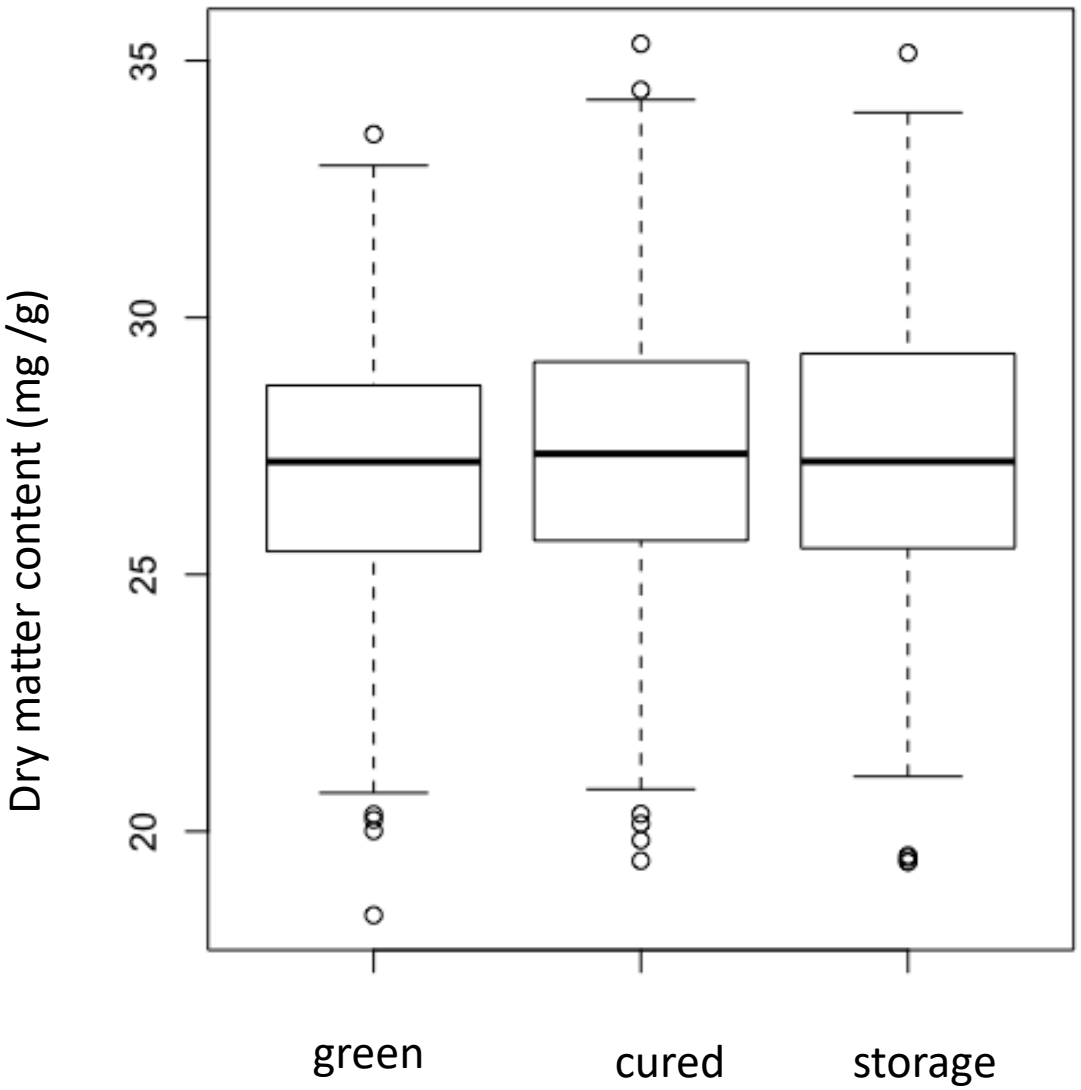
TB



BT



Distribution of dry matter content in TB samples



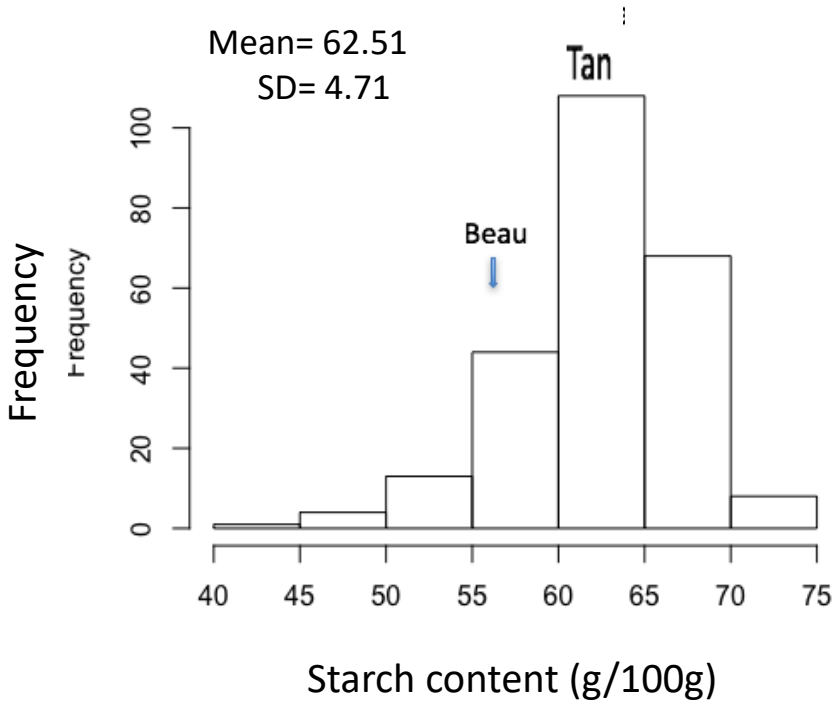
Dry matters content were stable over time and with different sampling categories

Population mean: Green samples ~ cured samples ~storage samples

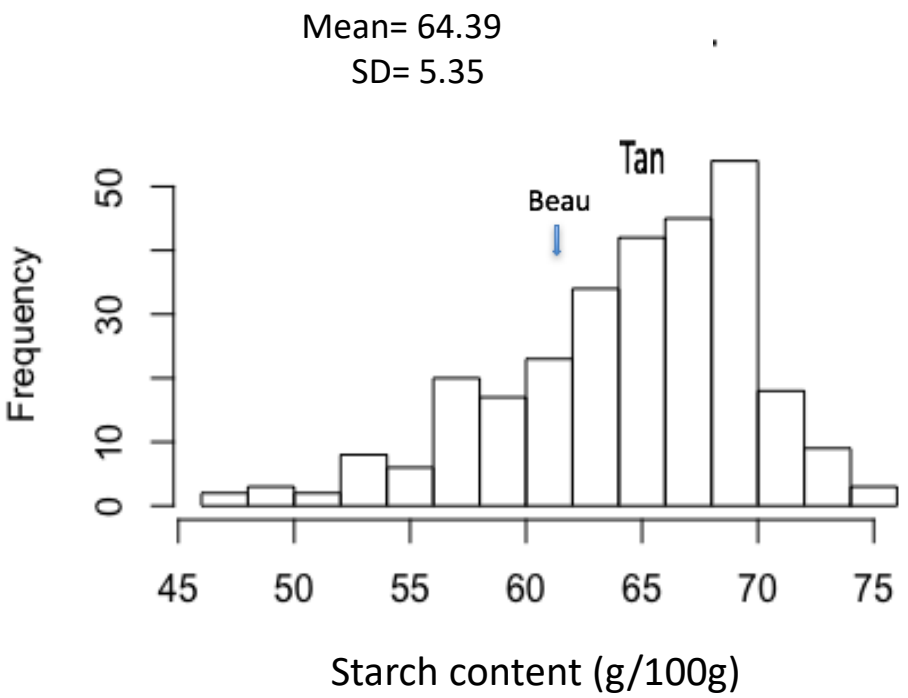
There were some outliers which is an indication of transgressive segragrants in relation to high dry matter content

Histogram of starch content of BT AND TB for green samples

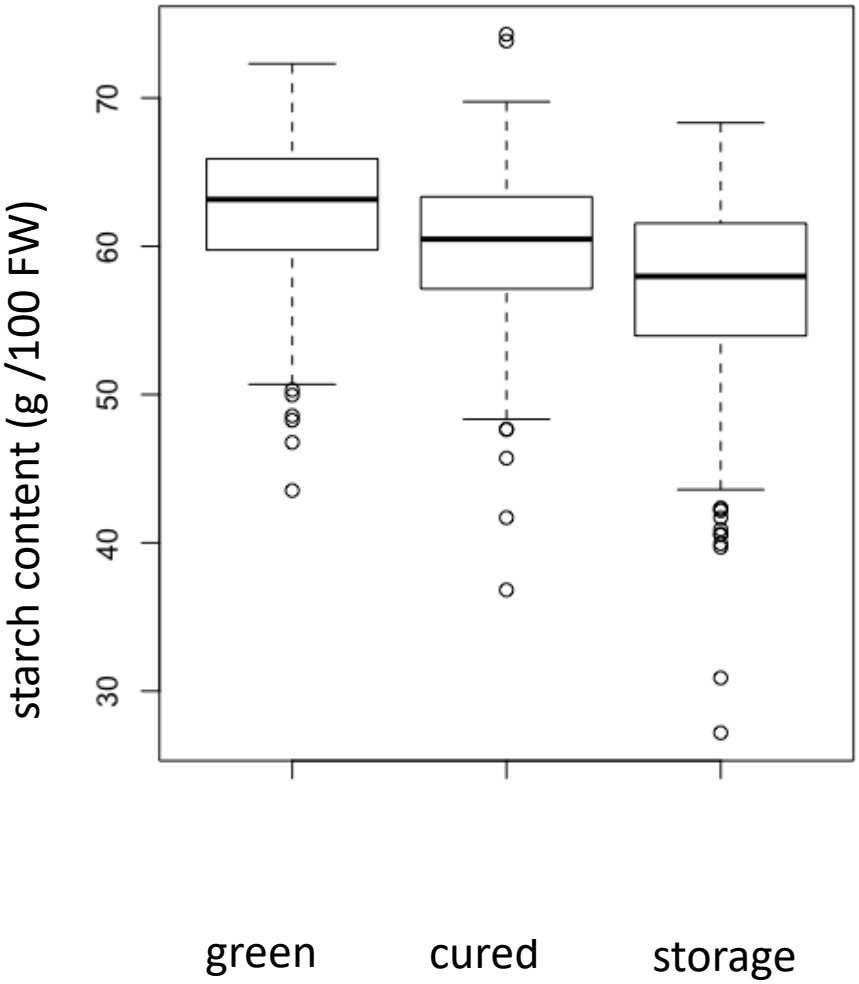
TB



BT



Distribution of starch content in green, cured and storage TB samples

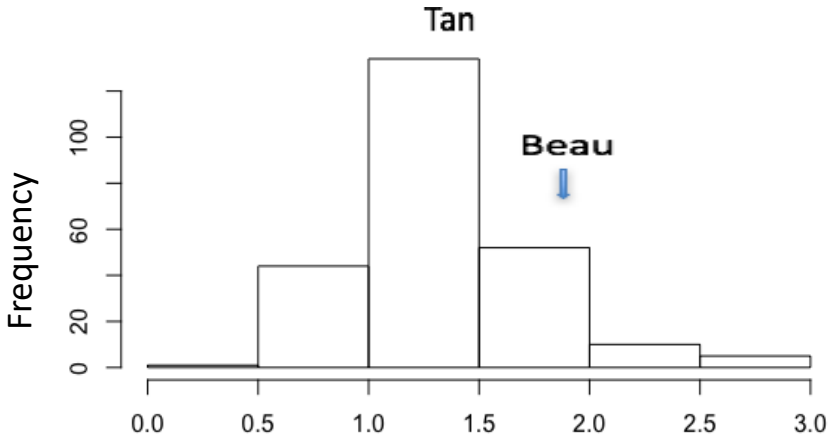


- Starch content decreased over time and with different sampling categories
- Population mean: Green samples > cured samples > storage samples
- There were some outliers which is an indication of transgressive segragrants in relation to starch content

Histogram of total sugars of green BT and TB samples

TB

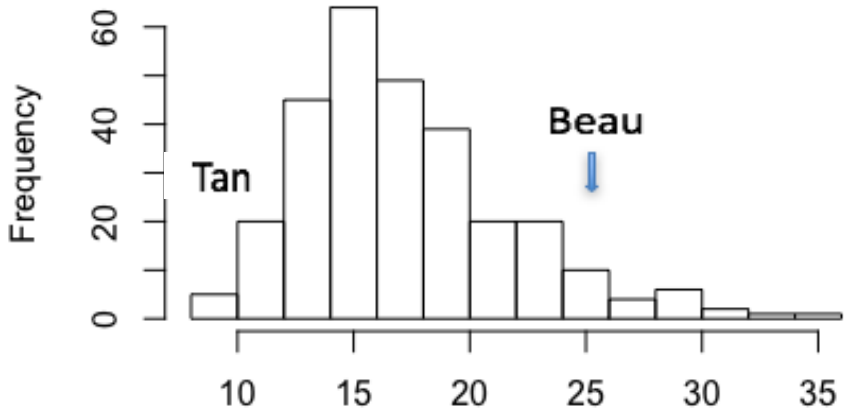
Mean= 1.31
SD= 0.41



Total sugar content (mg /g FW)

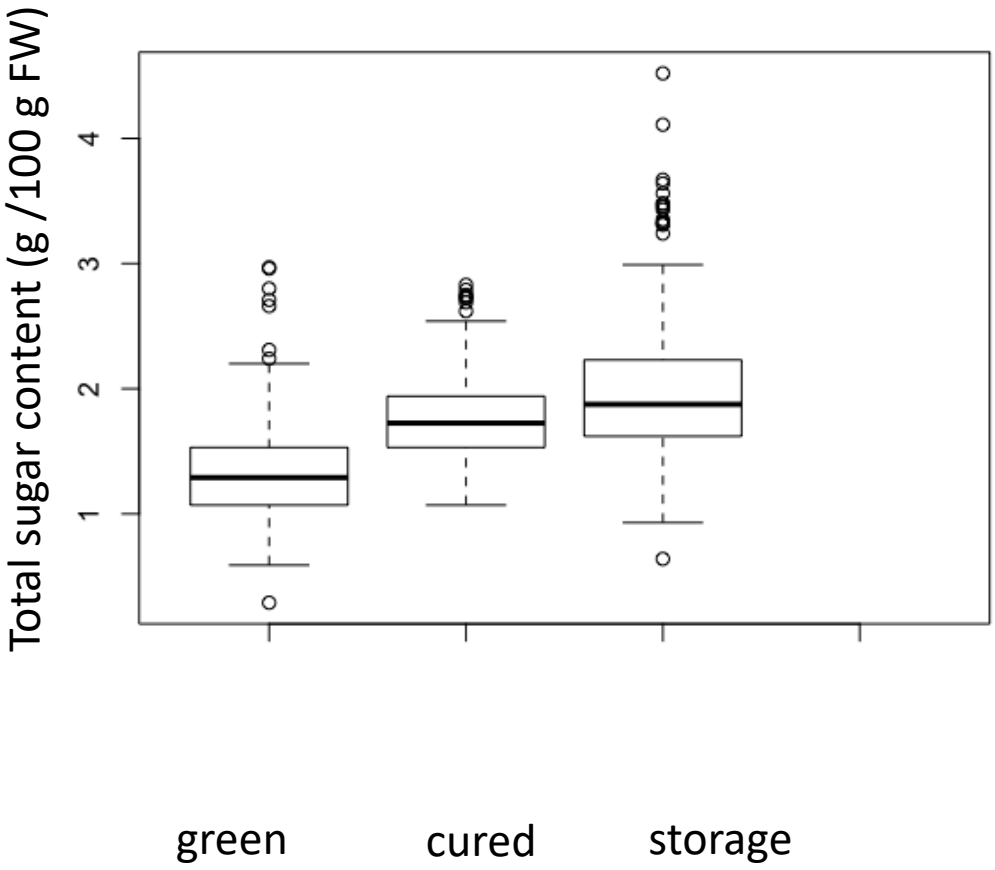
BT

Mean=17.184
SD= 4.6



Total sugar content (g /100g FW)

Distribution of total sugars in green, cured and storage in the TB samples

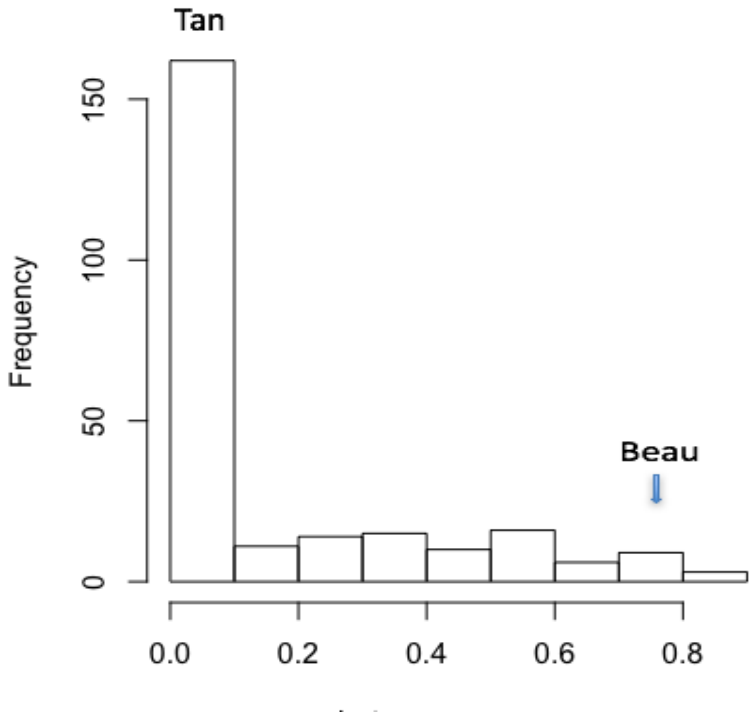


- Total sugar content population increased over time and with different sampling categories
- Population mean: Green samples < cured samples < storage samples
- There were some outliers which is an indication of transgressive segragrants in relation to high total sugars for different sampling categories

Histogram of beta carotene content of BT and TB green samples

TB

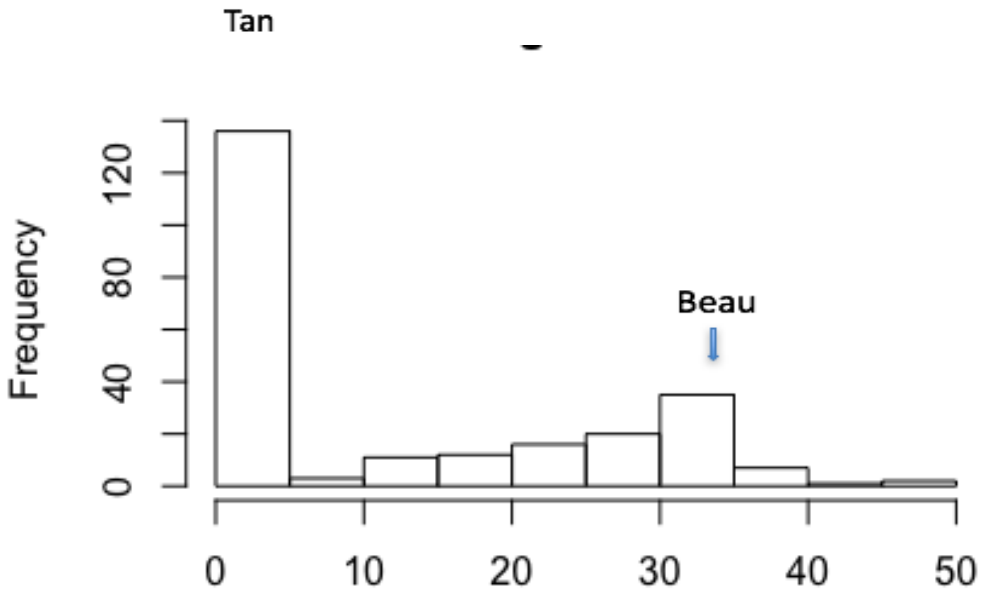
Mean= 0.15
SD= 0.24



Beta carotene content (mg /g)

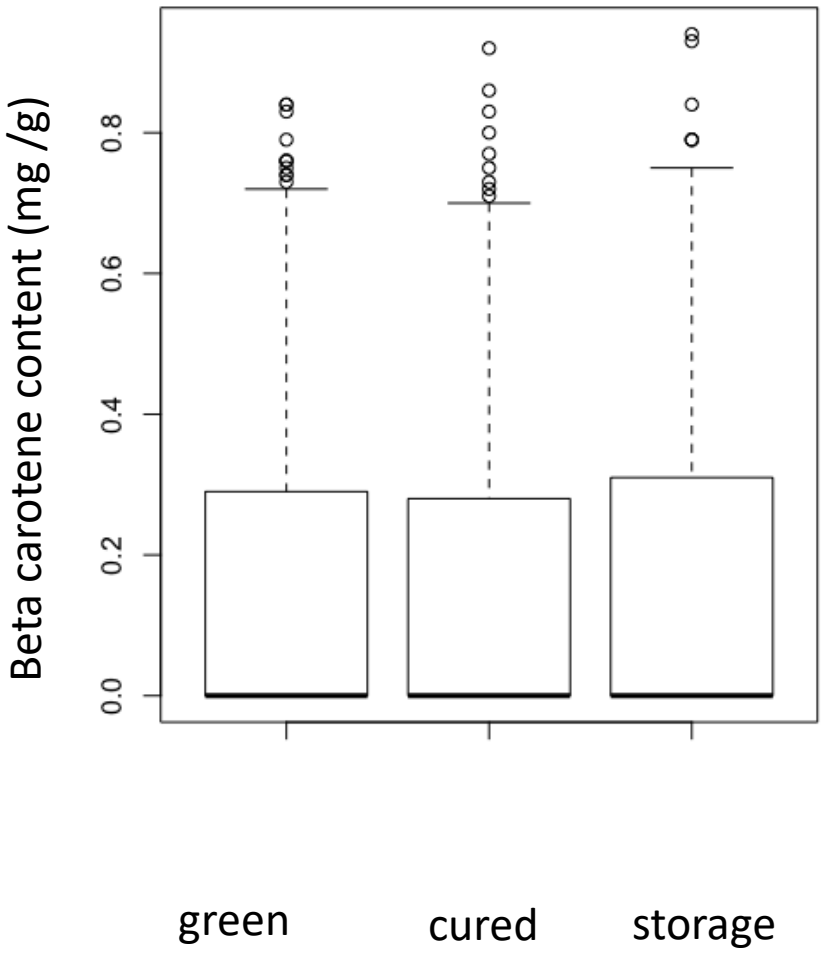
BT

Mean= 11.7
SD= 14.08



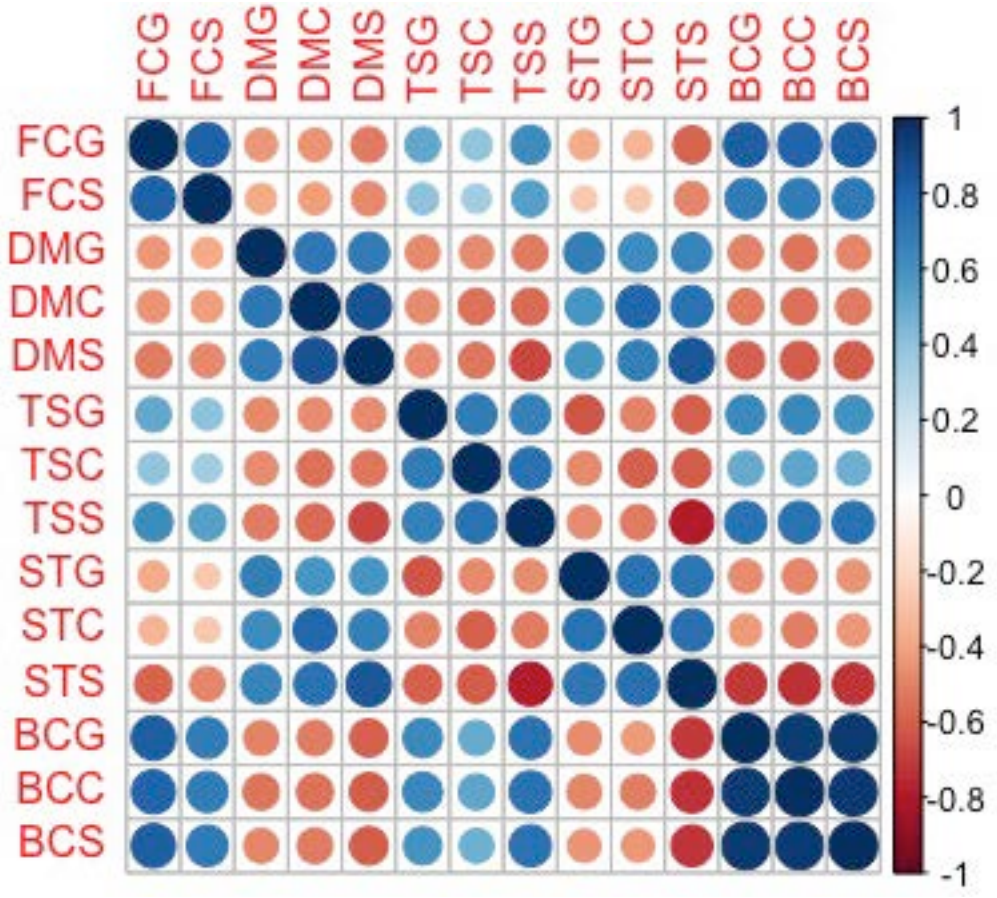
Beta carotene content (mg /100g)

Distribution of beta carotene content in TB samples



- Beta carotene content was stable over time and with different sampling categories
- Unlike starch and sugar contents, population mean: Green samples ~ cured samples ~storage samples
- There were some outliers which is an indication of transgressive segragrants in relation to high beta carotene content

Correlation matirx of traits for different sampling categories



- High positive moderate association between dry matter content and starch content for all sampling categories
- Beta carotene content is positively associated with with total sugars
- Association become stronger when samples are in storage for about 11 weeks
- There is a negative moderate association amongst sampling categories for beta carotene content and becomes stronger when samples are in storage

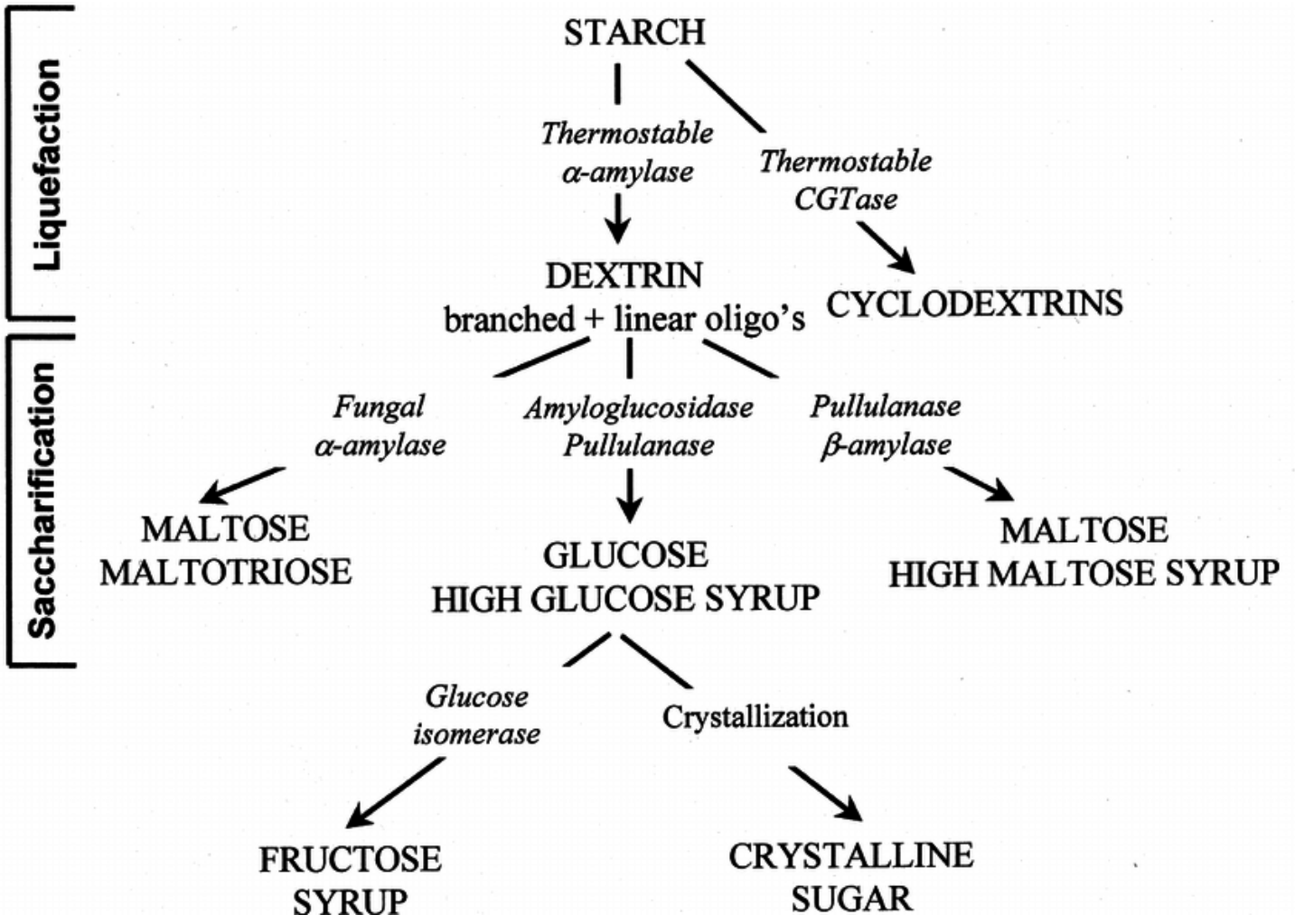
Evaluation of alpha and beta amylase activities

- The endogenous amylases have enormous effect on sweetpotato in storage and during processing because they facilitate the breakdown of starch

- Alpha amylase is known to exist in small quantities in sweetpotato whilst but has been reported to control significantly starch model systems.

- Beta amylase on the other has been reported to be abundant in sweetpotato storage root and greatly influence maltose formation

Biochemical pathway for maltose formation



https://www.researchgate.net/publication/11562967_Properties_and_applications_of_starch-converting_enzymes_of_the_-amylase_family/figures?lo=1

Previous research

- Sweetpotato germplasm can be separated into four classes based on initial sugar concentration and changes during cooking:

1. Low sugars/low starch hydrolysis
2. Low sugars/high starch hydrolysis
3. High sugars/low starch hydrolysis
4. High sugars/high starch hydrolysis

- It has been hypothesized that use of NON-SWEET, low flavor sweetpotatoes in breeding programs will promote a much broader range of sweetpotato flavors to be developed and can be used in more dishes.

- This will facilitate new uses and markets.

(Morrison et al., 1993)

Materials and methods
(Evaluation of alpha amylase activity)

From low throughput to medium throughput amylase assay

Test tube assay

- Bulky
- Time consuming
- More reagents and cost
- Less samples in a day (15)
- Not modern breeder friendly

MULTI-MODE PLATE READER ASSAY

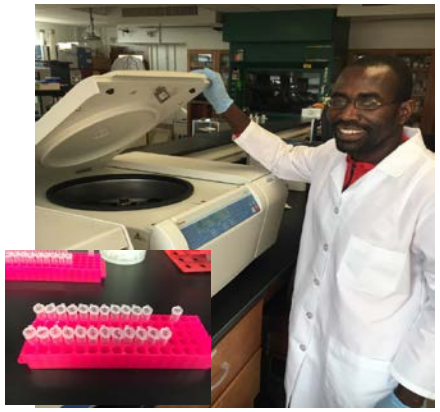
- NOTBULKY
- LESS TIME CONSUMING
- LESS REAGENTS AND COST
- MORE SAMPLES IN A DAY (120)
- MODERN BREEDER FRIENDLY

Alpha amylase enzyme extraction and microplate assay

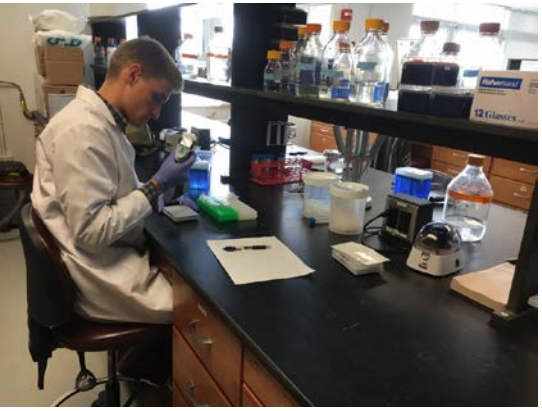
1. Weighing of freeze-dried samples

2. Incubation at 40 C after addition of extraction buffer

3. Centrifuging microfuge tubes containing enzyme extract at 1000 g for 10 minutes



4. Transfer of sweetpotato enzyme extract into 96-well cereal extract plate



5. Making reaction of mixture of enzyme extract and Amylase HR reagent substrate

6. Incubation at 40 C and stopping the reaction after 20 minutes

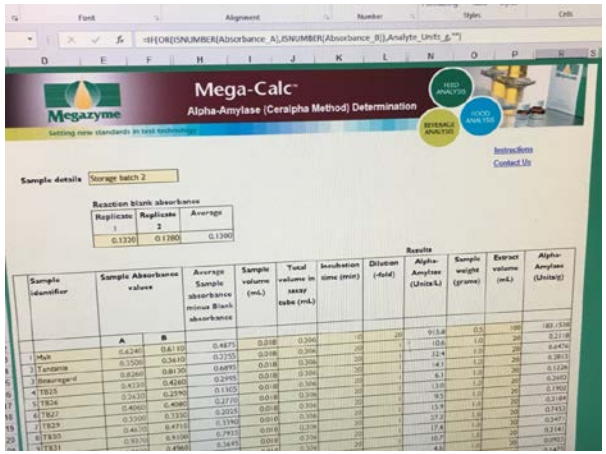
Alpha amylase enzyme activity calculated and data analysis

CALCULATION OF ACTIVITY:

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable α-glucosidase, required to release one micromole of *p*-nitrophenol from BPNPG7 in one minute under the defined assay conditions, and is termed a **Ceralpha Unit.**

Units/g Flour:

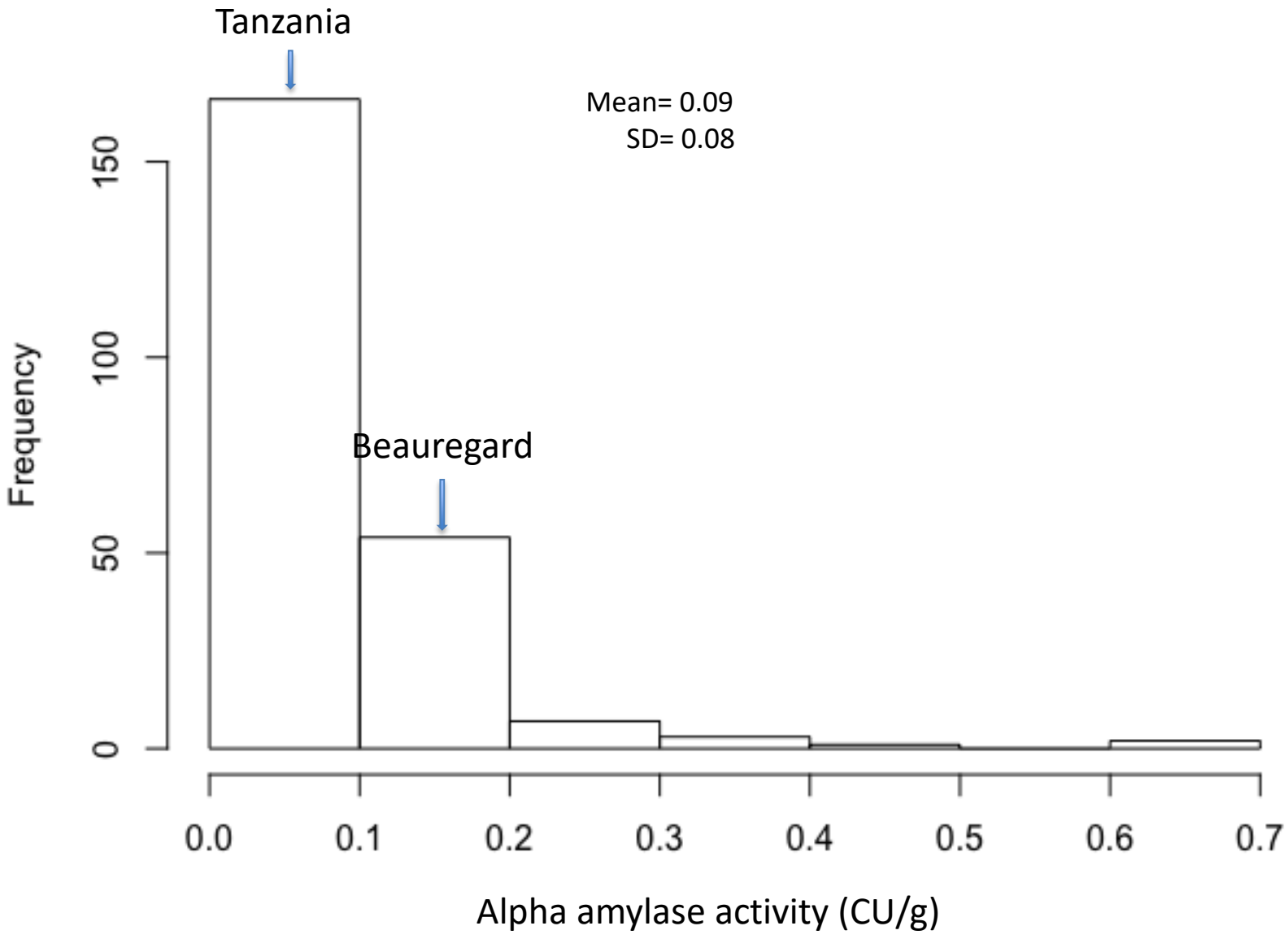
$$= \frac{\Delta E_{400}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{\varepsilon_{mM}} \times \frac{\text{Extraction Vol.}}{\text{Sample Weight}} \times \text{Dilution}$$



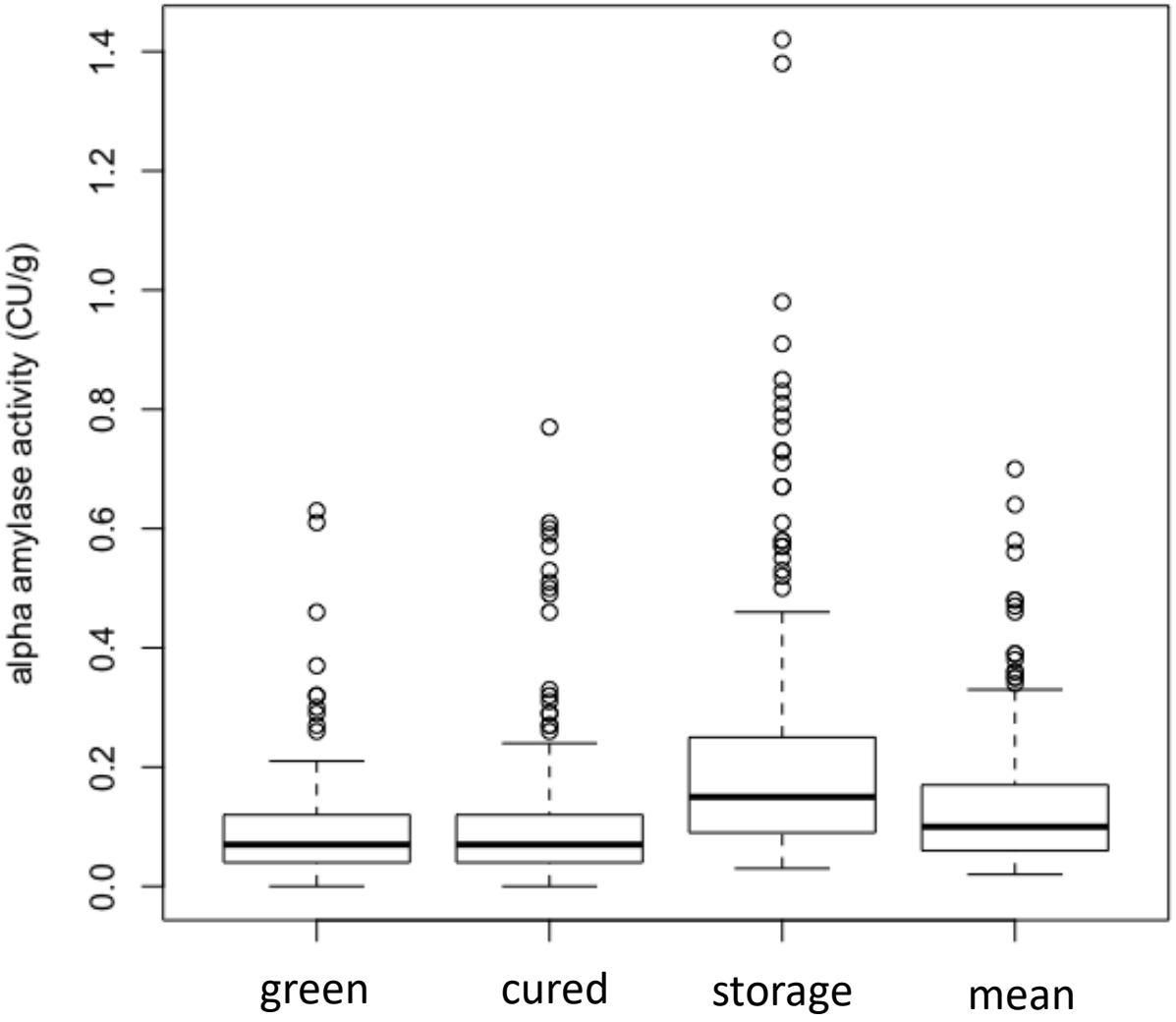
- Alpha amylase enzyme activity data was calculated for 2016 trial samples
- Boxplots and histogram were drawn using R version 3.4.1
- Simple correlations between traits were done using the corrplot package in r

Results
(Evaluation of alpha amylase activity)

Histogram of alpha amylase activity of TB green samples



Distribution of alpha amylase activity in raw, cured and storage TB samples



- Alpha amylase activity were stable over time and for green and cured samples and increased in storage
- Population mean: Green samples ~ cured samples < storage samples
- There were some outliers which is an indication of transgressive segragrants in relation to alpha amylase activity

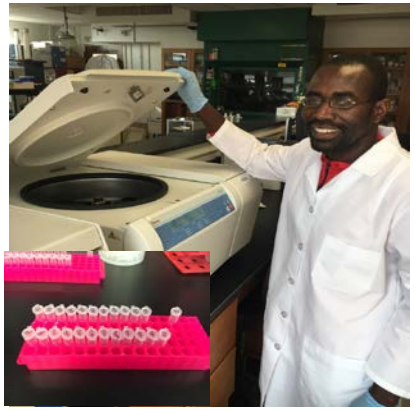
Materials and methods
(Evaluation of beta amylase activity)

Beta amylase enzyme extraction and microplate assay

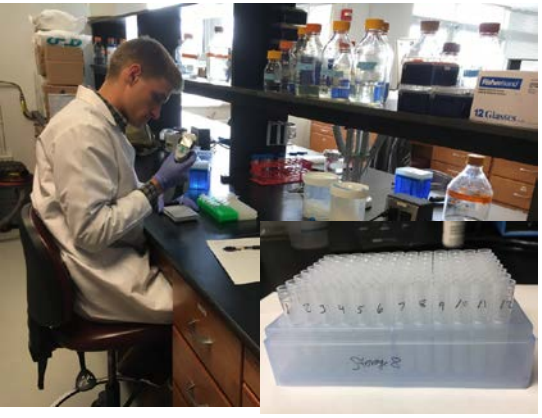
1. Weighing of freeze-dried samples

2. Incubation at 23 C for 1 hr and vortex at every 15 mins interval

3. Centrifuging microfuge tubes containing enzyme extract at 2000 g for 10 minutes



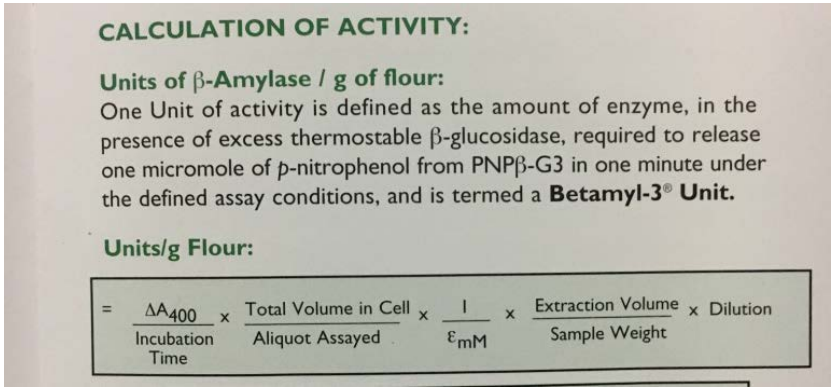
4. Dilution and transfer of sweetpotato enzyme extract into 96-well cereal extract plate



5. Making reaction of mixture of enzyme extract and Amylase HR reagent substrate

6. Incubation at 40 C and stopping the reaction at 10 minutes

Beta amylase enzyme activity calculated and data analysis



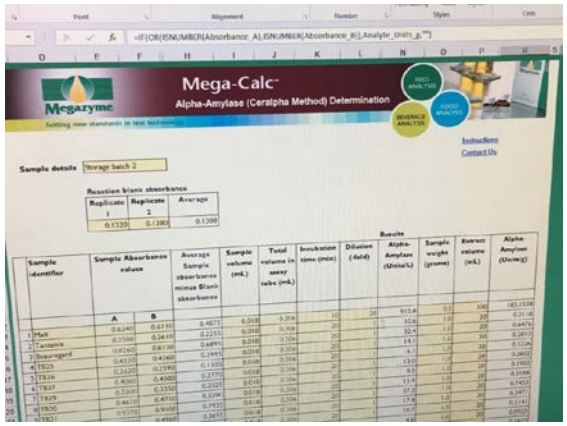
CALCULATION OF ACTIVITY:

Units of β-Amylase / g of flour:

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable β-glucosidase, required to release one micromole of *p*-nitrophenol from PNPβ-G3 in one minute under the defined assay conditions, and is termed a **Betamyl-3® Unit.**

Units/g Flour:

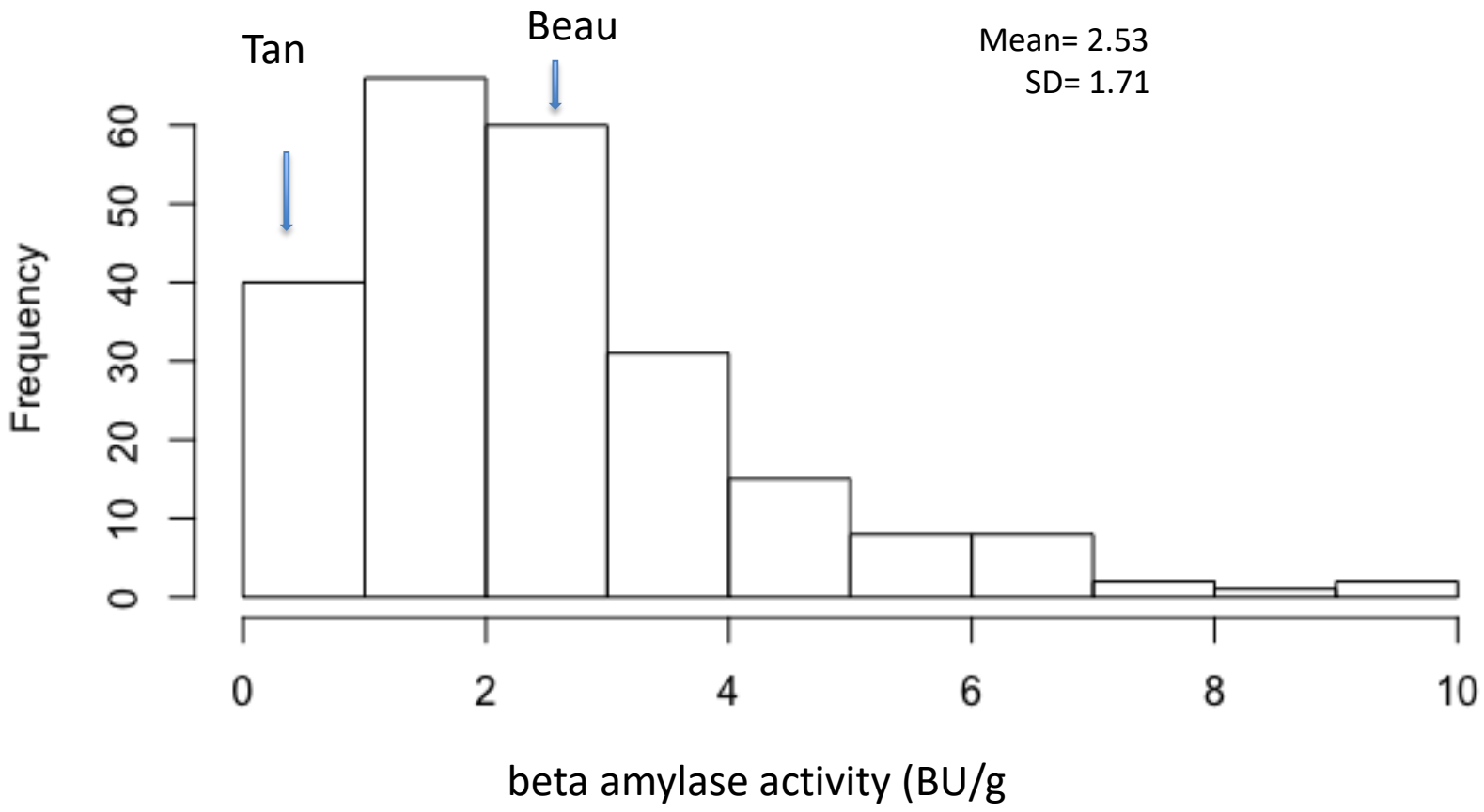
$$= \frac{\Delta A_{400}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{\varepsilon_{mM}} \times \frac{\text{Extraction Volume}}{\text{Sample Weight}} \times \text{Dilution}$$



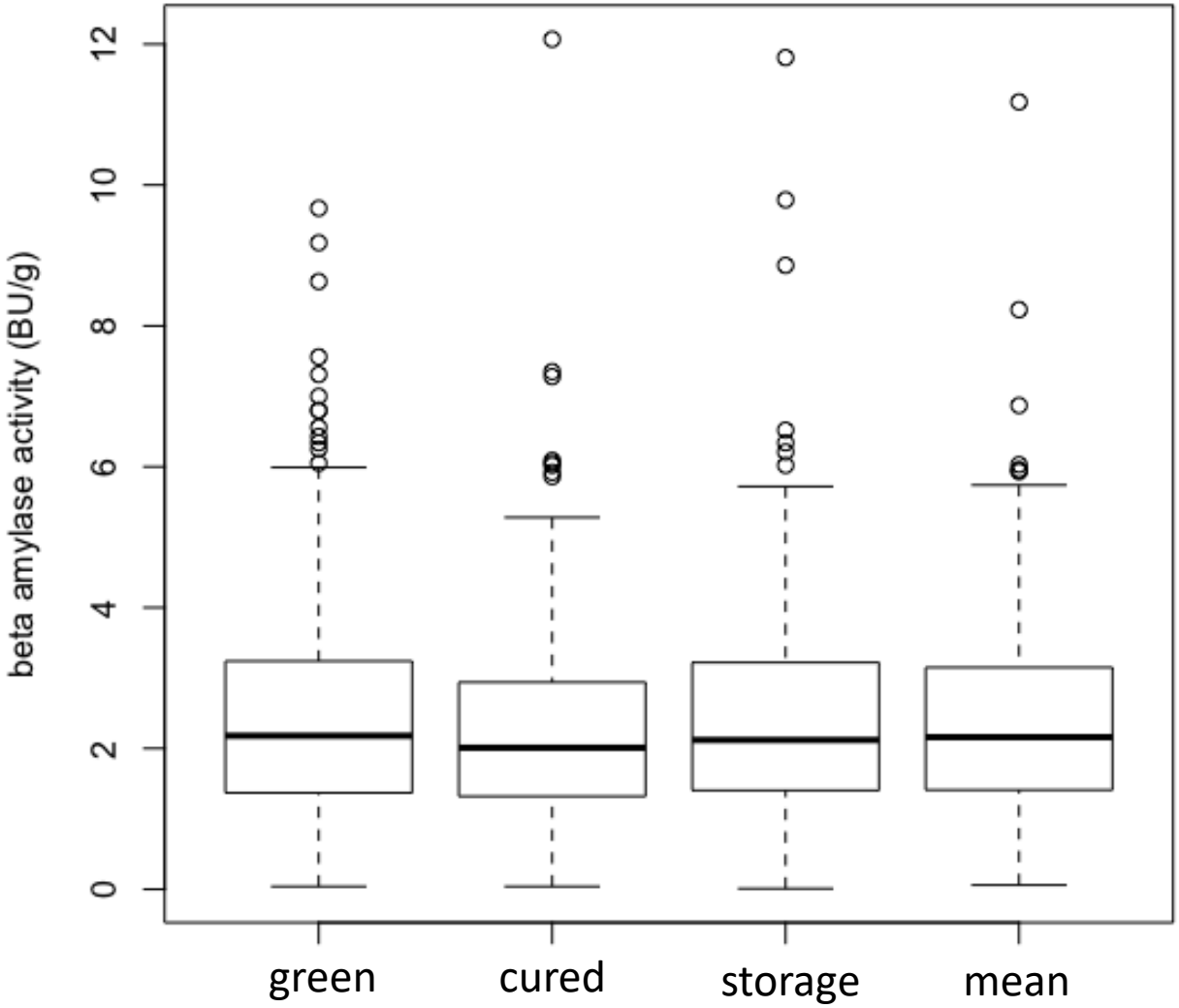
- Beta amylase enzyme activity data was calculated for 2016 trial samples
- Boxplots and histogram were drawn using R version 3.4.1
- Simple correlations between traits were done using the corrplot package in r

Results
(Evaluation of beta amylase activity)

Histogram of beta amylase activity of TB green samples

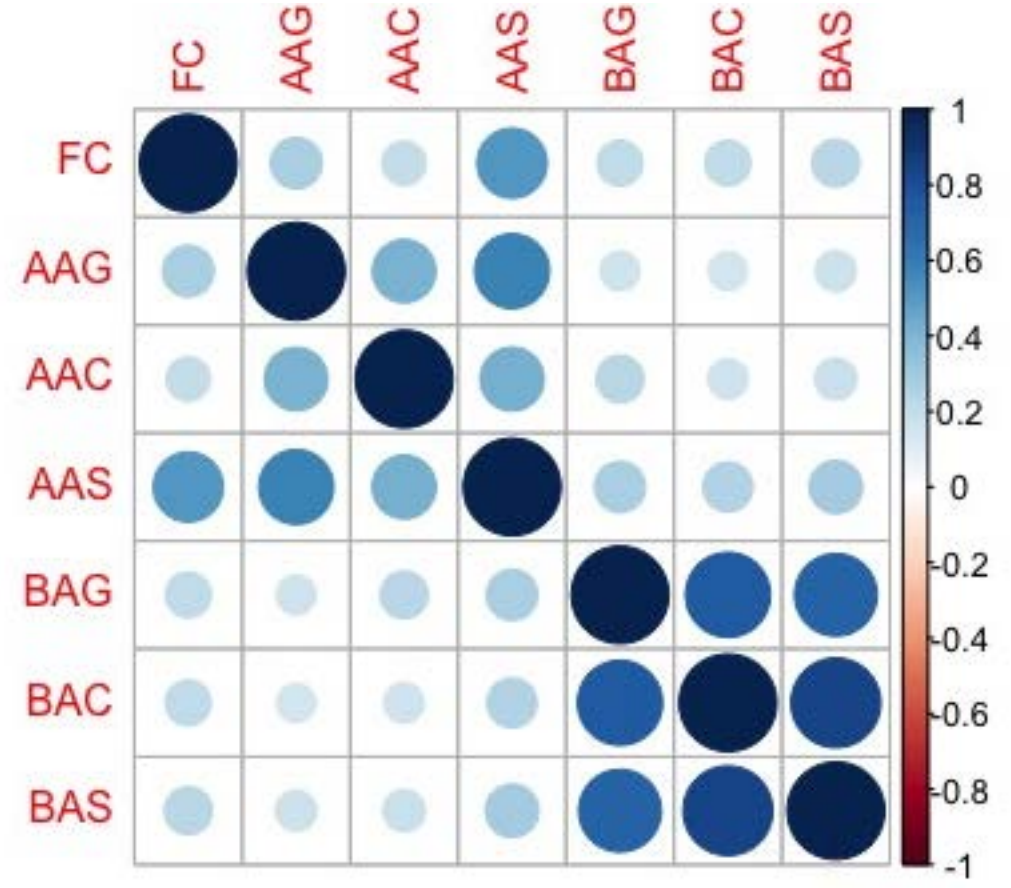


Distribution of beta amylase activity in raw, cure, green and their mean in the TB mapping population



- Beta amylase activity was stable over time and for green, cured and storage samples
- Population mean: Green samples ~ cured samples ~ storage samples
- There were some outliers which is an indication of transgressive segragrants in relation to beta amylase activity

Correlation matrix of different sampling categories and flesh color



- There was weak positive correlation between alpha and beta amylase activities for all sampling categories
- Different sampling categories for alpha amylase were moderately positively correlated
- Different categories for beta amylase were strongly positively correlated

Evaluation of cooked sugars and correlation of maltose amylase activity

- The hydrolysis of starch to maltose catalyzed by beta amylase confers to cooked roots sweetness characteristics (Morrison et al., 1993).

- Sugars that exist in the storage roots of sweetpotatoes are fructose, glucose, sucrose and maltose. Maltose is hardly detected in raw storage roots.

- Differences in varietal differences in maltose content in heated storage roots have been reported by some researchers

Materials and methods
(Evaluation of cooked sugars)

Evaluation of cooked sugars and correlation of maltose amylase activity

- The hydrolysis of starch to maltose catalyzed by beta amylase confers to cooked roots sweetness characteristics (Morrison et al., 1993)

- Sugars that exist in the storage roots of sweetpotatoes are fructose, glucose, sucrose and maltose. Maltose is hardly detected in raw storage roots.

- Differences in varietal differences in maltose content in heated storage roots have been reported by some researchers

Baking of sweetpotatoes and sugar extraction

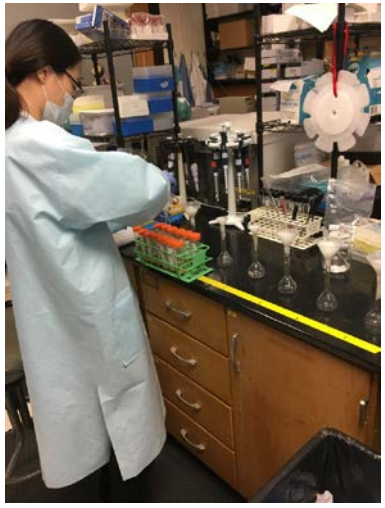
1. Pricked sweetpotato roots wrapped in aluminum foil

2. Baking in an oven at 204 C for 90 minutes

3. Baked sample cut into two longitudinal sections

4. Scooping, sampling into zip-lock bags and weighing

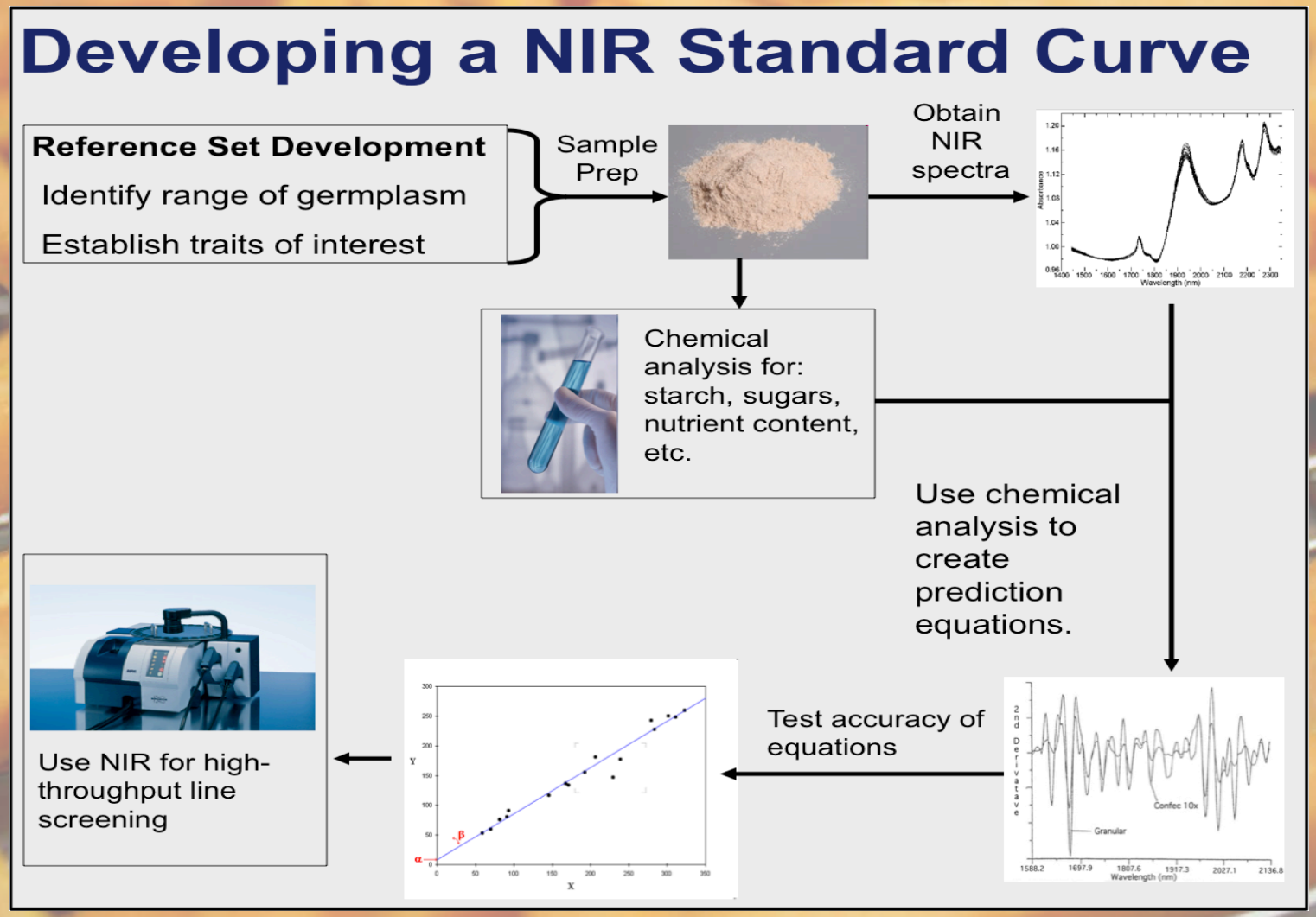
5. Sugar extraction using absolute ethanol



6. HPLC

High-throughput Chemical Analysis in Sweetpotato

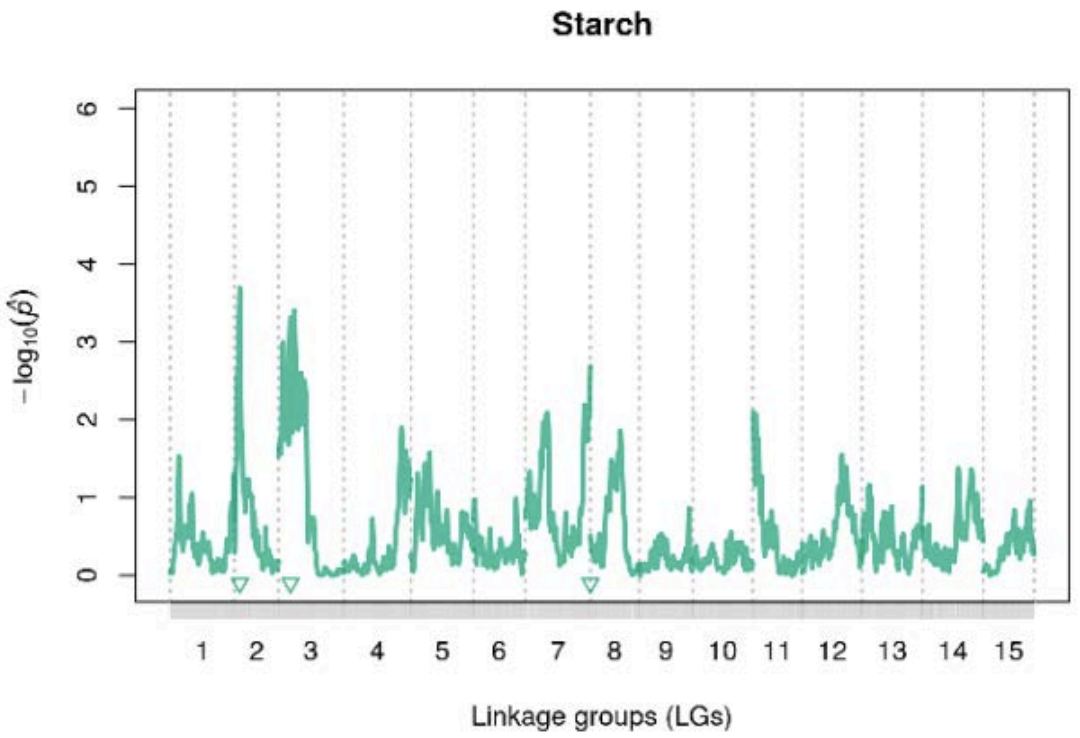
Use of Near Infrared Reflectance Spectroscopy (NIR) to predict amylase activity and cooked sugars



QTL Analysis for nutrition and Quality traits

- The overall goal will be detecting associations between the already measured nutrition and quality traits using a model.

- Identify molecular mechanisms underlying these traits



Summary

- Green samples for BT and TB showed similar phenotypic distribution
- Total sugar content and starch content changed for different sampling categories
- Dry matter content and beta carotene content remained stable over time and with different sampling categories
- Alpha amylase activity at 11 weeks of storage in the TB was higher than at harvest whilst beta amylase remained stable
- Sweetpotato genotypes in the TB have low alpha amylase activity compared with beta amylase
- The TB mapping population has transgressive segregants which could be selected as parents to be used for improvement of quality traits

THANK YOU