

Population diversity and GWAS of sweetpotato varieties in Burundi



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17th Sweetpotato SpeedBreeders' and Genomics Community of Practice Meeting Swiss Lenana Mount Hotel, Nairobi, Kenya, 07th June 2018

Background

- ❑ Sweetpotato belongs to the family Convolvulaceae, originated in central America.
- ❑ Grown in 100 countries and ranked 7th most important food crop after wheat, rice, maize, potato, barley and cassava in the world (FAOSTAT, 2007)
- ❑ Main sweetpotato producers in the world and in EAC.

Rank	Country	Production (MMT)	Country	Production (MMT)
1	China	71.1	Tanzania	34.5
2	Nigeria	38.3	Uganda	20.4
3	Tanzania	34.5	Kenya	12.3
4	Indonesia	23	Rwanda	9.3
5	Uganda	20.4	Burundi	5.8

Source: FAOSTAT, 2015

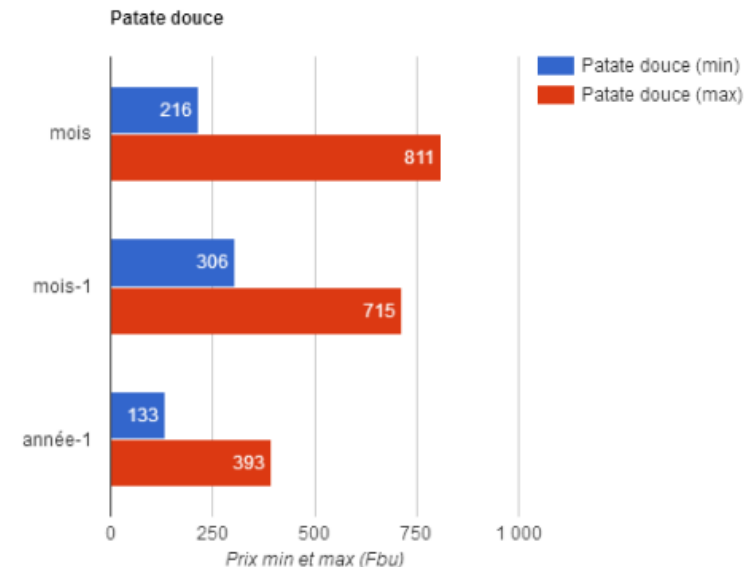
- ❑ Burundi annual per capita sweetpotato consumption is estimated at 102 kg and is ranked second in the world after Rwanda

Background cont.

- ❑ Sweetpotato is **source of protein**, dietary fiber and **vitamins** (β -carotene a precursor of vitamin A)
- ❑ It is used in household daily consumption and its absence/shortage caused food insecurity
- ❑ Provides 20% of calories taken by Burundians' populations
- ❑ Grown in all agroecological zones and is the 3rd important food crop after banana and cassava
- ❑ Matures early (4 months) in low land (700-1300 m) while it is harvested after 6 months in middle and high land (more than 1500 m).

Evolution of sweetpotato Area, Production and Yield in Burundi

Year	Area (ha)	Production (Tonnes)	Yield (T/ha)
2006	125000	837311	6.67
2007	131000	373663	6.67
2013	89333	839715	9.4
2014	66752	664217	10.06
2015	58620	580848	10
2016	127654	726048	5.6
Mean	114349.1	766003.9	7.43



Sweetpotato price in April 2017:

<http://www.sip.minagrie.gov.bi/web/home>

Statement of the problem

- ❑ sweetpotato production and seed system are constrained by diseases including *Alternaria* spp and virus (Simbashizweko and Perreaux, 1987)
- ❑ *Alternaria* blight disease reduce sweetpotato root yield up to 60%
- ❑ Low adoption of introduced sweetpotato varieties (diseases, low dry matter)
- ❑ High vit A deficiency: Unavailability of sweetpotato varieties with high yielding, beta-carotene content and resistant to diseases



SPVD



Alternaria blight



Weevil disease



Pest: Agrius convolvuli

Justification

- ❑ Need to identify farmers's preferred traits in sweetpotato
- ❑ Use that information to breed the most preferred sweetpotato variety for Burundian.
- ❑ That information will also help in designing sweetpotato program and will increase the farmers' adoption.

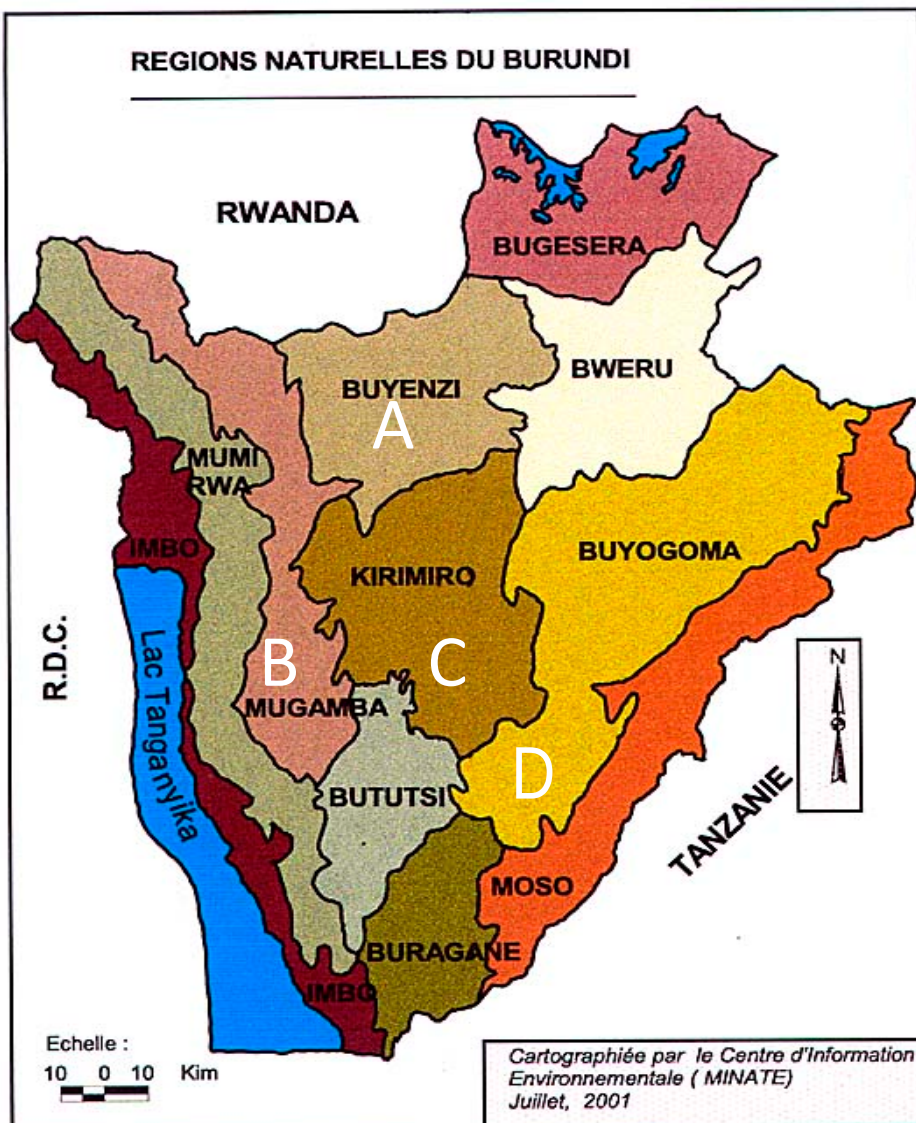
Broad objective

To develop **farmer preferred sweetpotato genotypes resistant to Alternaria spp.** for enhanced sweetpotato productivity, income generation and health improvement among the small holder farmers in Burundi.

Specific objectives

1. To investigate **farmer preferred sweetpotato traits, production constraints** and Alternaria spp. awareness in Burundi;
2. To analyze the country wide occurrence, **prevalence and distribution of Alternaria blight** affecting sweetpotato in Burundi;
3. To study the **genetic diversity of Alternaria** spp. affecting sweetpotato in Burundi;
4. To characterize sweetpotato germplasm and develop markers for resistance to Alternaria spp.;
5. To evaluate F1 hybrids produced from germplasm with farmer preferred traits and resistant to Alternaria spp.

1. Germplasm collection sites: Buyenzi, Kirimiro, Mugamba and Buyogoma



2. Plant material

☐ 172 genotypes: Farmers' varieties (85), CIP varieties (27), ISABU collection (57) and RAB varieties (3)

☐ 145 white to cream flesh color

☐ 25 Orange flesh color

☐ 2 yellow flesh color

3. Field evaluation

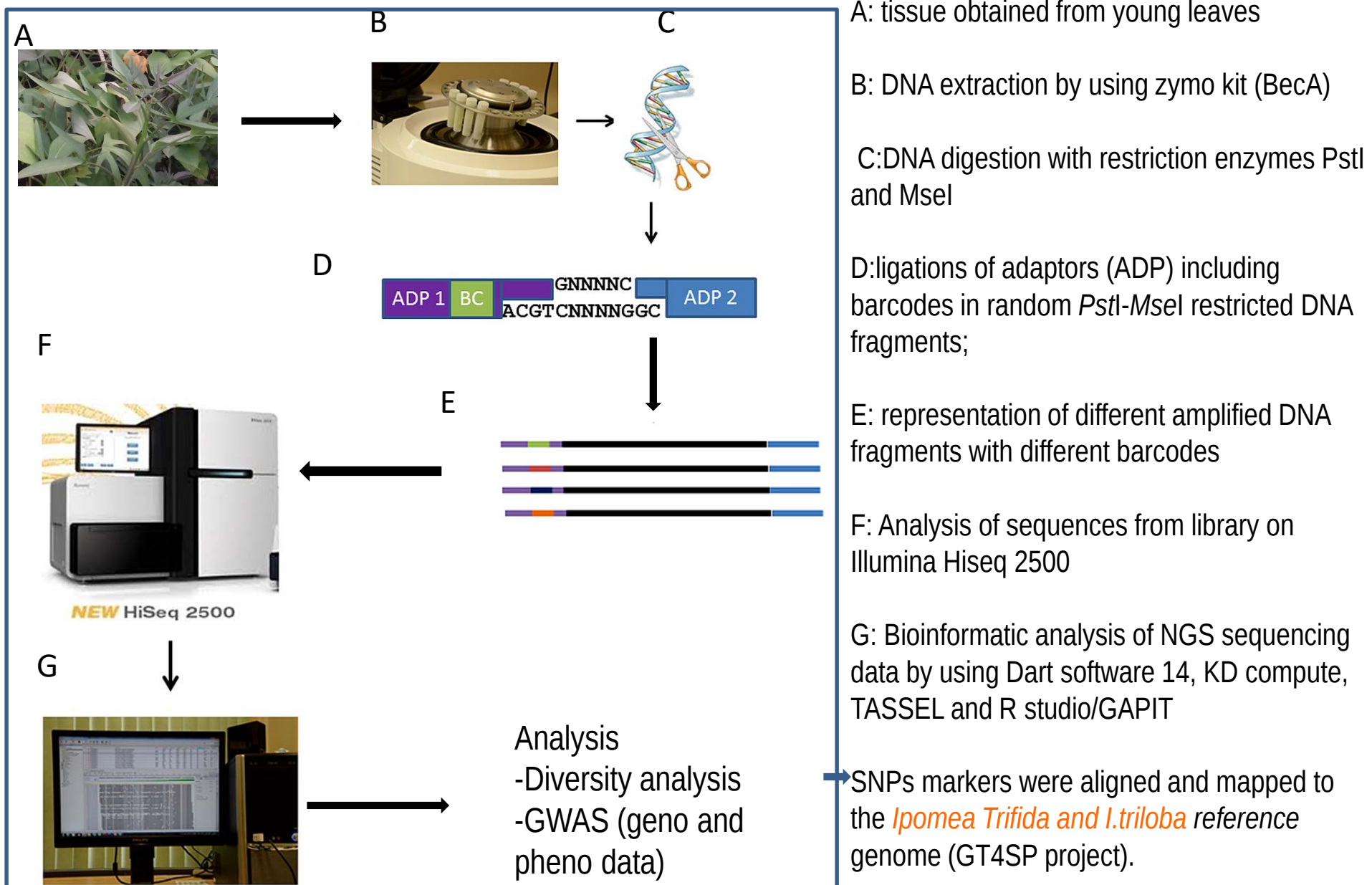
☐ Sites: Gisozi (high altitude: B) and Bukemba (low altitude: Moso)

☐ Experimental design: RCBD (5 cuttings in single rows on ridges 1 m apart and 0.3 m within the row), 2 replications

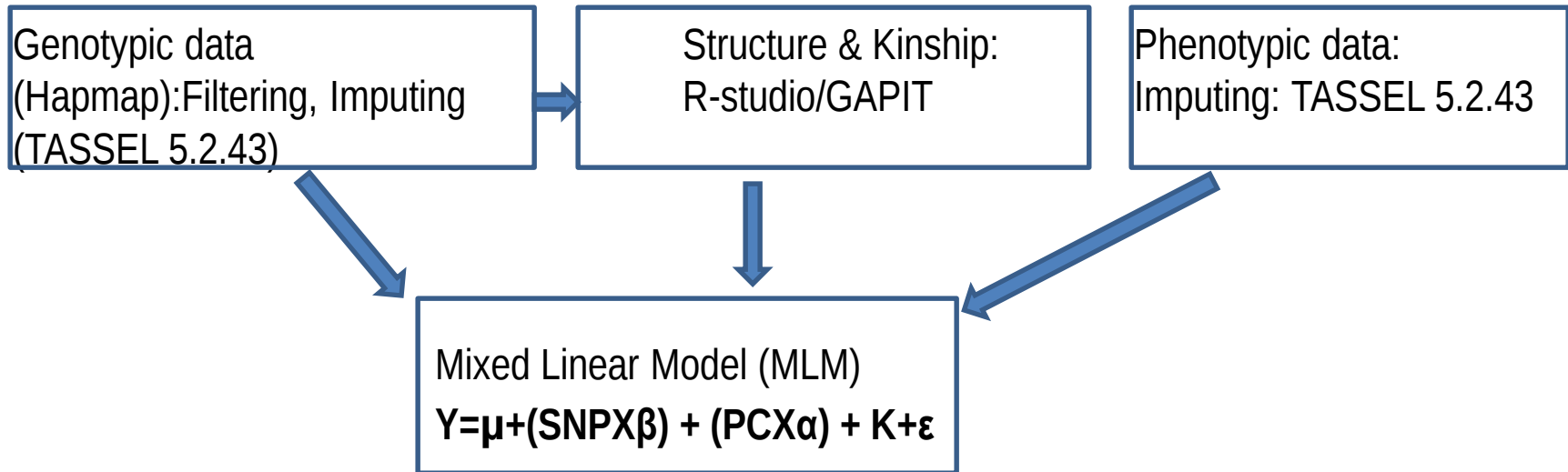
☐ Morphological traits recorded according to CIP (1991) descriptors

4. Molecular characterization

4.1.DNA extraction and Genotyping by sequencing



4.2.Data analysis-GWAS



Y = phenotypic response of the dependent variable

μ = Average phenotypic response

SNP = SNP marker (fixed effect)

β = fixed effect model coefficient for SNP

PC = population structure (fixed effect)

α = fixed effect model coefficient for PC (population structure)

K = kinship matrix (random effect)

ϵ = residual.

Control of False positive

Number of marker: 23000

Significance level at 0.05 Bonferoni $(0.05/23000) = 2.08 \cdot 10^{-6}$

Results

1. Heritability

	MRW	NMRW	VW	CC	SPVD	ALT	WEEV
Vg	28.7	0	58.2	6.65	0.14	0.28	0.0006
Vgxe	55.8	0.63	38	0.89	1.99	0.82	0.312
Verror	86.7	5.65	212.3	1.27	2.33	2.82	1.01
#envs	2	2	2	2	2	2	2
#reps	2	2	2	2	2	2	2
h^2	0.367	0	0.447	0.897	0.082	0.201	0.001

Legend: MRW: marketable root weight, NMRW: Non marketable root weight, VW: vine weight, CC: beta carotene content, ALT: Alternaria blight, WEEV: weevil

☐ High heritability for beta-carotene content

☐ Moderate heritability for marketable root and vine weight

☐ Low heritability for non marketable root weight, SPVD, Alternaria blight and weevil

Results

2.Diversity analysis

Markers	Number	PICsnp	Call rate (%)	Reproducibility (%)	Heterozygosity	Homozygosity	Rare allele
SNPs_1row	35286/ 34109	0/0.01-0.5	>40	95-100	0 - 80	0 - 99	4557
Silico markers	51986	0.01-0.5	>80	95-100	0.5- 99	1-99.5	6633

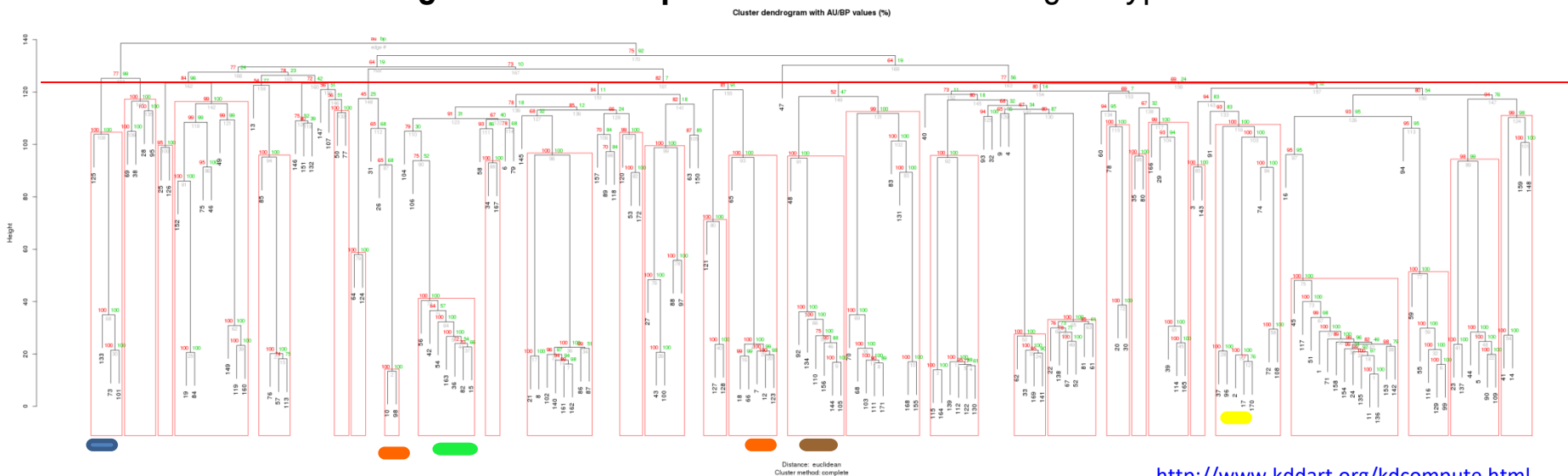
☐ SNPs and silico markers generated

☐ High reproducibility and call rate

☐ Revealed high PIC

Results: Diversity analysis,cont.

3.Hierarchical Clustering with Bootstrap P-Values based on the genotypic marker data



<http://www.kddart.org/kdcompute.html>

Legend

Origin		Flesh color	Identity
Rwanda/RAB	Burundi/ISABU	98. SPK004	163. Tanzania
101. Gihingumukungu	110. 5496 cordes vertes	10. NASPOT 9 Vitaa	56. Kemb 10
73. 97062	105. 1022 Nsenyakaniga	123. Gloria	17. Mugande
125. Terimbere	144. 977 Divirovisky/6R5/6	7. Inamushinwa	170. Kajondi
133. Cacearpedo	134. MJ karama	12. Pepiris	D=0.02
	92. N19	18. Amelia	D=0.01
D=0.01 (101 & 73)	D=0.01 (110 & 144)	66. Zadeni	

4. Population Genetics Fs: Fixation Index (F) within population

	F-statistics
Fis	0.161542
Fit	0.295749
Fst	0.160064

- High differentiation among individuals with respect to total population ($F_{it} > 0.25$)
- Moderate differentiation of individuals within respect to subpopulation ($0.15 < F_{is} < 0.25$)
- Moderate differentiation of subpopulation with respect to total population ($0.15 < F_{st} < 0.25$)

Fixation Index

	Fixation_Index_F
Buyenzi	0.21408892
Mugamba	0.129777309
Bukemba-ISABU	0.262211436
Kirimiro	0.194794055
CIP	0.244396871
Imbo	0.17453271
unknown	0.245066416
RAB	-1
Moso	0.159666518

- 9 population according to the origins of germplasm

Relatedness by using Wang (2007) estimators

- ☐ 96%: unrelated
- ☐ 2.4% less related
- ☐ 1.6% more related

} High diversity: good for breeding

5. Genome wide association: Beta-carotene content

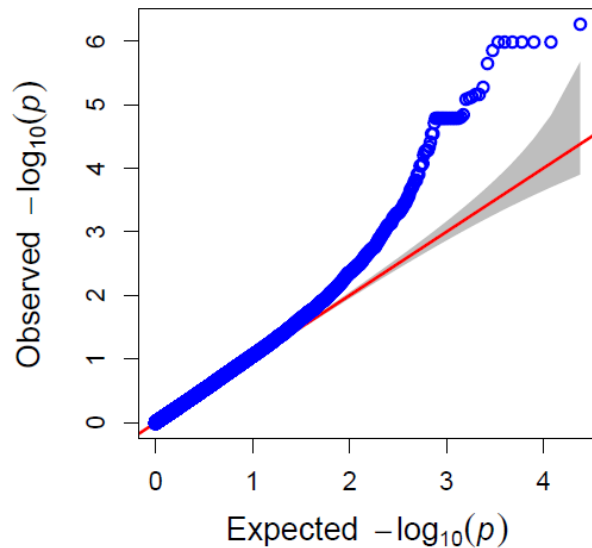


Figure1: QQplot

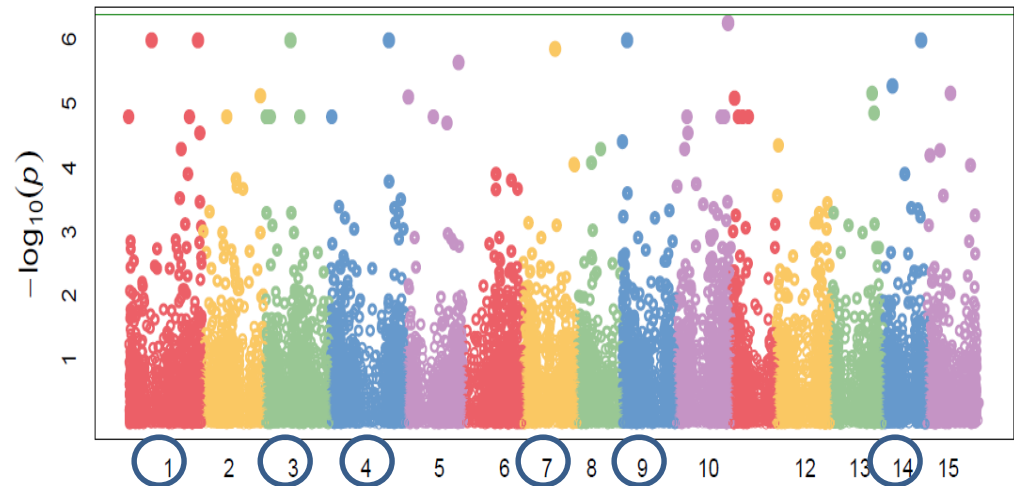


Figure2: Manhattan plot

Table1: SNPs markers associated with beta-carotene content (P-value<2.08. 10⁻⁶)

Markers	SNP	size (bp)	Chr	Position	P.value	nobs	R2_Model	FDR_Adj_PV	Allele effect
7538693 F 0-55:G>C-55:G>C	G/C	69	1	10046068	1.04E-06	172	0.31	0.004	5.597
7538891 F 0-64:G>A-64:G>A	G/A	69	1	30393881	1.04E-06	172	0.31	0.004	5.597
7550771 F 0-17:A>G-17:A>G	A/G	69	3	11097723	1.04E-06	172	0.31	0.004	5.597
7537928 F 0-41:T>C-41:T>C	T/C	69	4	25671183	1.04E-06	172	0.31	0.004	5.597
7548185 F 0-55:G>T-55:G>T	G/T	69	9	2292906	1.04E-06	172	0.31	0.004	5.597
100033370 F 0-44:G>C-44:G>C	G/C	69	14	16793062	1.04E-06	172	0.31	0.004	5.597
100067305 F 0-48:G>A-48:G>A	G/A	69	7	14010979	1.40E-06	172	0.31	0.004	2.841

6. Genome wide association: weevil resistance

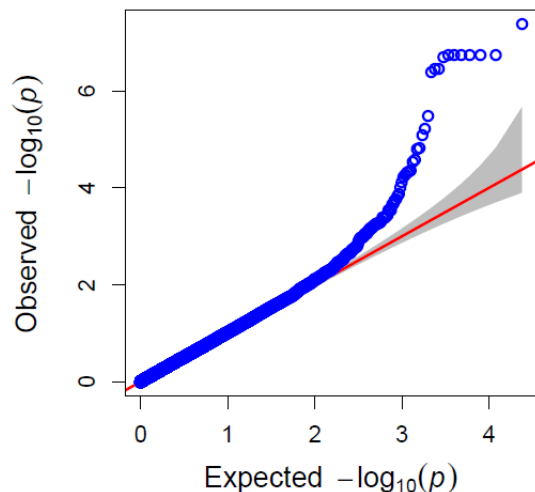


Figure3:QQplot

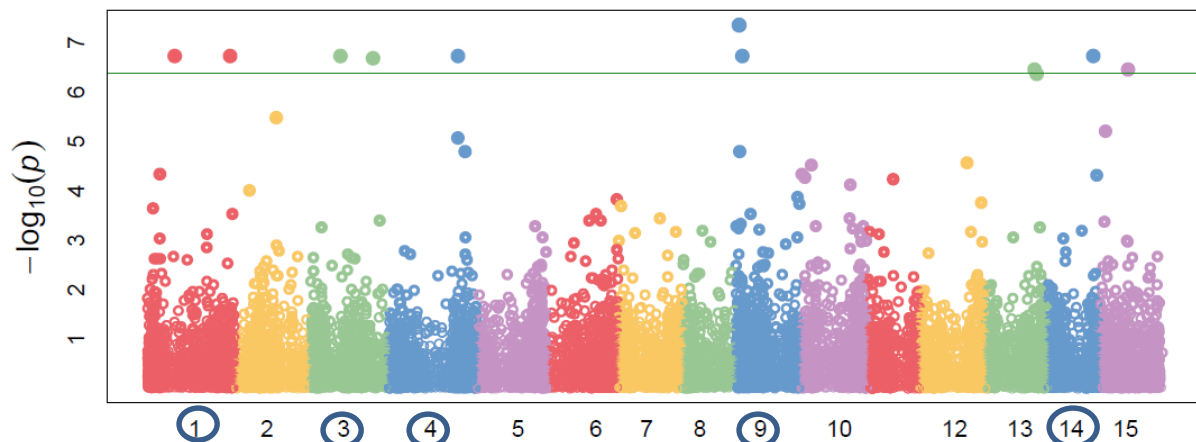


Figure4:Manhattan plot

Table 2: SNPs markers associated with weevil resistance (P-value<2.08. 10⁻⁶)

Markers	SNPS	Chr	Pos	P.value	nobs	R2_Model	FDR_Adj_PV	Allele effect
7610663 F 0-37:G>A-37:G>A	G/A	9	1085679	4.22294E-08	172	0.22	0.001	1.63
7538693 F 0-55:G>C-55:G>C	G/C	1	10046068	1.83167E-07	172	0.20	0.001	1.63
7538891 F 0-64:G>A-64:G>A	G/A	1	30393881	1.83167E-07	172	0.20	0.001	1.63
7550771 F 0-17:A>G-17:A>G	A/G	3	11097723	1.83167E-07	172	0.20	0.001	1.63
7537928 F 0-41:T>C-41:T>C	T/C	4	25671183	1.83167E-07	172	0.20	0.001	1.63
7548185 F 0-52:A>T-52:A>T	A/T	9	2292906	1.83167E-07	172	0.20	0.001	1.15
100033370 F 0-44:G>C-44:G>C	G/C	14	16793062	1.83167E-07	172	0.20	0.001	1.63
9844423 F 0-9:T>A-9:T>A	T/A	3	23071452	2.03381E-07	172	0.20	0.001	1.17
9841567 F 0-14:G>A-14:G>A	G/A	13	18117673	3.51516E-07	172	0.19	0.001	1.14
7558013 F 0-16:A>T-16:A>T	A/T	15	10480571	3.51516E-07	172	0.19	0.001	1.14
7618537 F 0-8:A>G-8:A>G	A/G	13	18925853	4.18881E-07	172	0.19	0.001	1.19

7. Genome wide association: Alternaria Blight resistance

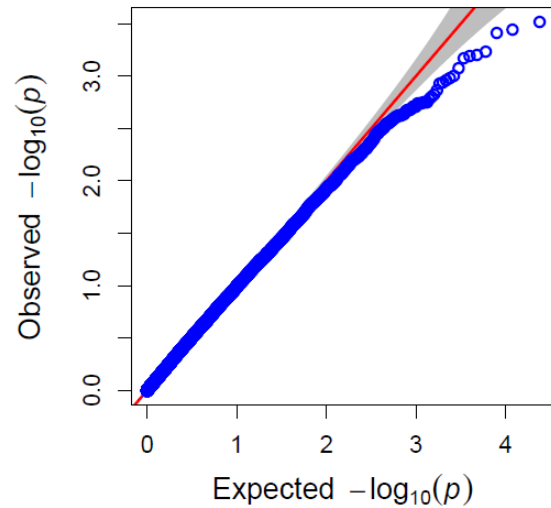


Figure 5: QQplot

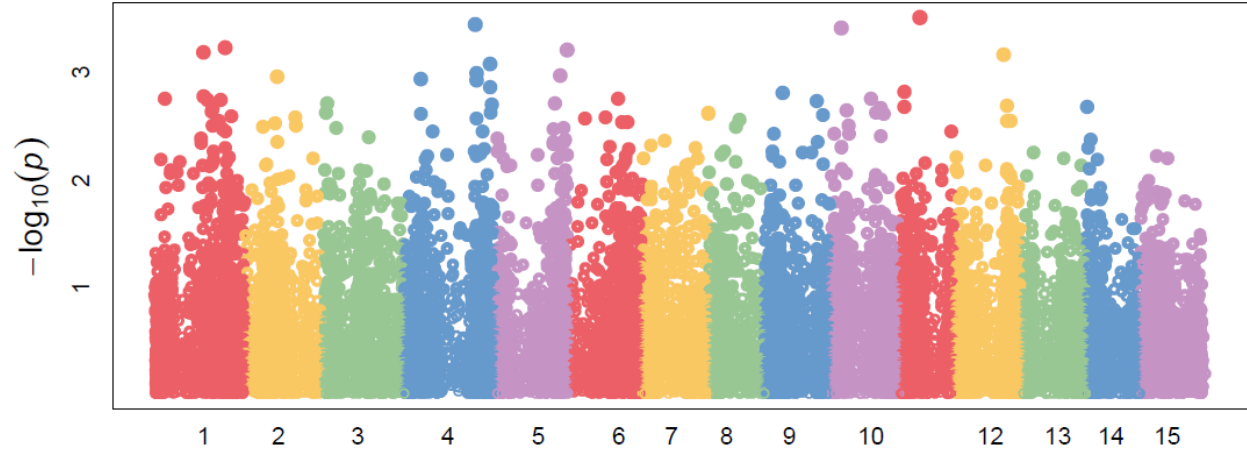


Figure 6: Manhattan plots

☐ P-values of tested SNPs $> 2.08 \cdot 10^{-6}$

☐ Any associated SNPs

☐ **Hypotheses:** Trait which was not intensively phenotyped, Contribution of data collected in low land (less disease pressure)

Conclusion and way forward

- ❑ Genotypic data for sweetpotato grown in Burundi was availed
- ❑ New SNPs markers were found for the traits associated with high heritability: beta carotene content and weevil resistance
- ❑ Phenotype the same germplasm in high disease pressure for Alternaria blight resistance and perform GWAS
- ❑ Validate the identified SNPs markers
- ❑ Introgression the Alternaria blight resistance markers to the current used farmer's sweetpotato varieties
- ❑ Evaluate the offspring and select the farmers's preferred sweetpotato varieties in Burundi

Acknowledgement

BecA-ILRI Hub, CIP, Supervisors, IGSS team



Thank you!!!