Genetic diversity and association mapping for brix in Brazilian Panel of Sugarcane Genotypes (BPSG)

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Introduction

Molecular markers may be helpful for identification of marker-trait associations.

Two approaches can be used: bi-parental or association mapping.

The goals of this study were to:

i) evaluate the phenotypic variation on a panel of 258 sugarcane accessions, and

ii) estimate the genetic diversity and population structure among a collection of 258 sugarcane germplasm accessions, and

iii) identify SSR markers associated with brix in sugarcane

Plant material

Brazilian Panel of Sugarcane Genotypes (BPSG)

Full Panel : 258 accessions

Criteria

Saccharum species

Wild species

most cultivated varieties in Brazil

genotypes using bi- parental mapping programs

genotypes from breeding stations around the world

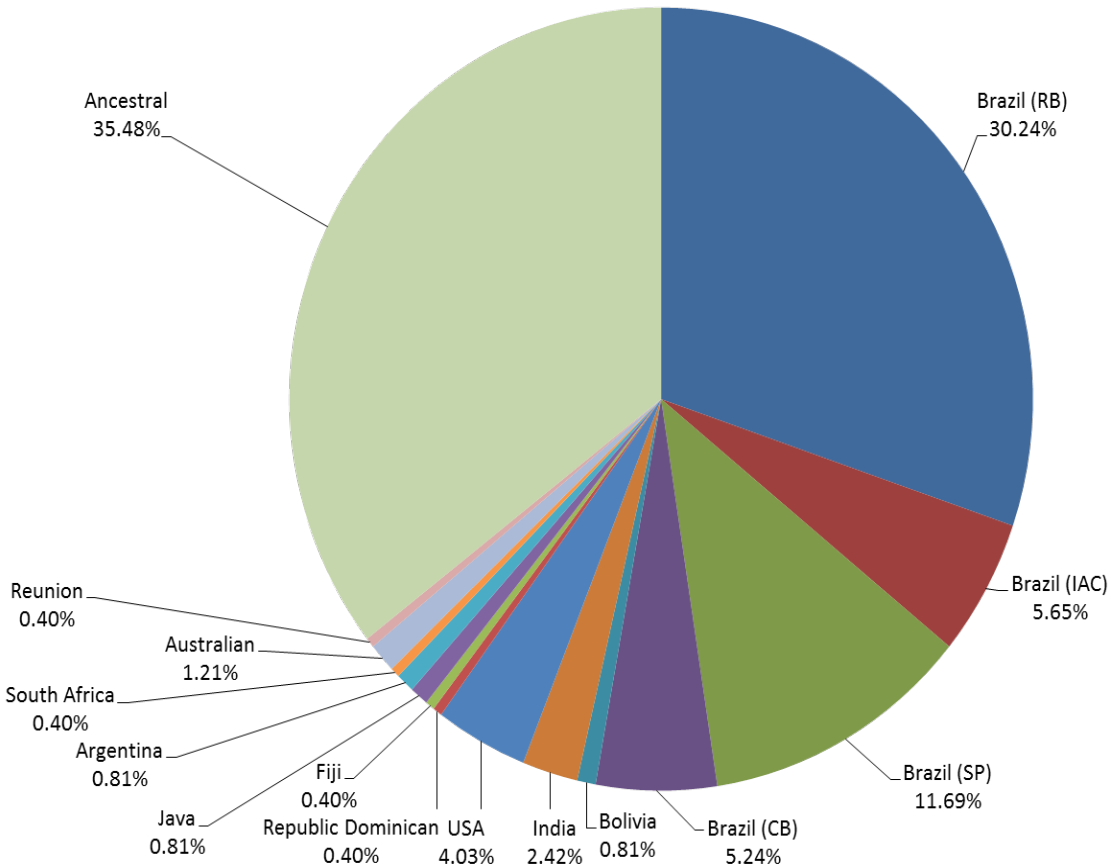


Figure 1. Pie chart of the population of accession within the diversity panel

Field trials and phenotyping

The field trial was set in a randomized complete block design with four replications in March 2013 at UFSCAR/Araras – SP

The elementary plot was composed of two rows three meters in length, with 0.5 meter inter-row spacing

The panel was evaluated for 10 traits:

1- Stalk diameter (SD, in mm)
2- Stalks number (SN)
3- Stalk height (SH, in cm)
4- Stalk weight (SW)
5- Fiber (%FIB)

BIOMASS

6- Soluble solid content (°BRIX)
7- Sucrose content of Juice (POL %J)
8- Sucrose content of cane (POL %C)
9 - Purity (PUR)

SUGAR

10- Brown rust severity (a fungal disease caused by Puccinia melanocephala)

DISEASE

Genotyping – DNA extraction

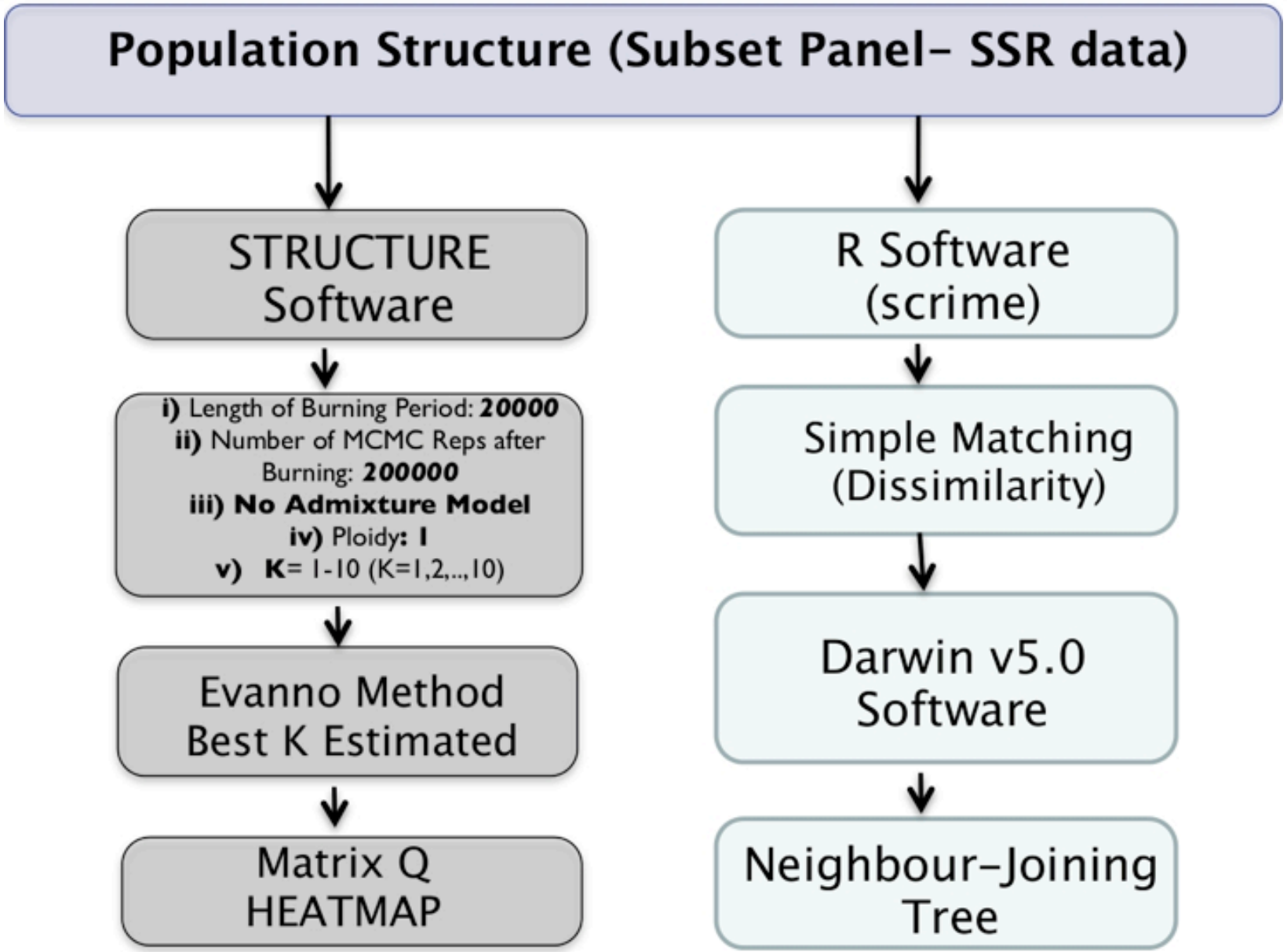


Figure 2. DNA extraction steps using the Al-Janabi et al. method (1999)

Genotyping – SSR analysis

- Subset Panel – 134 Genotypes
- SSR
  - 100 microsatellites primers
  - 84 EST-SSR (Pinto et al., 2004, 2006; Oliveira et al., 2009; Marconi et al., 2011).
  - 16 genomic-SSR (Cordeiro et al., 2000; Raboin et al., 2006).
  - Visualization of amplified products by polyacrylamide gel (6%).
  - Staining with silver nitrate (Creste et al., 2001).

Population structure analysis



Association Analysis

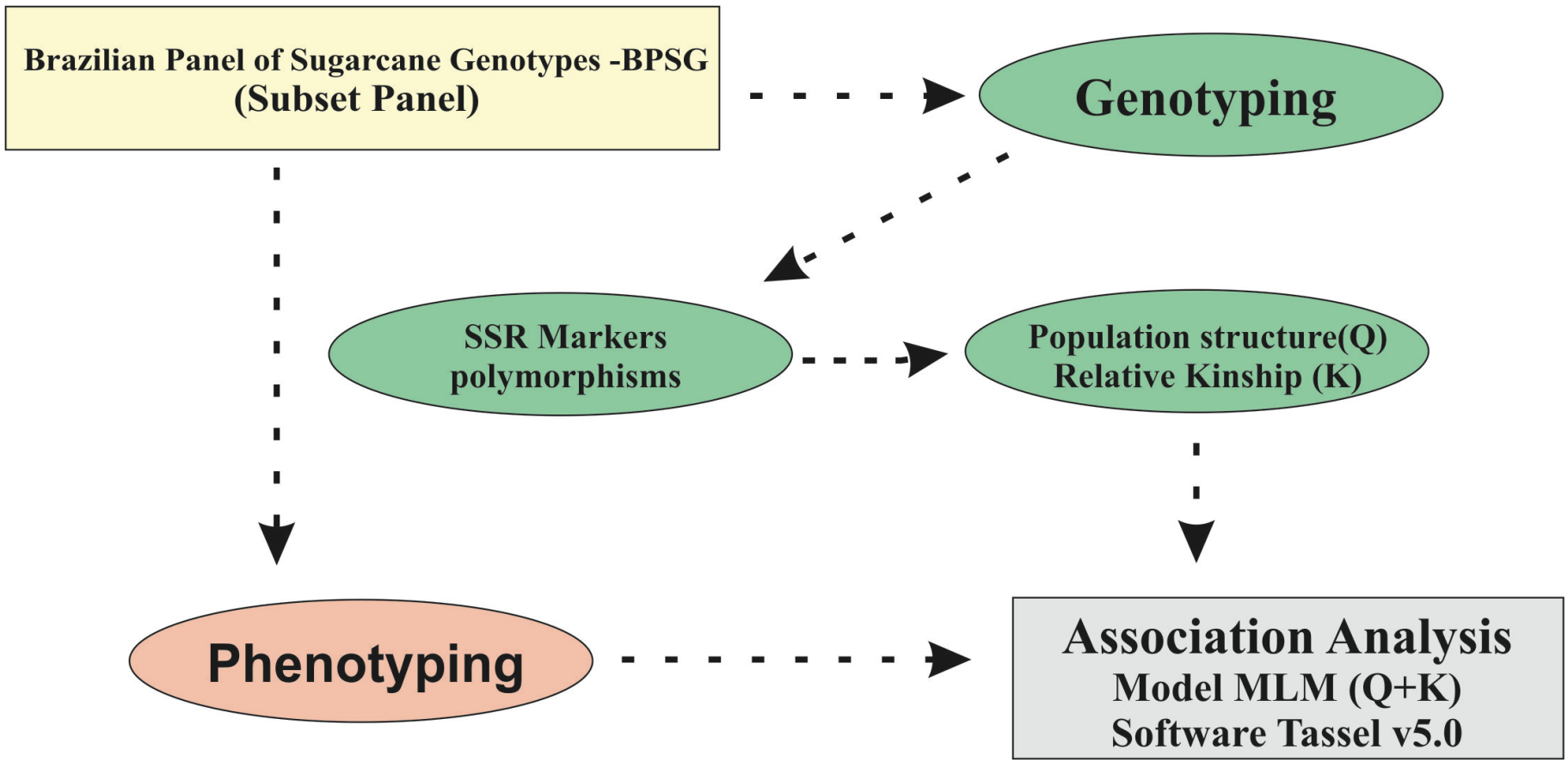


Figure 3. Data pipeline for association analysis between SSR markers and brix

Results

The Linear Mixed Model was obtained for SD, SH, SN, SW, BRIX, POL%C, POL%J, PUR, FIB

$$Y_{ij} = \mu + b_j + g_i + e_{ij}$$

Y_{ij}= phenotype treatment i in the repetition j;
μ = Intercept;
b_j = effect for block (fixed);
g_i = effect for the genotypes (random);
e_{ij}= error term.

Use of covariables (C_{ij}) to model adjustment

-**Adjusted means:**

- Genotypic Correlations
- Principal Component Analysis (PCA)

Results

Table 1. Phenotypic variation of traits analyzed in the full panel (* plant cane).

Trait*	Mean	Min	Max	CV(%)	H^2
Stalk number (SN)	90.8	8.0	438.0	22.9	0.87
Stalk weight (SW, in kg)	130.4	3.0	404.1	24.7	0.70
Stalk height (SH, in m)	2.5	1.0	3.8	10.1	0.66
Stalk diameter (SD, in mm)	26.6	9.9	44.3	7.4	0.79
Soluble solid content(°BRIX)	16.8	8.0	22.9	8.3	0.73
Sucrose content of juice (POL %J)	15.0	2.4	21.6	9.8	0.81
Sucrose content of cane (POL %C)	13.5	1.5	18.6	10.4	0.83
Purity (PUR)	82.2	24.7	102.6	5.2	0.84
Fiber(%)	12.6	4.4	31.9	11.8	0.86

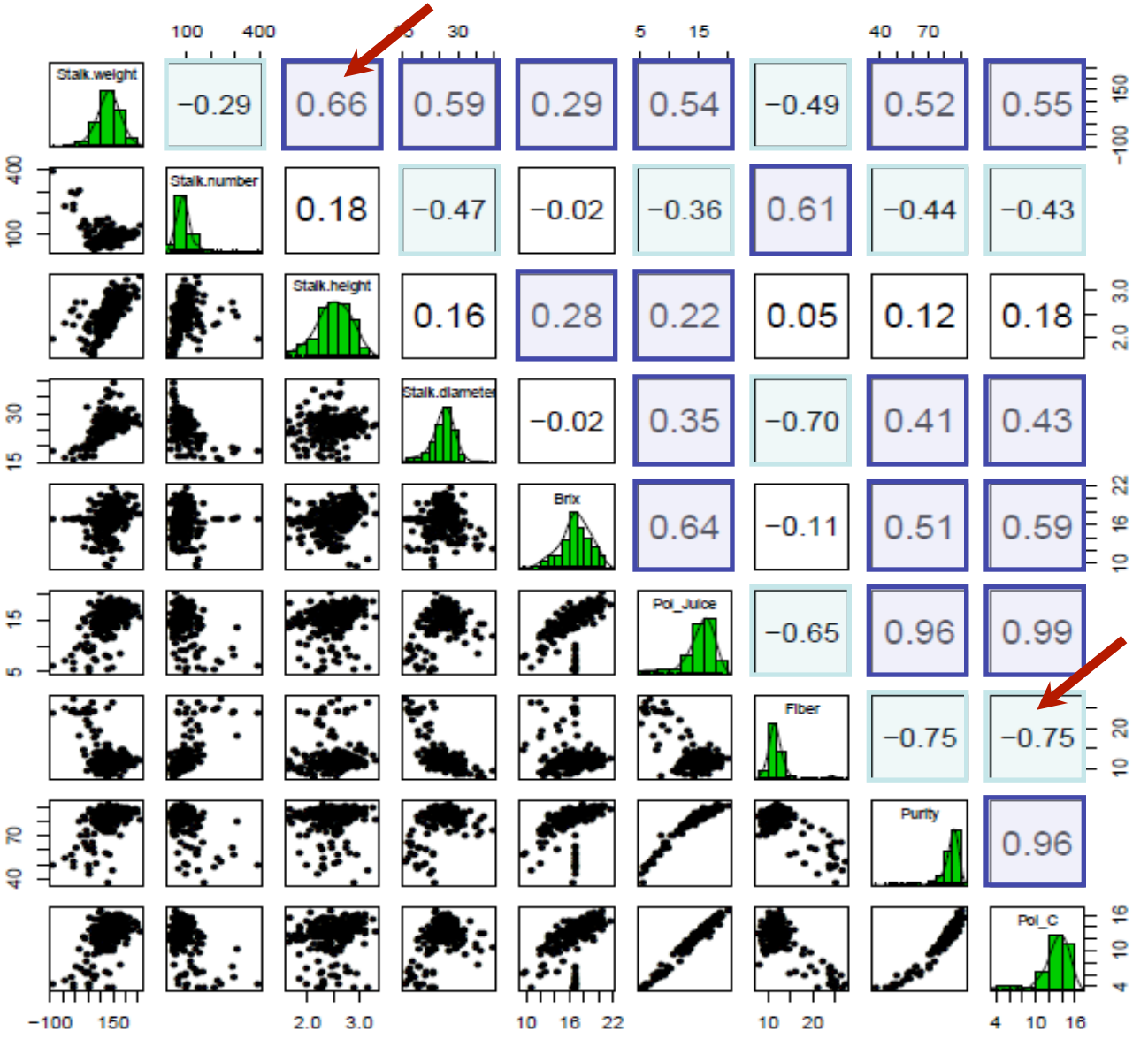
The minimum and maximum values showed a large phenotypic variation of the panel, which was expected.

For all traits, broad sense heritabilities (H^2) were moderate to high, ranging from 0.66 for Stalk height to 0.87 for stalk number.

These Brix data were used in the association analysis

Results

Figure 4. Estimates of the genotypic correlation for agronomic traits in sugarcane for plant cane (Araras-Brazil). Highlighted the significant negative correlations (green) and positive (blue)



28 genotypic correlations were considered significant (p< 0.01)

10 negative correlations (fiber x pol cane)

18 positive correlations (height x weight stalks)

Results

Green – Ancestral

Blue – Cultivars

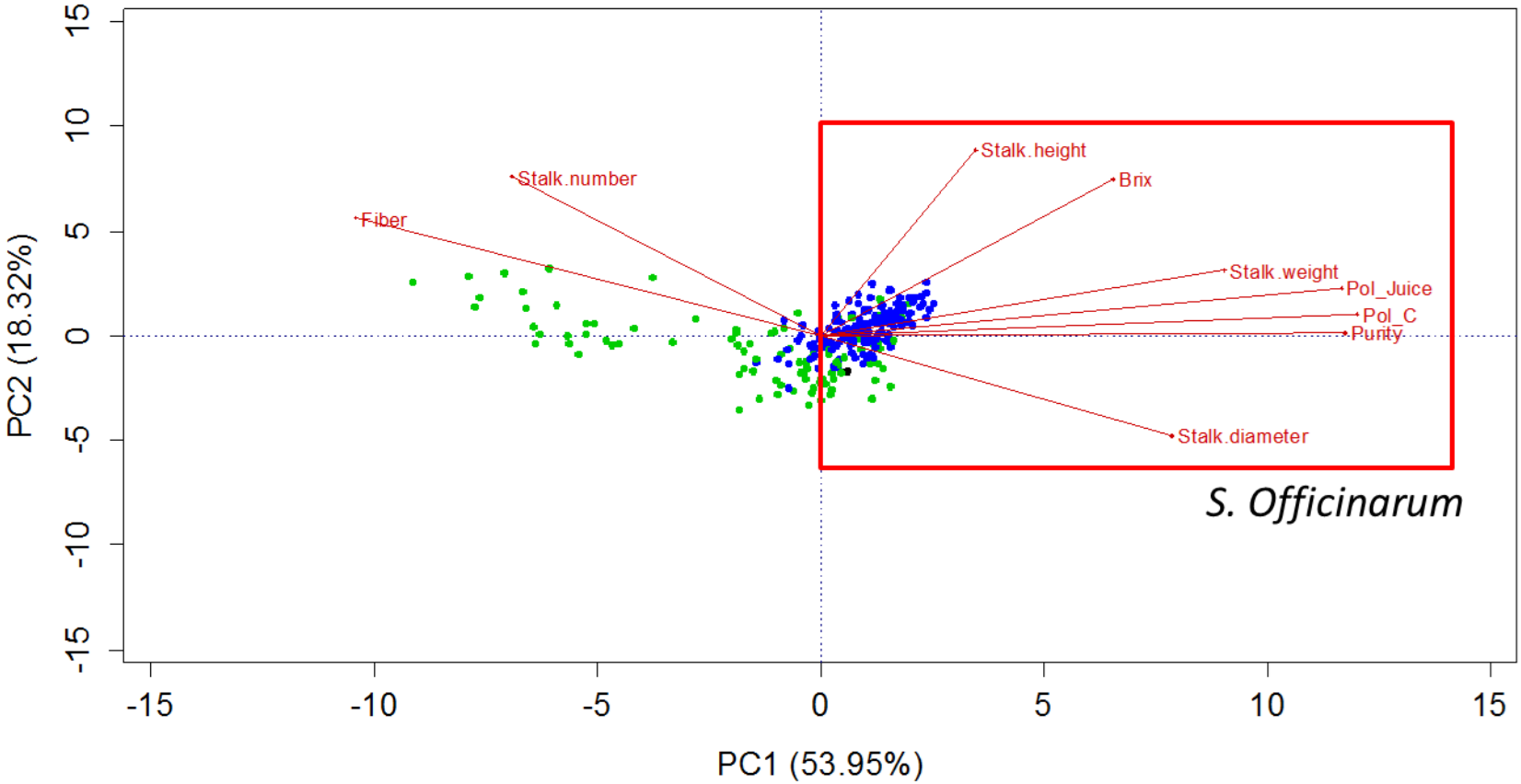


Figure 5. Bi-plot of the first and second components (PC1 and PC2) of the principal coordinate analysis on the adjusted means matrix obtained for BPSG accessions using agronomics traits

Results

Subset Panel

SSR markers – 1,476 polymorphic fragments

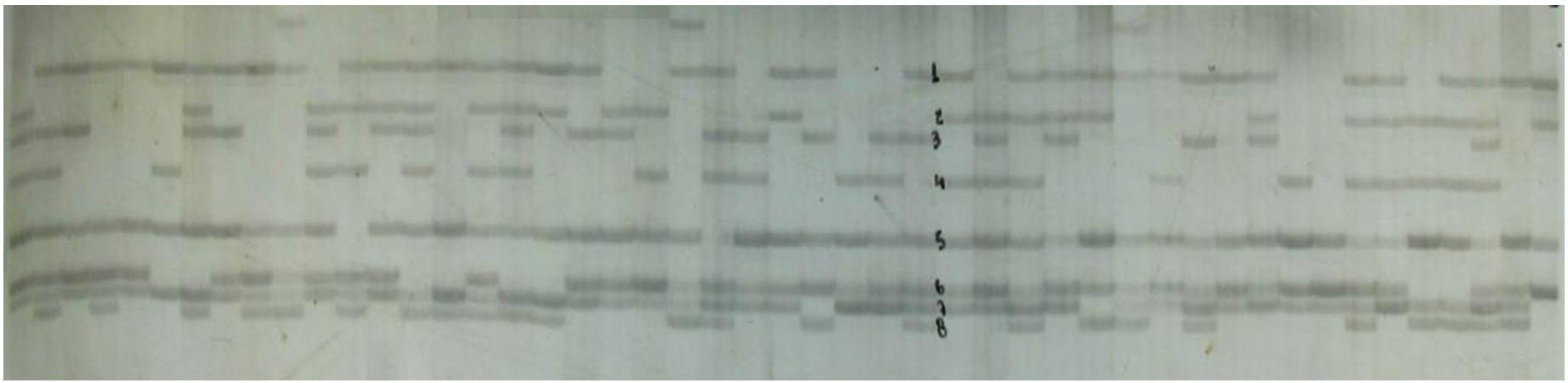
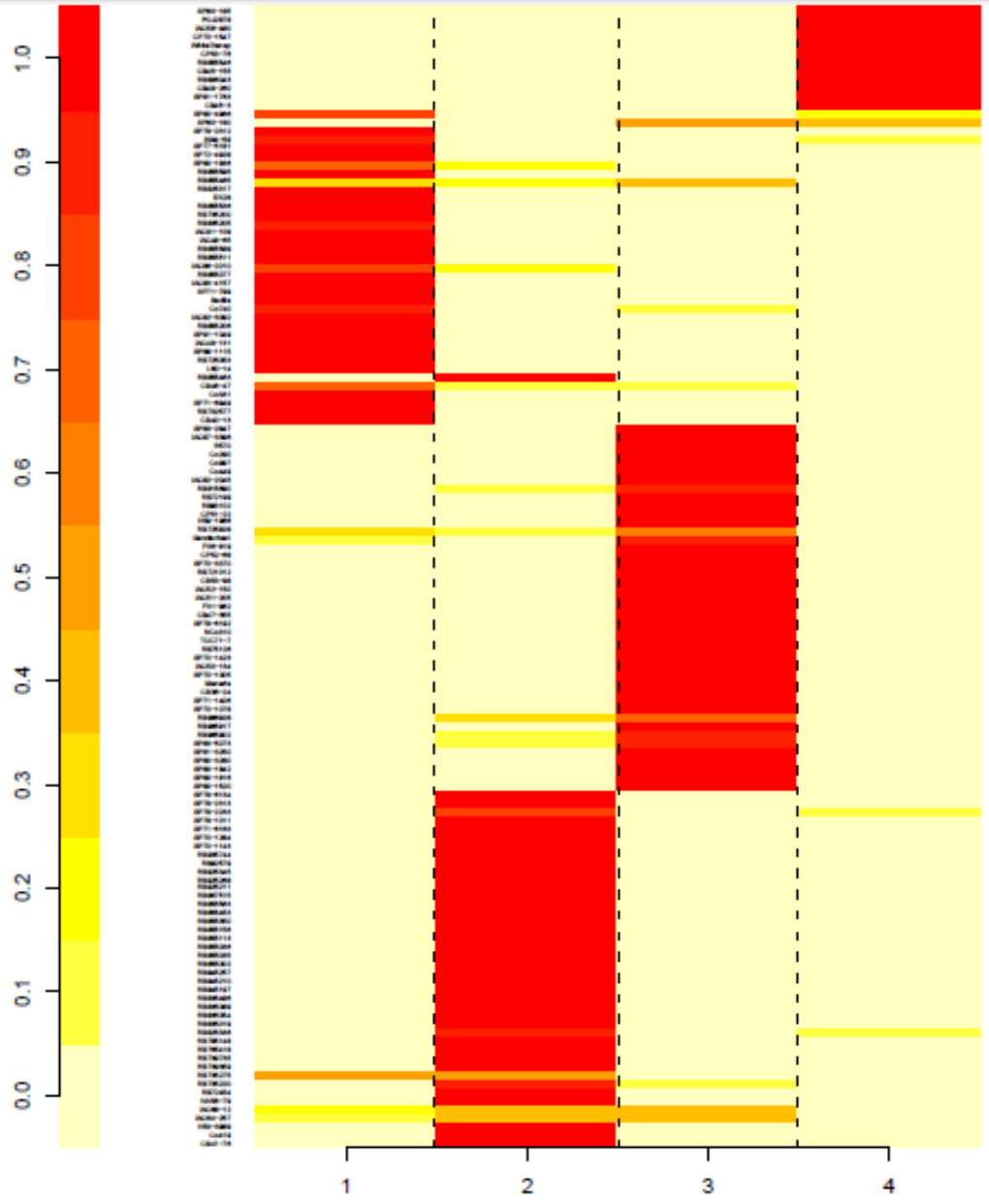


Figure 6 - DNA amplified products for the SSR marker (SCB 190).

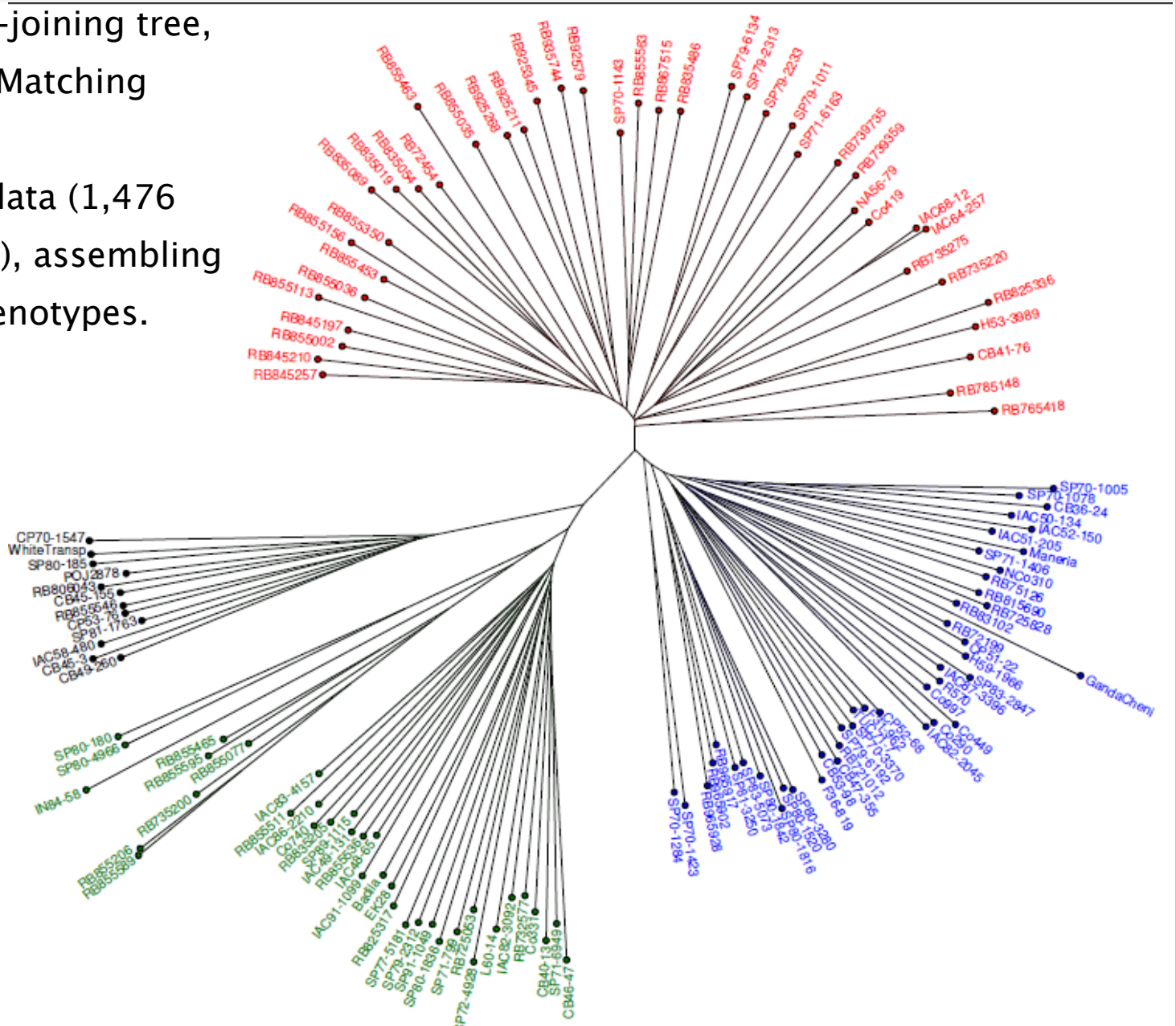
Results

Figure 7. Plot of 134 individuals BPSG (y-axis) and 4 different subpopulations (x-axis) obtained by probabilistic method implemented in STRUCTURE .



Results

Figure 8. Neighbour-joining tree, based on the Simple Matching dissimilarity index calculated from SSR data (1,476 polymorphic markers), assembling the 134 sugarcane genotypes.



Results

Table 2. Associations between SSR markers and brix in sugarcane using TASSEL software

Trait	Marker	p-value	marker R^2	Effect	Trait	Marker	p-value	marker R^2	Effect
Brix	M01	6.88E-08	0.23	13.61	Brix	M19	6.54E-08	0.24	13.61
Brix	M02-EST	8.72E-10	0.09	5.13	Brix	M20-EST	8.80E-08	0.23	13.49
Brix	M03-EST	8.22E-08	0.22	13.44	Brix	M21	9.51E-08	0.23	13.39
Brix	M04-EST	8.22E-08	0.22	13.44	Brix	M22	8.49E-08	0.23	13.43
Brix	M05-EST	8.22E-08	0.22	13.44	Brix	M23-EST	5.81E-08	0.24	13.67
Brix	M06	1.44E-12	0.12	2.38	Brix	M24-EST	2.31E-12	0.12	7.01
Brix	M07	1.44E-12	0.13	4.76	Brix	M25-EST	1.02E-10	0.23	13.38
Brix	M08	8.22E-08	0.22	13.44	Brix	M26-EST	1.02E-10	0.23	13.38
Brix	M09	8.22E-08	0.22	13.44	Brix	M27-EST	8.22E-08	0.22	13.44
Brix	M10-EST	2.5E-11	0.12	3.46	Brix	M28-EST	8.13E-10	0.14	-7.51
Brix	M11-EST	5.73E-11	0.10	18.32	Brix	M29-EST	7.97E-11	0.10	6.31
Brix	M12	6.2E-11	0.10	6.48	Brix	M30	8.22E-08	0.22	13.44
Brix	M13-EST	1.32E-09	0.23	13.22	Brix	M31-EST	9.68E-07	0.22	13.49
Brix	M14-EST	1.32E-09	0.23	13.22	Brix	M32-EST	1.57E-11	0.12	-17.42
Brix	M15-EST	1.32E-09	0.23	13.22	Brix	M33-EST	8.47E-11	0.12	2.46
Brix	M16-EST	5.12E-11	0.11	6.33	Brix	M34	3.1E-12	0.11	6.14
Brix	M17-EST	6.87E-08	0.22	1.36	Brix	M35	3.1E-12	0.11	6.14
Brix	M18-EST	1.92E-12	0.13	13.59	Brix	M36	8.52E-08	0.22	13.43

Genes involved in the cell wall

Genes involved in the sugar accumulation

Next steps

Phenotypic analysis in the ratoon crops (in progress)

Field assessments in other locations

Evaluation of the panel through Genotyping-by-Sequencing (GBS) (in progress): 15,163 SNPs (alignment with sorghum genome)

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