



# KASP Markers for Efficiency in *Musa* Improvement

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# Musa improvement

Matooke (3x - AAA)

Uganda (Namulonge)  
*East Africa*



Mchare (2x - AA)

Tanzania (Arusha)  
*East Africa*



Plantain (3x - AAB)

Nigeria (Ibadan)  
*Central and West Africa*



# Traits in the Target Product Profiles



## Resistance to pests and diseases

- Weevils
- Nematodes
- Fusarium wilt
- Black Sigatoka
- Banana bacterial wilt
- Banana bunchy top

## Agronomic traits

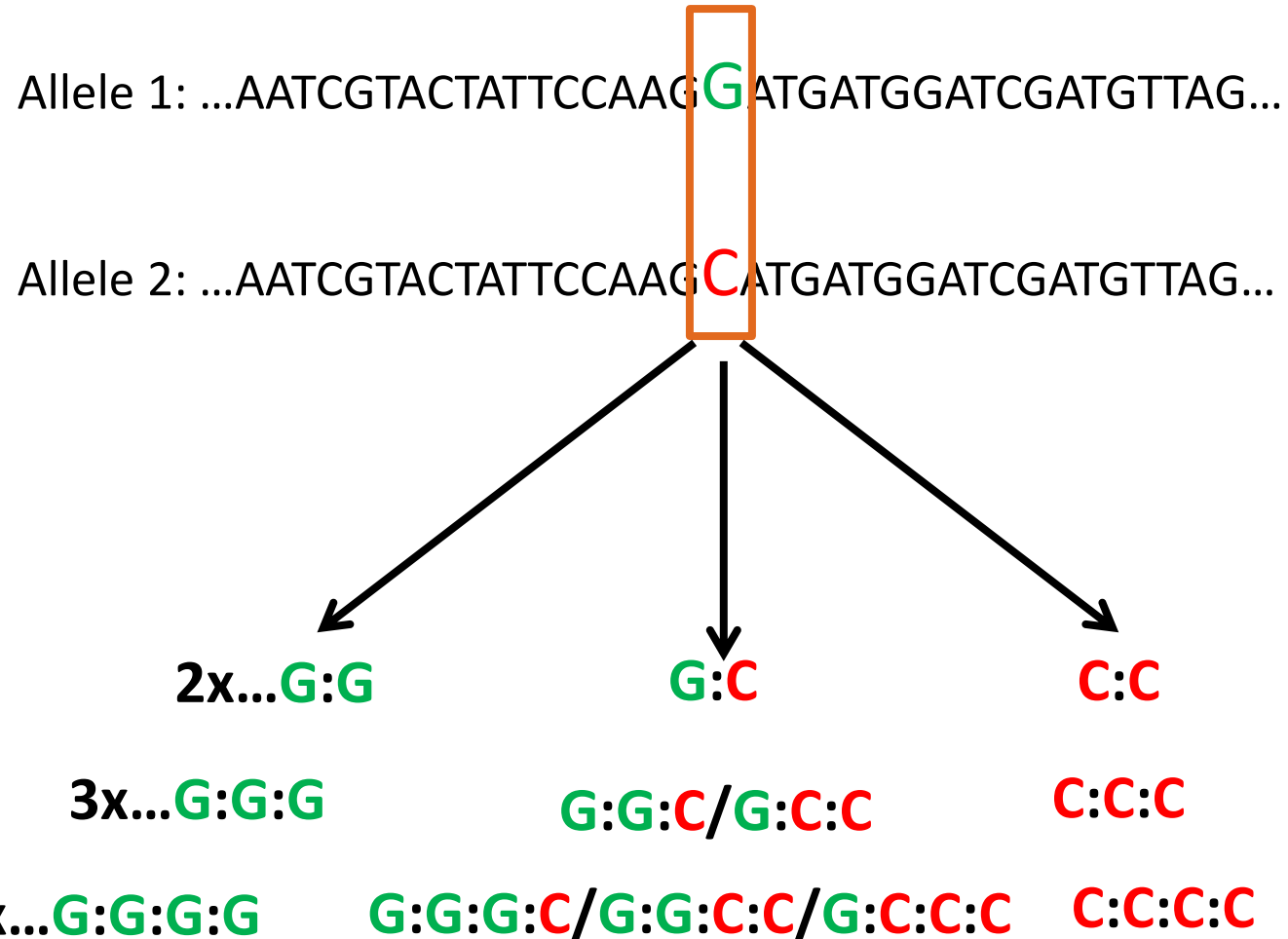
- Yield traits
- Fruit filling
- Number of hands
- Number of fingers
- Ratooning
- Parthenocarp
- Bunch orientation
- Plant stature

## Consumer's traits (quality)

- Taste
- Colour
- Texture
- pVAC

# KASP markers for MAS

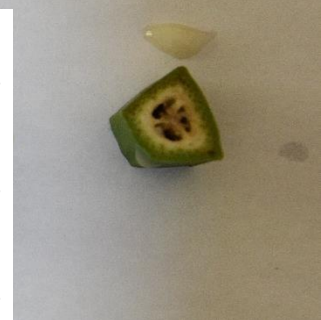
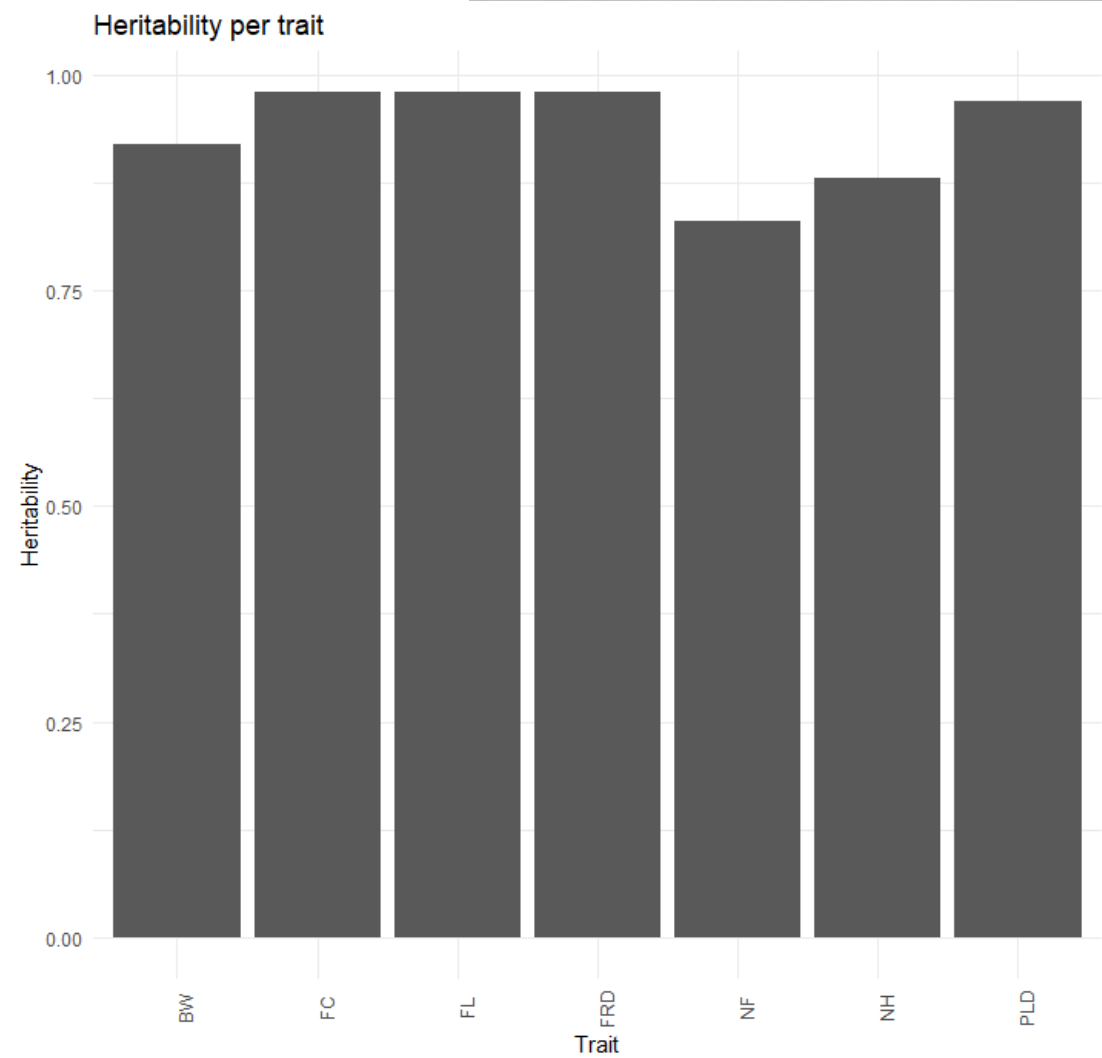
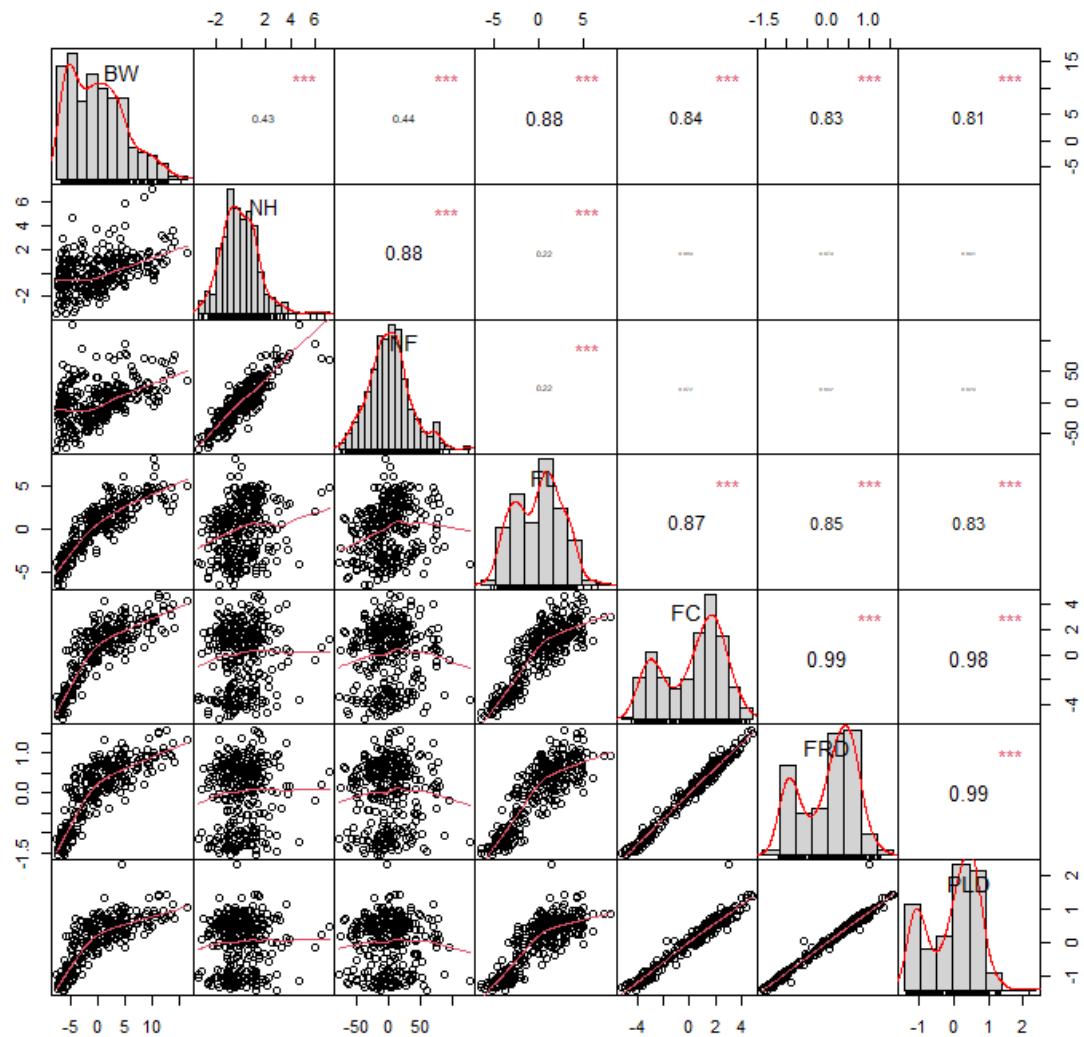
# SNPs in Polyploids



# 1. KASP markers for MAS: fruit-filling



# Fruit-filling = fruit circumference, fruit diameter, pulp diameter



# QTL for fruit filling

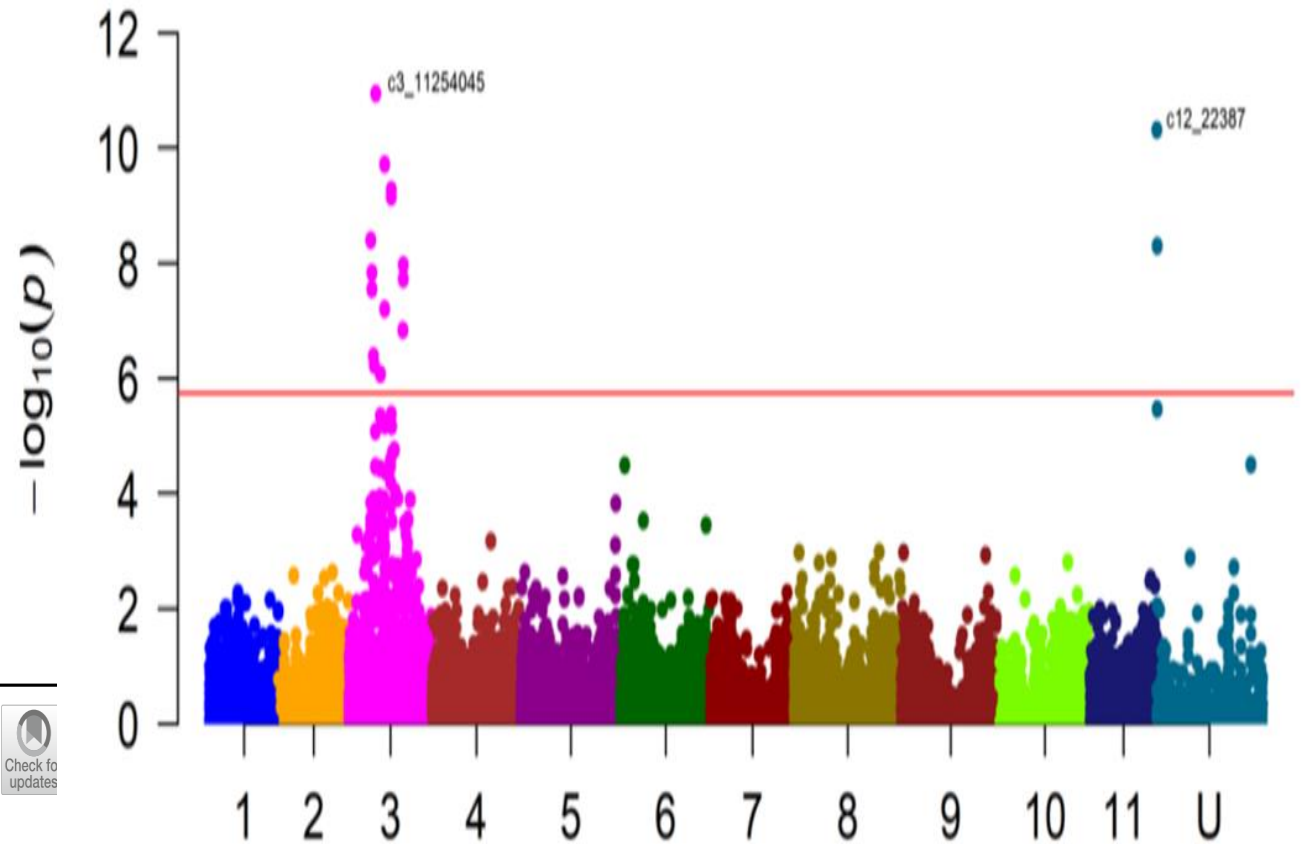


ORIGINAL ARTICLE



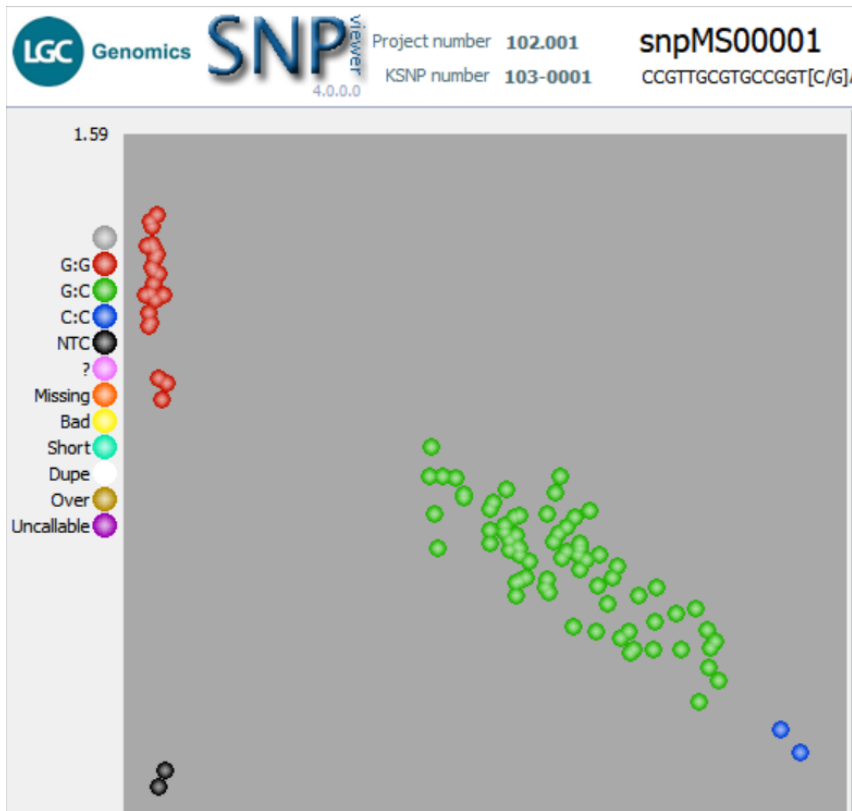
Association genetics of bunch weight and its component traits in East African highland banana (*Musa* spp. AAA group)

Moses Nyine<sup>1,7</sup> · Brigitte Uwimana<sup>1</sup> · Violet Akech<sup>1</sup> · Allan Brown<sup>2</sup> · Rodomiro Ortiz<sup>3</sup> · Jaroslav Doležel<sup>4</sup> ·

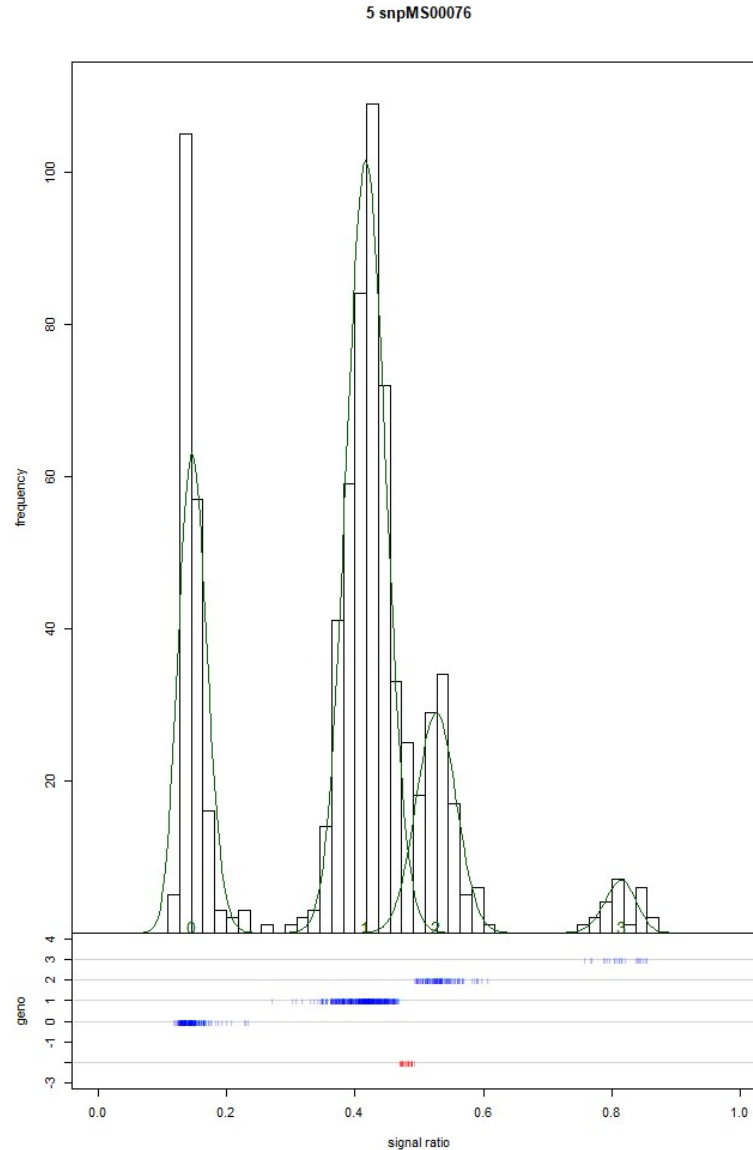




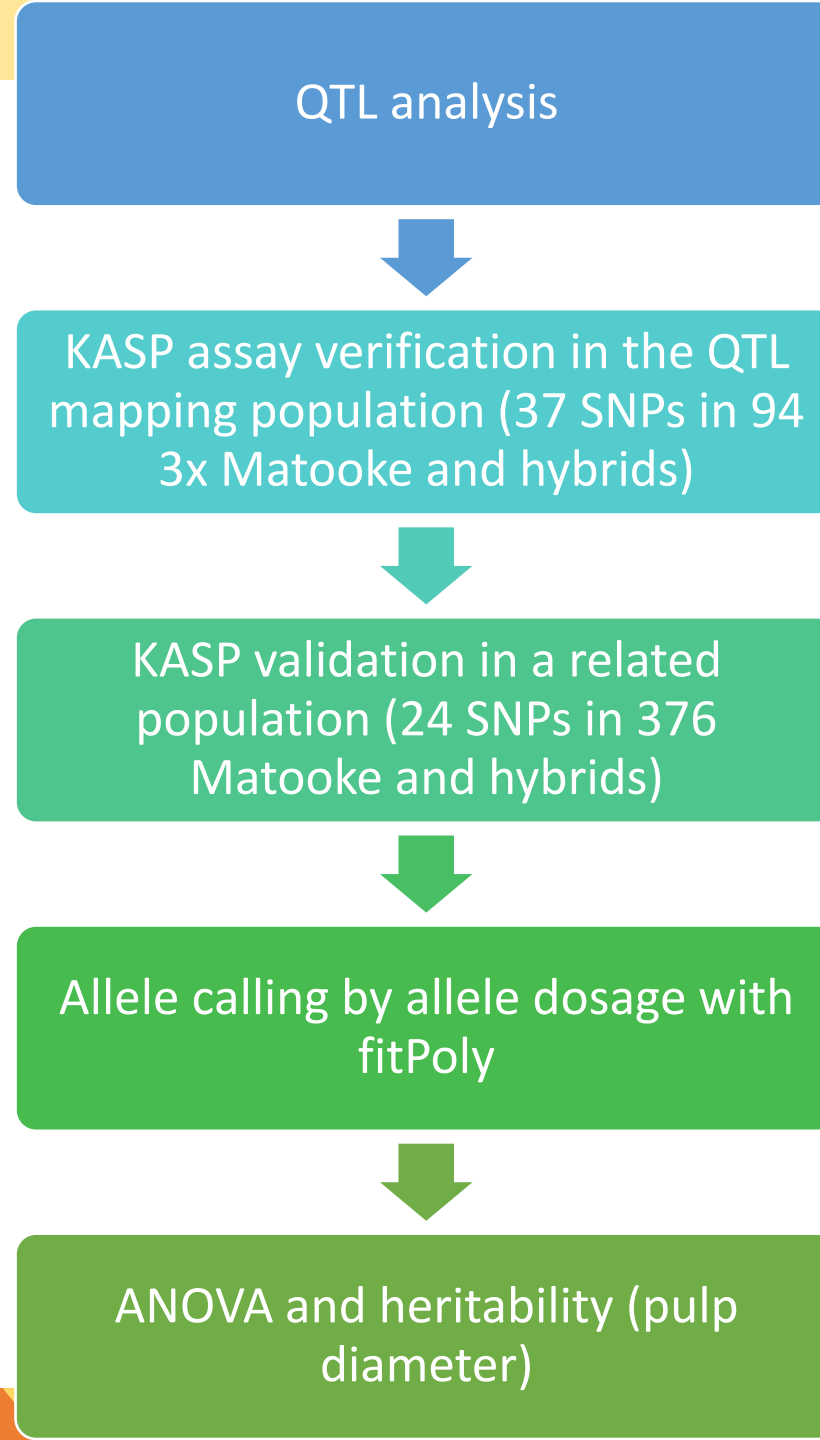
# KASPs for the SNPs associated with fruit-filling



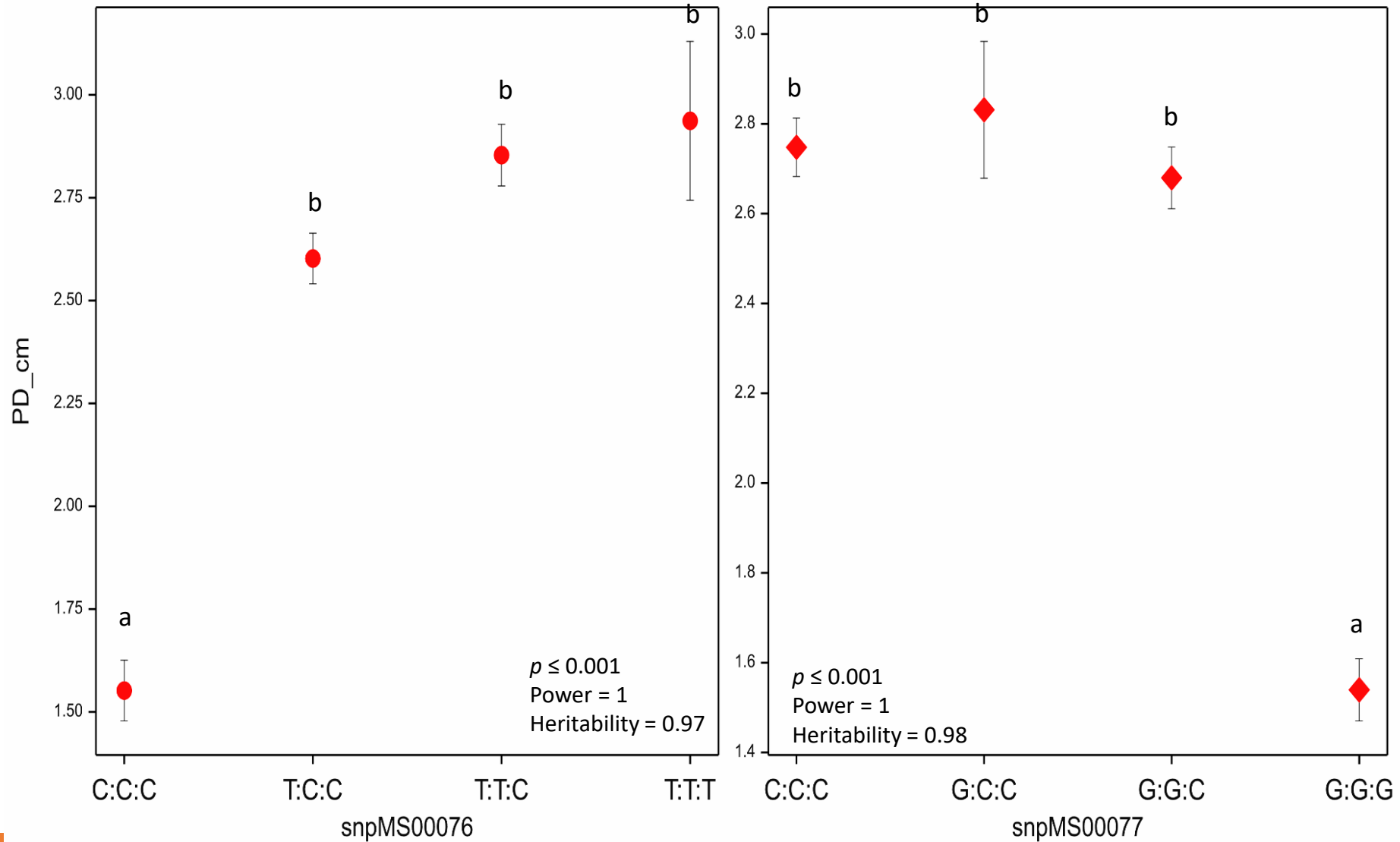
Standard calling as 2x



Calling per allele dosage with fitPoly

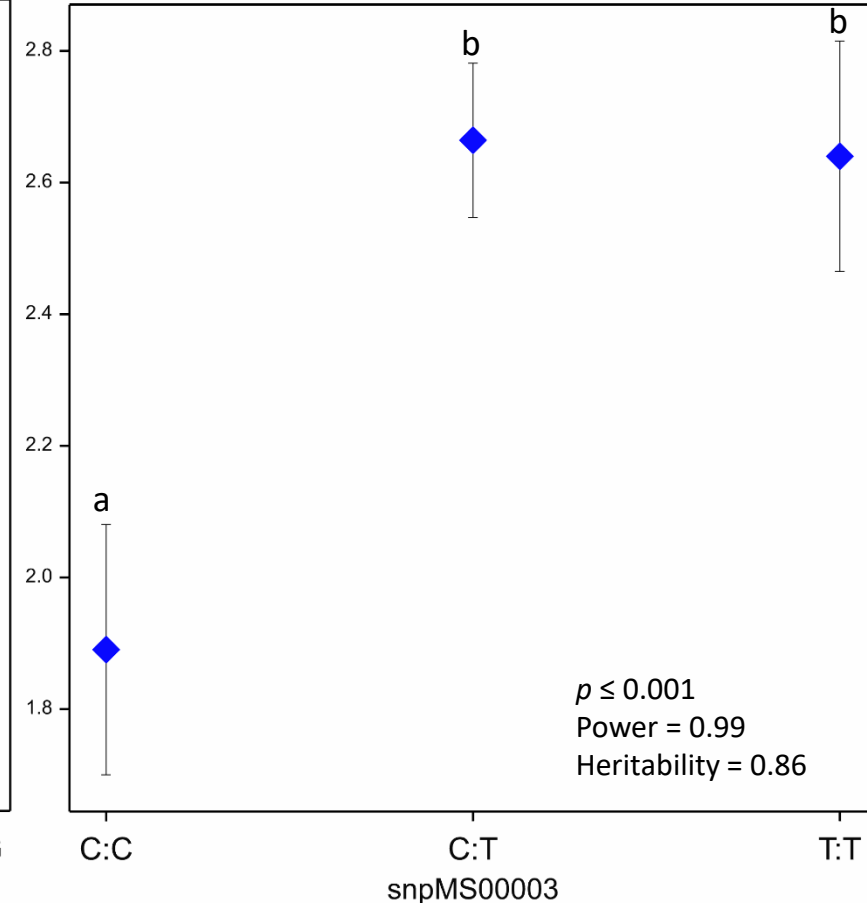
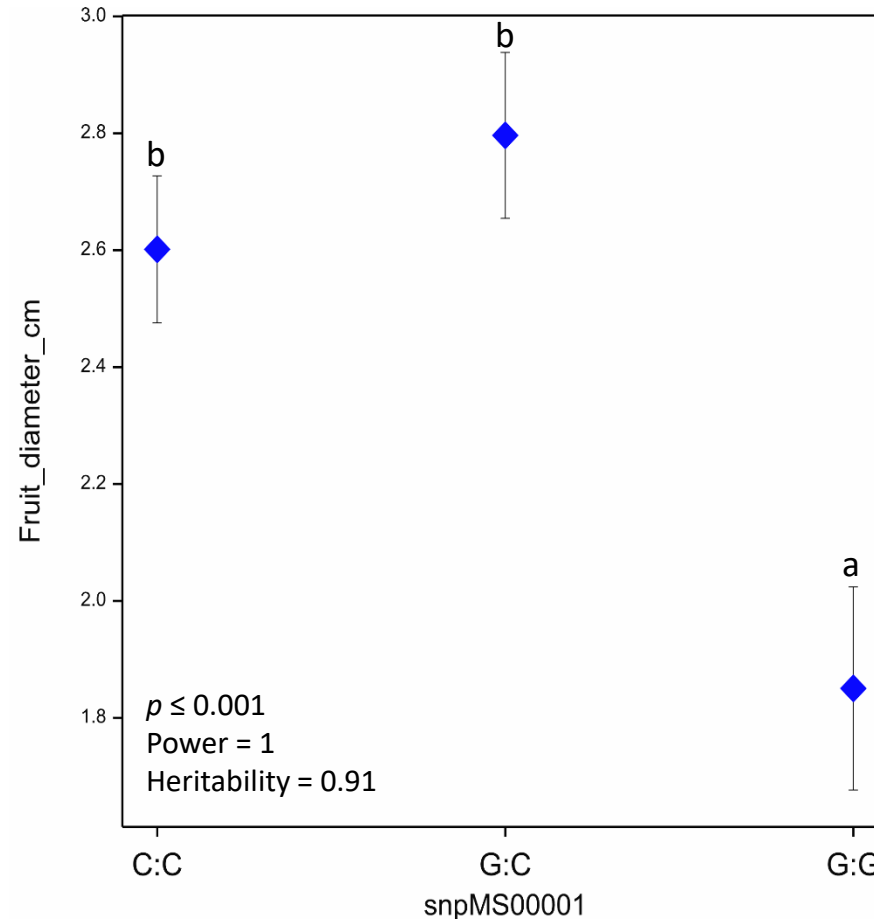


# KASP validation and MAS in 3x Matooke hybrids



# Validation in other genetic backgrounds

- 53 Mchare landraces and hybrids (EET 1)
- Fruit diameter
- Mchare EET2 genotyped, phenotyping ongoing
- Plantain PYT and EET genotyped, phenotyping ongoing
- 2x parents genotyped, phenotyping ongoing



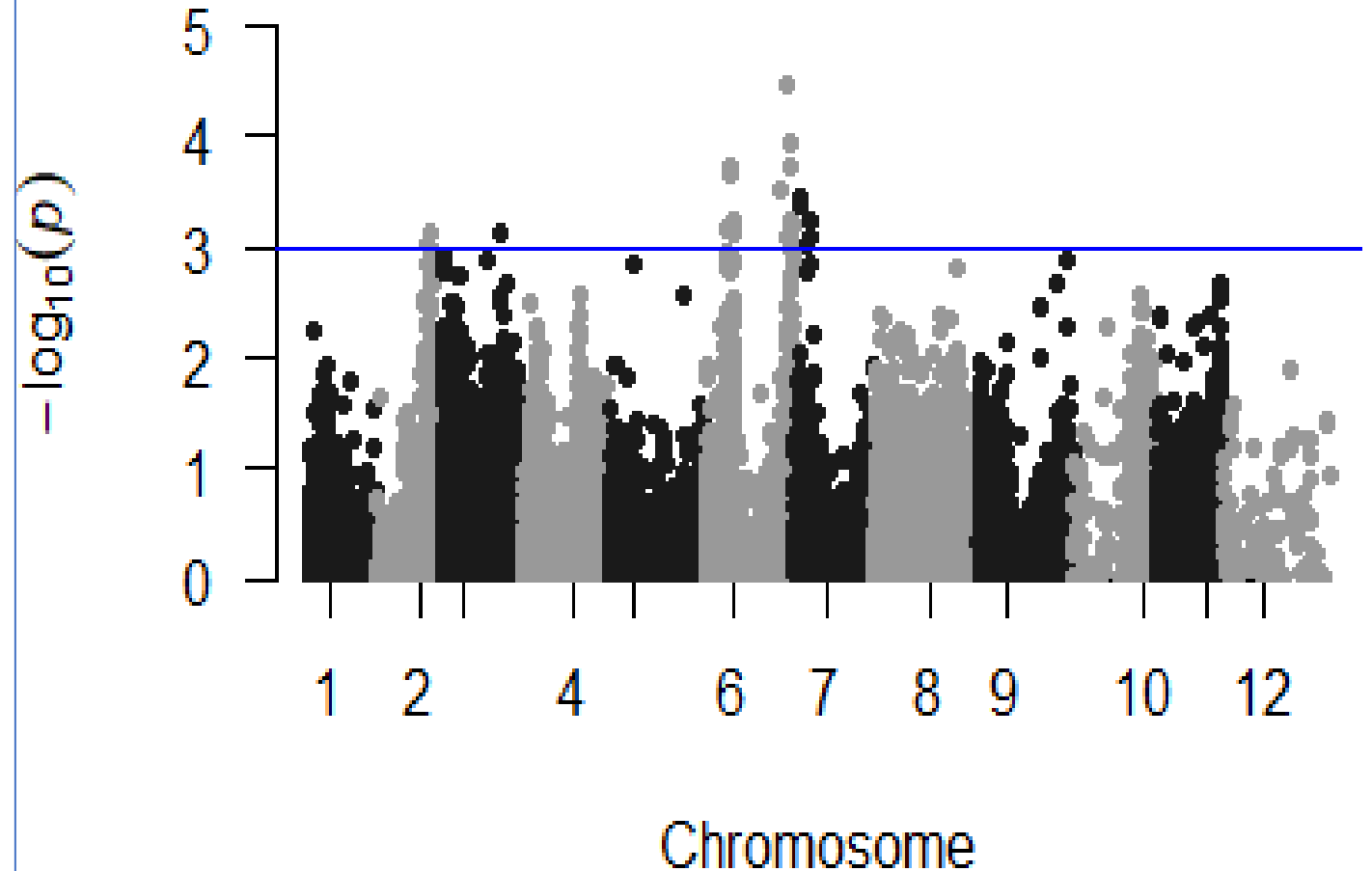
## 2. Banana *Xanthomonas* Wilt (*Xanthomonas vasicola* pv. *musacearum* )

- Prevalent in East and Central Africa
- Affected plants produce non-edible fruits
- Resistance found in *Musa balbisiana*
- Resistance limited in *M. acuminata*
- So far resistance identified in *M. acuminata* subsp. *zebrina*
- <https://doi.org/10.1111/ppa.12945>



## QTL for Banana *Xanthomonas* Wilt

- ‘Monyet’ (4x) x ‘Kokopo’ (2x)
- 147 3x progeny phenotyped
- 0.62 heritability AUDPC
- 18,009 SNPs – DArTseq
- Continuous mapping QTL analysis



# 26 KASP markers verified for BXW

Intertek SNP ID	SNP	Trait
snpMS00179	G/A	maxAUDPC for Xvm
snpMS00180	A/G	maxAUDPC for Xvm
snpMS00181	A/G	maxAUDPC for Xvm
snpMS00182	A/G	maxAUDPC for Xvm
snpMS00183	T/A	maxAUDPC for Xvm
snpMS00184	G/T	maxAUDPC for Xvm
snpMS00185	A/G	maxAUDPC for Xvm
snpMS00186	G/T	maxAUDPC for Xvm
snpMS00187	G/T	maxAUDPC for Xvm
snpMS00188	A/G	maxAUDPC for Xvm
snpMS00189	T/C	maxAUDPC for Xvm
snpMS00190	A/T	maxAUDPC for Xvm
snpMS00191	G/A	maxAUDPC for Xvm
snpMS00192	A/G	maxAUDPC for Xvm
snpMS00193	G/C	maxAUDPC for Xvm
snpMS00194	T/C	maxAUDPC for Xvm
snpMS00195	G/T	maxAUDPC for Xvm
snpMS00196	G/T	maxAUDPC for Xvm
snpMS00197	T/C	maxAUDPC for Xvm
snpMS00198	A/G	maxAUDPC for Xvm
snpMS00199	C/T	maxAUDPC for Xvm
snpMS00200	G/C	maxAUDPC for Xvm
snpMS00201	T/A	maxAUDPC for Xvm
snpMS00202	A/G	maxAUDPC for Xvm
snpMS00203	C/A	maxAUDPC for Xvm
snpMS00204	A/C	maxAUDPC for Xvm

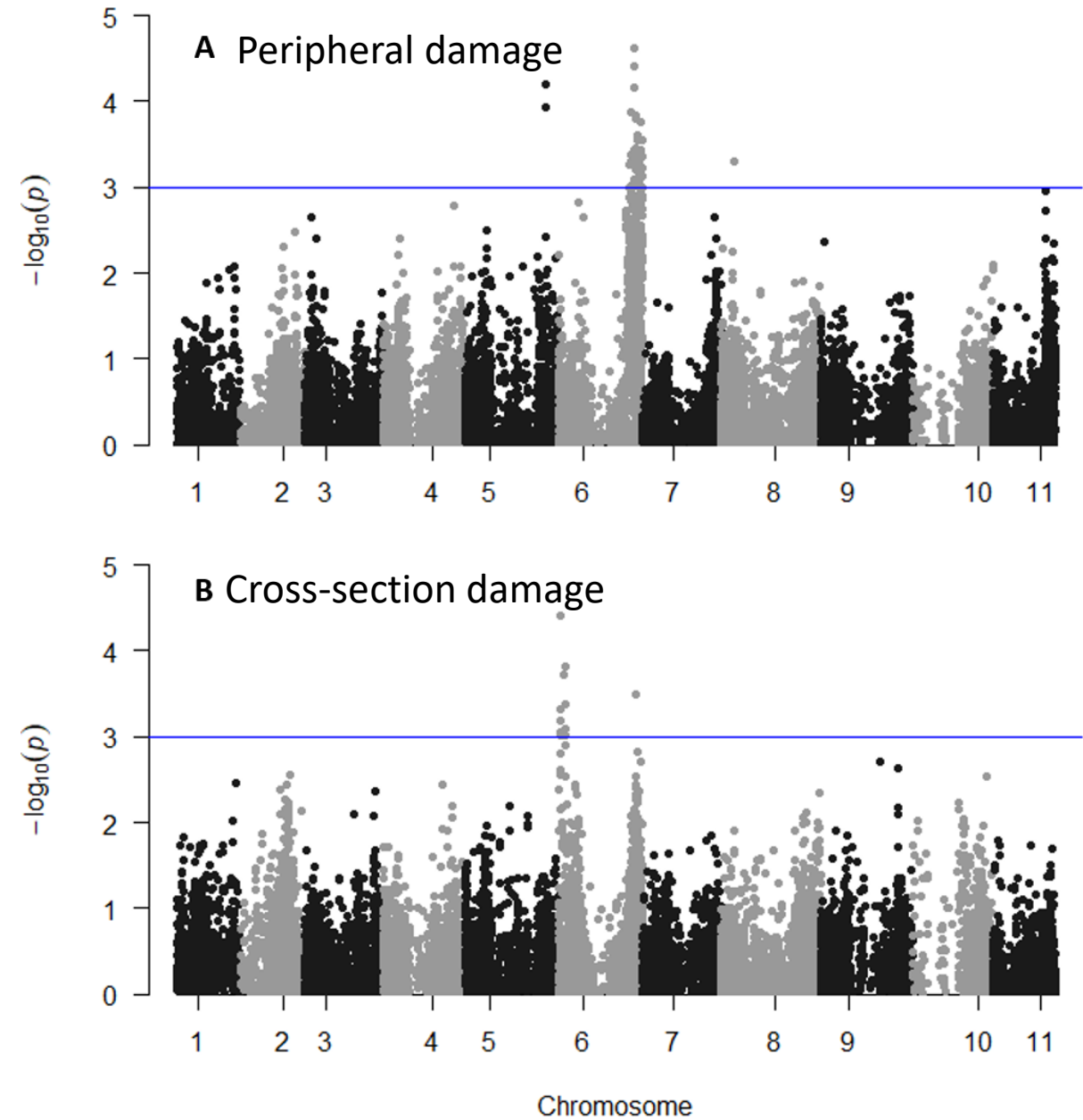
### 3. Banana weevils (*Cosmopolites sordidus*)

- One of the two major pests of bananas
- Matooke and plantains susceptible
- Gets worse over time as the weevil population builds up
- Up to 100% yield loss due to mats disappearance
- QTL mapping in 2 unrelated bi-parental populations



# QTL for weevil resistance

- 'Monyet' x 'Kokopo'
- Continuous mapping in 3x
- <https://doi.org/10.3389/fpls.2021.753241>





44 + 11 KASP  
markers  
verified for  
weevil  
resistance

Intertek SNP ID *	SNP	Trait
snpMS00100	C/A	logit_PD weevil resistance
snpMS00101	G/A	logit_PD weevil resistance
snpMS00102	C/T	logit_PD weevil resistance
snpMS00103	T/C	logit_PD weevil resistance
snpMS00104	T/C	logit_PD weevil resistance
snpMS00105	C/T	logit_PD weevil resistance
snpMS00106	C/T	logit_PD weevil resistance
snpMS00107	A/T	logit_PD weevil resistance
snpMS00108	A/C	logit_PD weevil resistance
snpMS00109	G/T	logit_PD weevil resistance
snpMS00110	A/C	logit_PD weevil resistance
snpMS00111	G/A	logit_PD weevil resistance
snpMS00112	C/G	logit_PD weevil resistance
snpMS00113	C/T	logit_PD weevil resistance
snpMS00114	G/C	logit_PD weevil resistance
snpMS00115	G/T	logit_PD weevil resistance
snpMS00116	G/C	logit_PD weevil resistance
snpMS00117	T/A	logit_PD weevil resistance
snpMS00118	A/G	logit_PD weevil resistance
snpMS00119	G/T	logit_PD weevil resistance
snpMS00120	T/A	logit_PD weevil resistance
snpMS00121	A/C	logit_PD weevil resistance
snpMS00122	C/A	logit_PD weevil resistance
snpMS00123	T/C	logit_PD weevil resistance
snpMS00124	C/T	logit_PD weevil resistance
snpMS00125	A/G	logit_PD weevil resistance
snpMS00126	G/A	logit_PD weevil resistance
snpMS00127	G/T	logit_PD weevil resistance
snpMS00128	G/A	logit_PD weevil resistance
snpMS00129	G/A	logit_PD weevil resistance
snpMS00130	A/T	logit_PD weevil resistance
snpMS00131	A/G	logit_PD weevil resistance
snpMS00132	T/G	logit_PD weevil resistance
snpMS00133	A/T	logit_PD weevil resistance
snpMS00134	G/A	logit_PD weevil resistance
snpMS00135	T/G	logit_PD weevil resistance
snpMS00136	C/A	logit_PD weevil resistance
snpMS00137	A/G	logit_PD weevil resistance
snpMS00138	A/C	logit_PD weevil resistance
snpMS00139	G/T	logit_PD weevil resistance
snpMS00140	T/C	logit_PD weevil resistance
snpMS00141	T/C	logit_PD weevil resistance
snpMS00142	G/C	logit_PD weevil resistance
snpMS00143	A/T	logit_PD weevil resistance
snpMS00144	C/G	logit_TXD weevil resistance
snpMS00145	G/T	logit_TXD weevil resistance
snpMS00146	A/T	logit_TXD weevil resistance
snpMS00147	G/C	logit_TXD weevil resistance
snpMS00148	A/T	logit_TXD weevil resistance
snpMS00149	A/C	logit_TXD weevil resistance
snpMS00150	G/A	logit_TXD weevil resistance
snpMS00151	A/G	logit_TXD weevil resistance
snpMS00152	G/T	logit_TXD weevil resistance
snpMS00153	C/A	logit_TXD weevil resistance
snpMS00154	G/C	logit_TXD weevil resistance

## 4. Fusarium wilt (*Fusarium oxysporum* f.sp. *cubense* )



- Foc R1 affects Mchare production
- Does not affect Matooke and Plantain landraces
- Susceptibility can be introduced through breeding
- Pre-emptive breeding for Foc TR4
- Phenotyping for TR4 can't be done in Africa
- Partnership with Wageningen University, KeyGene and Queensland University
- <https://www.doi.org/10.3390/pathogens12020289>
- <https://www.doi.org/10.3390/pathogens12060820>

24 KASP  
markers  
verified for  
Foc TR4

sn	Intertek SNP ID	SNP	Trait
1	snpMS00155	C/A	Rhizome discoloration due to Foc TR4/STR4
2	snpMS00156	T/G	Rhizome discoloration due to Foc TR4/STR4
3	snpMS00157	T/A	Rhizome discoloration due to Foc TR4/STR4
4	snpMS00158	G/C	Rhizome discoloration due to Foc TR4/STR4
5	snpMS00159	C/T	Rhizome discoloration due to Foc TR4/STR4
6	snpMS00160	G/A	Rhizome discoloration due to Foc TR4/STR4
7	snpMS00161	T/G	Rhizome discoloration due to Foc TR4/STR4
8	snpMS00162	G/A	Rhizome discoloration due to Foc TR4/STR4
9	snpMS00163	G/A	Rhizome discoloration due to Foc TR4/STR4
10	snpMS00164	A/G	Rhizome discoloration due to Foc TR4/STR4
11	snpMS00165	A/T	Rhizome discoloration due to Foc TR4/STR4
12	snpMS00166	A/T	Rhizome discoloration due to Foc TR4/STR4
13	snpMS00167	C/G	Rhizome discoloration due to Foc TR4/STR4
14	snpMS00168	G/A	Rhizome discoloration due to Foc TR4/STR4
15	snpMS00169	T/C	Rhizome discoloration due to Foc TR4/STR4
16	snpMS00170	T/C	Rhizome discoloration due to Foc TR4/STR4
17	snpMS00171	T/C	Rhizome discoloration due to Foc TR4/STR4
18	snpMS00172	C/T	Rhizome discoloration due to Foc TR4/STR4
19	snpMS00173	C/T	Rhizome discoloration due to Foc TR4/STR4
20	snpMS00174	G/C	Rhizome discoloration due to Foc TR4/STR4
21	snpMS00175	A/G	Rhizome discoloration due to Foc TR4/STR4
22	snpMS00176	G/A	Rhizome discoloration due to Foc TR4/STR4
23	snpMS00177	C/T	Rhizome discoloration due to Foc TR4/STR4
24	snpMS00178	C/T	Rhizome discoloration due to Foc TR4/STR4

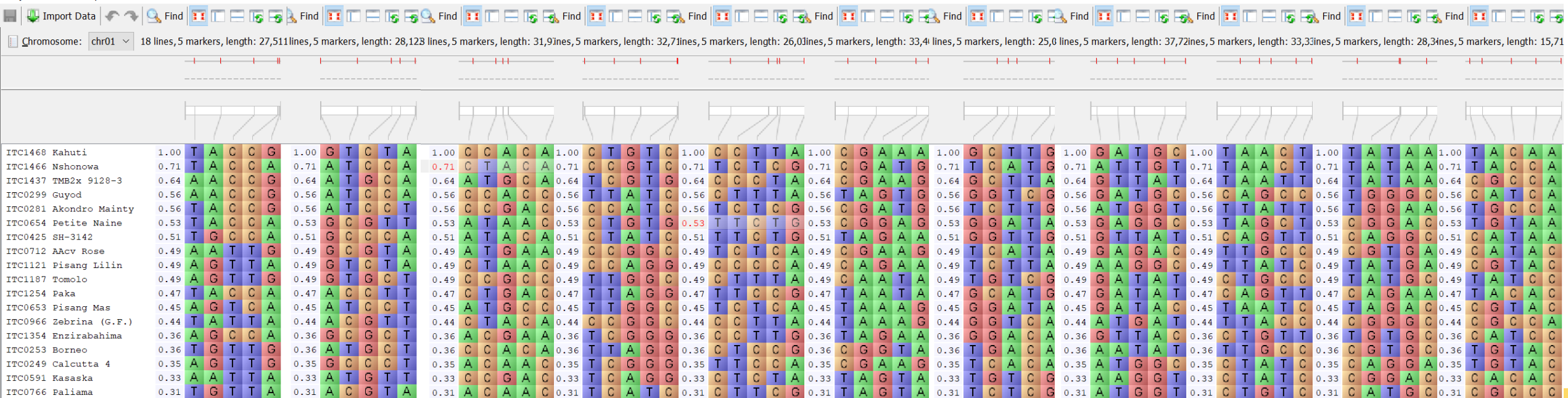
# Markers for QC/QA

# QA/QC markers for the A genome

- Re-sequence data of 18 genotypes
- DH Pahang reference genome
- 5.5M SNPs
- 5 markers x 11 chromosome

apjack - 1.21.02.04

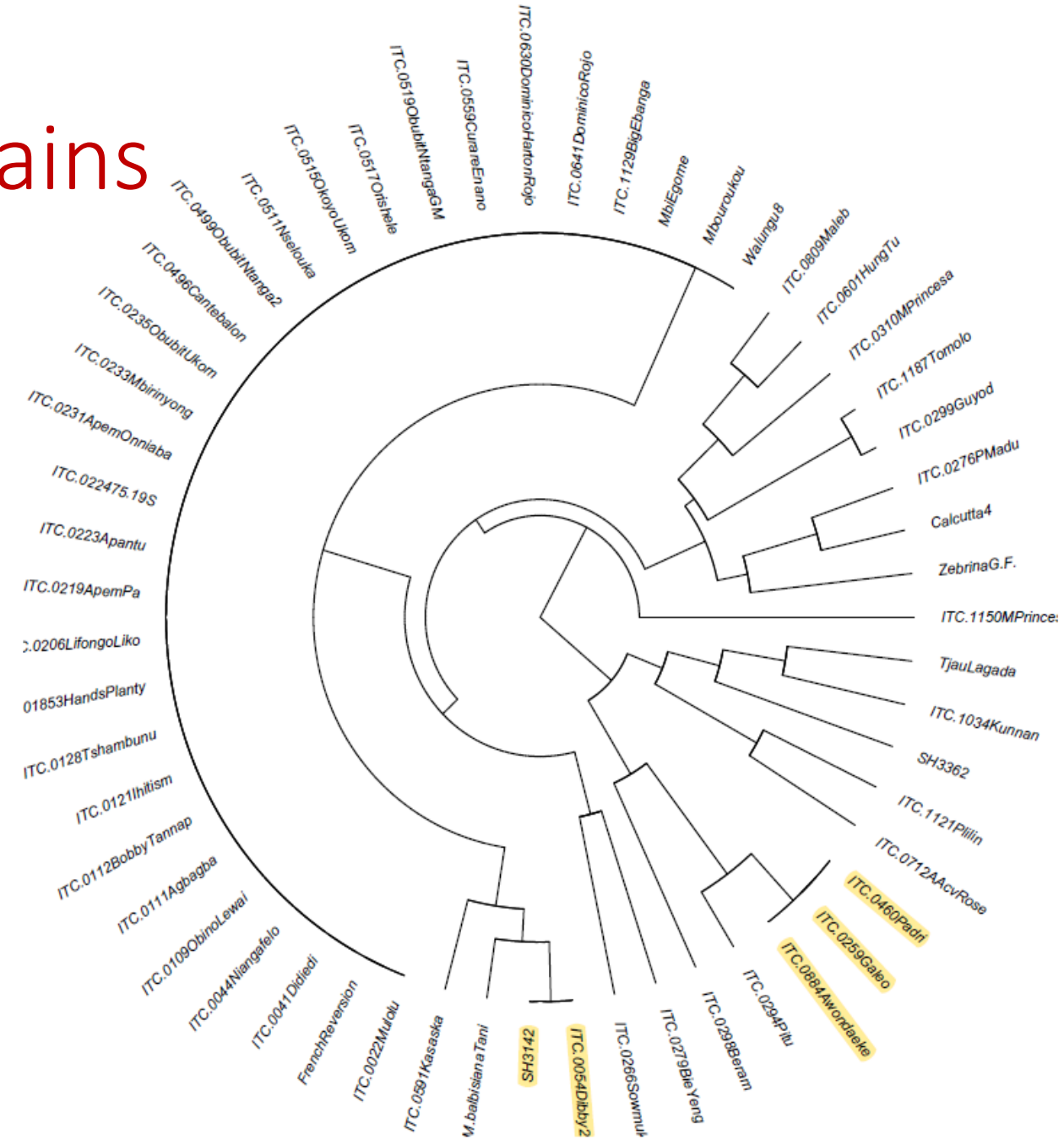
Analysis Data Help





# KASPs for QA/QC in plantains

- All 55 KASP assays
- 376 samples
  - Landrace plantains
  - 2x accessions
  - Hybrids
  - 1 Balbisiana
- 24 KASP markers successful
- 13 KASPs amplified in the B-genome
- Need for B-genome-specific SNPs



# B-genome specific KASPs for QA/QC in plantains

- Plantain genome (Institute of Experimental Botany)
- B-specific super-scaffolds
- From 3428 SNPs to 55 SNPs (5 x 11)
- Verification in 94 genotypes
  - Landrace plantains (AAB)
  - AB accessions
  - BB (*M. balbisiana*) accessions
  - AA controls

sn	SNP ID	Alternative ID	SNP*
1	snpMS00205	musaB_QC_snp_chr01B_19874038	T/C
2	snpMS00206	musaB_QC_snp_chr01B_28041718	A/C
3	snpMS00207	musaB_QC_snp_chr01B_37343370	G/A
4	snpMS00208	musaB_QC_snp_chr01B_45039445	T/C
5	snpMS00209	musaB_QC_snp_chr01B_48775012	A/G
6	snpMS00210	musaB_QC_snp_chr02B_769171	G/A
7	snpMS00211	musaB_QC_snp_chr02B_4044071	G/A
8	snpMS00212	musaB_QC_snp_chr02B_7506532	C/T
9	snpMS00213	musaB_QC_snp_chr02B_12924468	A/G
10	snpMS00214	musaB_QC_snp_chr02B_31110798	T/C
11	snpMS00215	musaB_QC_snp_chr03B_512388	C/T
12	snpMS00216	musaB_QC_snp_chr03B_4047768	A/G
13	snpMS00217	musaB_QC_snp_chr03B_10186000	T/C
14	snpMS00218	musaB_QC_snp_chr03B_16171431	C/T
15	snpMS00219	musaB_QC_snp_chr03B_25329414	C/T
16	snpMS00220	musaB_QC_snp_chr04B_475695	G/A
17	snpMS00221	musaB_QC_snp_chr04B_5940641	C/T
18	snpMS00222	musaB_QC_snp_chr04B_11588536	T/C
19	snpMS00223	musaB_QC_snp_chr04B_22759118	G/A
20	snpMS00224	musaB_QC_snp_chr04B_33404643	G/T
21	snpMS00225	musaB_QC_snp_chr05B_14210146	G/A
22	snpMS00226	musaB_QC_snp_chr05B_22365656	A/G
23	snpMS00227	musaB_QC_snp_chr05B_23839700	C/T
24	snpMS00228	musaB_QC_snp_chr05B_27019922	G/A
25	snpMS00229	musaB_QC_snp_chr05B_36969423	A/G
26	snpMS00230	musaB_QC_snp_chr06B_1774321	T/C
27	snpMS00231	musaB_QC_snp_chr06B_5979616	C/T
28	snpMS00232	musaB_QC_snp_chr06B_16104175	G/A
29	snpMS00233	musaB_QC_snp_chr06B_20177651	G/A
30	snpMS00234	musaB_QC_snp_chr06B_22820524	G/A
31	snpMS00235	musaB_QC_snp_chr07B_745259	G/C
32	snpMS00236	musaB_QC_snp_chr07B_2938033	C/T
33	snpMS00237	musaB_QC_snp_chr07B_15576714	C/T
34	snpMS00238	musaB_QC_snp_chr07B_24910853	G/A
35	snpMS00239	musaB_QC_snp_chr07B_35144551	C/T
36	snpMS00240	musaB_QC_snp_chr08B_1412172	T/G
37	snpMS00241	musaB_QC_snp_chr08B_5602039	A/G
38	snpMS00242	musaB_QC_snp_chr08B_10526087	A/C
39	snpMS00243	musaB_QC_snp_chr08B_23629516	C/T
40	snpMS00244	musaB_QC_snp_chr08B_35890298	C/T
41	snpMS00245	musaB_QC_snp_chr09B_2956888	A/G
42	snpMS00246	musaB_QC_snp_chr09B_10551880	A/G
43	snpMS00247	musaB_QC_snp_chr09B_17930782	G/A
44	snpMS00248	musaB_QC_snp_chr09B_22652596	C/T
45	snpMS00249	musaB_QC_snp_chr09B_37274057	C/T
46	snpMS00250	musaB_QC_snp_chr10B_11671	C/T
47	snpMS00251	musaB_QC_snp_chr10B_10586783	G/A
48	snpMS00252	musaB_QC_snp_chr10B_23222159	T/C



# Application: same name but different genotypes?

Genotype	snpMS00018	snpMS00023	snpMS00024	snpMS00046	snpMS00054	snpMS00061	snpMS00066
Calcutta-4_Sendusu_B	T:T	?:?	T:T	C:C	C:C	A:A	A:A
Calcutta-4_Sendusu	T:T	C:C	T:T	C:C	C:C	A:A	A:A
Calcutta 4_Kawanda	C:C	T:T	T:A	G:G	T:C	G:G	G:G
Calcutta 4_Arusha	T:T	?:?	T:T	C:C	C:C	A:A	A:A

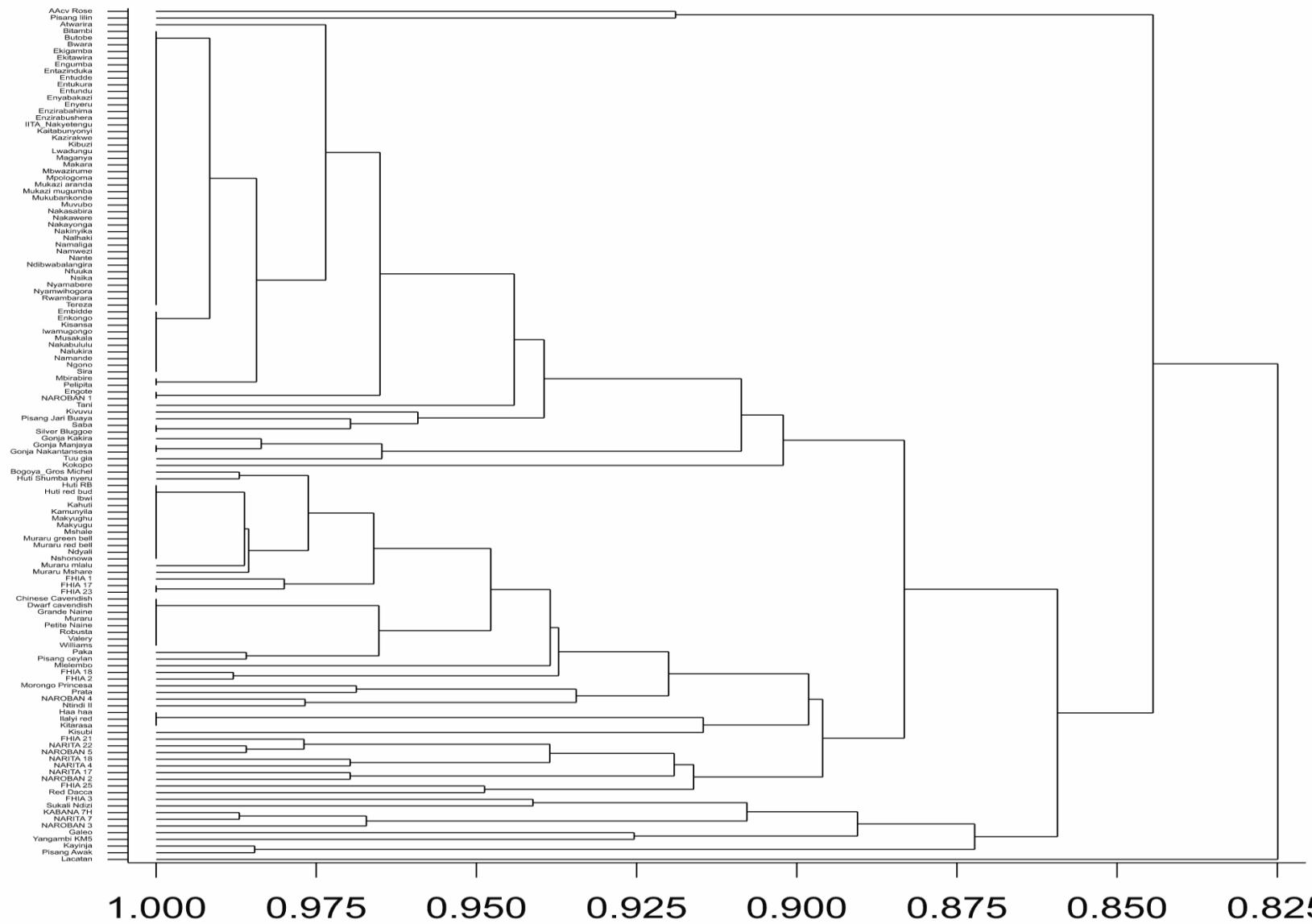
➤ Genotyping of all the shared parents across stations

# F<sub>1</sub> verification

	A	B	C	D	E	F	G	H	I	J	K	L
	Genotype	Female Parent	Male Parent	Field	Breeding program	Location	Number of markers	Considered markers	Non-expected genotype	As expected for F1	% conformit	Remark
2	25291-S62	2829-62	9128-3	2x-2x EET	IITA Plantain	Ibadan	18	18	0	18	100%	
3	25291-S62_B	2829-62	9128-3	2x-2x EET	IITA Plantain	Ibadan	19	18	0	18	100%	
4	IB3985-S3	DT23-15	ITC.0259Galeo	2x-2x EET	IITA Plantain	Ibadan	15	14	0	14	100%	
5	IB3985-S4	DT23-15	ITC.0259Galeo	2x-2x EET	IITA Plantain	Ibadan	19	18	0	18	100%	
6	IB3985-S6	DT23-15	ITC.0259Galeo	2x-2x EET	IITA Plantain	Ibadan	18	18	0	18	100%	
7	IB3966-S1	DT61-15	ITC.0259Galeo	2x-2x EET	IITA Plantain	Ibadan	12	11	0	11	100%	few number of markers
8	IB2400-2	EKUL1142	ITC.0294Pitu	2x-2x EET	IITA Plantain	Ibadan	11	11	0	11	100%	few number of markers
9	DT49-5	ITC.0259Galeo	Calcutta4	2x-2x EET	IITA Plantain	Ibadan	16	15	0	15	100%	
0	DT49-7	ITC.0259Galeo	Calcutta4	2x-2x EET	IITA Plantain	Ibadan	17	16	0	16	100%	
	DT106-1	ITC.0276PMadu	Calcutta4	2x-2x EET	IITA Plantain	Ibadan	15	10	0	10	100%	few number of markers
2	IB2479-1	ITC.0884Awondaeke	Calcutta4	2x-2x EET	IITA Plantain	Ibadan	18	11	0	11	100%	few number of markers
3	IB2479-8	ITC.0884Awondaeke	Calcutta4	2x-2x EET	IITA Plantain	Ibadan	12	9	0	9	100%	few number of markers
4	ON864-S1	PITA 12	1297-3	PYT trial genotype	IITA Plantain	Ibadan	19	19	0	19	100%	
5	EKUL1142	ZEBRINA GF	Calcutta4	2x-2x EET	IITA Plantain	Ibadan	18	12	0	12	100%	
6	IB4094-S2	1297-3	9128-3	2x-2x EET	IITA Plantain	Ibadan	18	18	1	17	94%	
7	IB4094-S1	DT23-2	ITC.0259Galeo	2x-2x EET	IITA Plantain	Ibadan	18	17	1	16	94%	
8	IB4010-S3	DT44-1	ITC.0712 Aacv Rose	2x-2x EET	IITA Plantain	Ibadan	13	10	1	9	90%	few number of markers
9	IB3996-S1	DT61-15	ITC.0259Galeo	2x-2x EET	IITA Plantain	Ibadan	16	15	1	14	93%	
0	DT23-2	ITC.0259Galeo	Calcutta4	2x-2x EET	IITA Plantain	Ibadan	17	15	1	14	93%	
1	DT61-15_B	ITC.0259Galeo	Calcutta4	2x-2x EET	IITA Plantain	Ibadan	16	15	1	14	93%	
2	DT51-13	ITC.0259Galeo	Calcutta4	2x-2x EET	IITA Plantain	Ibadan	17	16	1	15	94%	

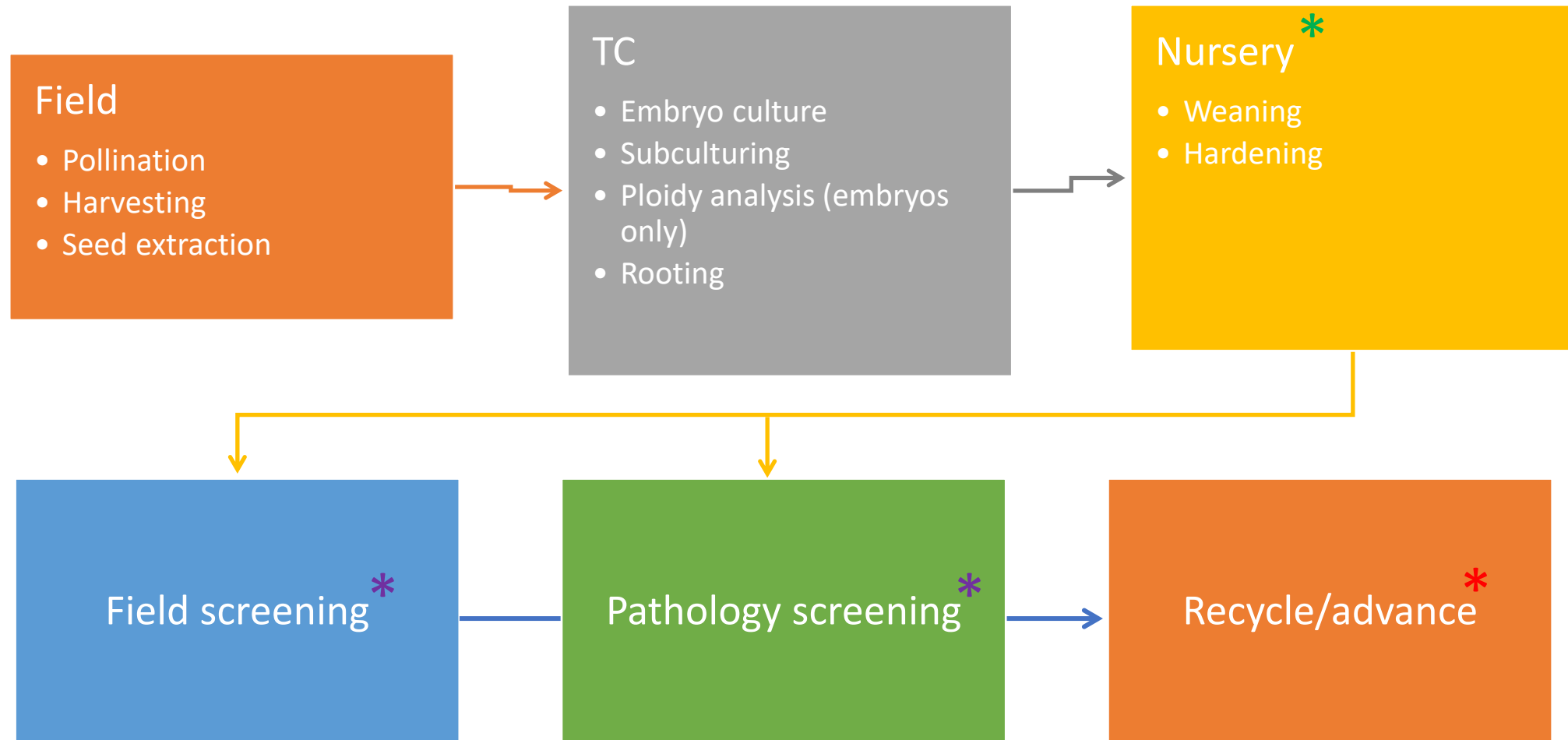
# Impact study on banana varieties in Uganda

- Impact study with SPIA in Uganda
- Reference library of 133 banana genotypes
- Field sampling of 3,290 samples
- Genotyping with 33 QC/QA markers and LD DArTSeq



# Application of QA/QC markers

## Parental Improvement (2x and 4x)

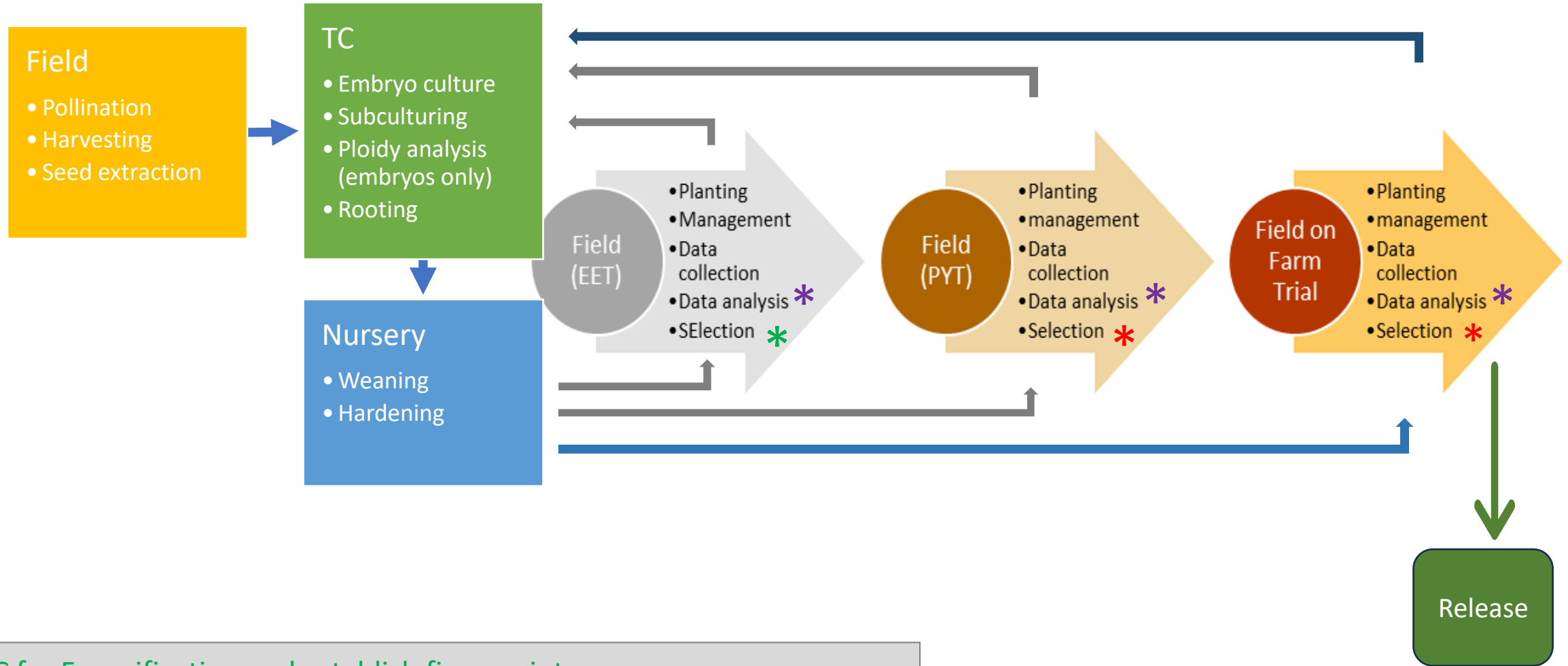


\*QC for F<sub>1</sub> verification and establish fingerprint

\*QC for true-to-typeness/identity

\*Leaf sampling, QC on outliers or inconsistent plants

# Product Development and Selection



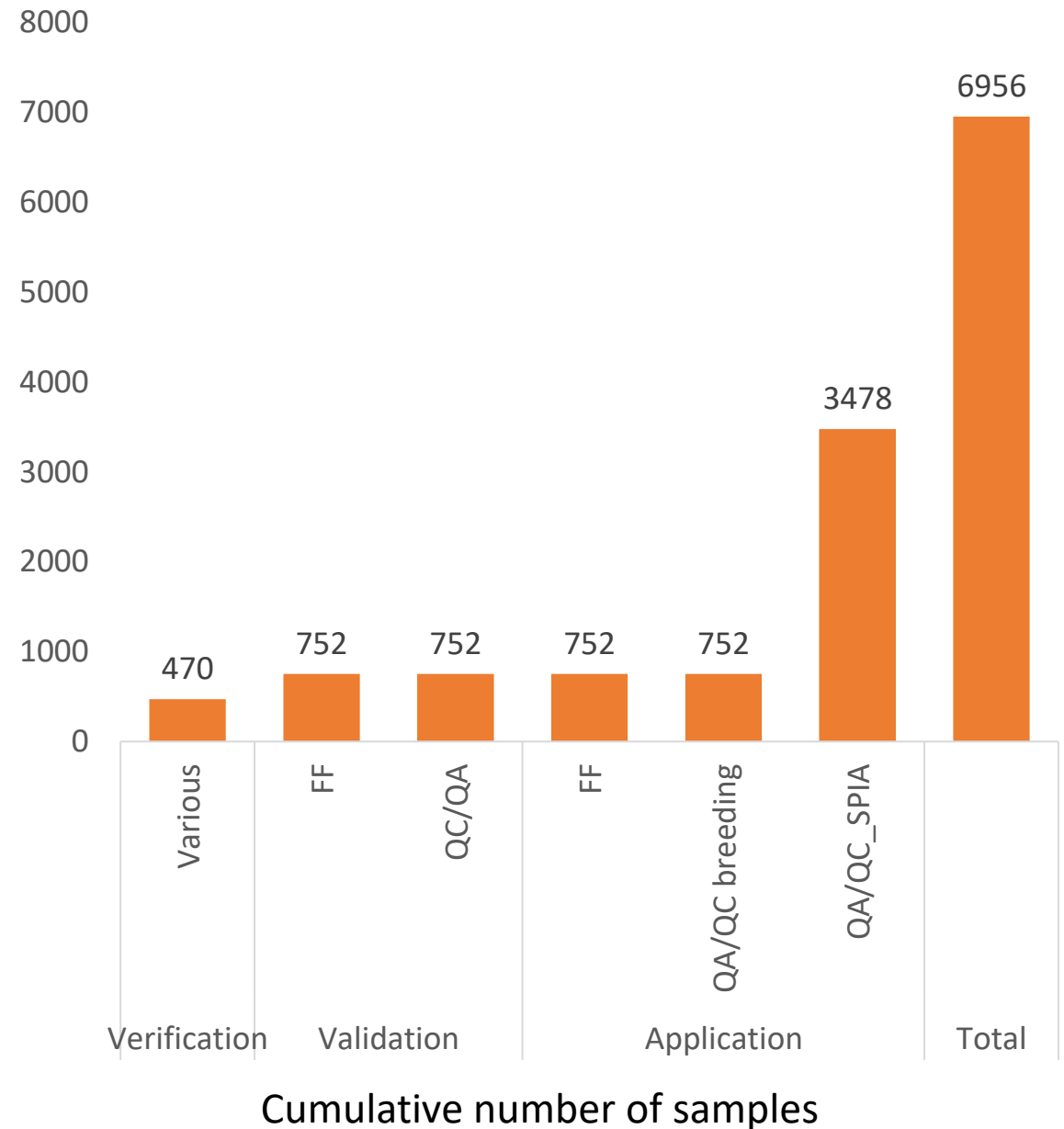
\*QC for F<sub>1</sub> verification and establish fingerprint

\*QC for true-to-typeness/identity

\*Leaf sampling, QC on outliers or inconsistent plants

# Challenges

- Calling as 2x
  - Use of fitPoly for 3x and 4x (extra time)
- Low number of samples per station per season
  - Pooling samples across stations
- Need to streamline QA/QC markers in the breeding process



# Conclusion

- KASP markers applied for MAS for fruit-filling
- KASPs verified for more traits, validation in 2024
  - Resistance to weevils
  - Resistance to banana bacterial wilt
  - Resistance to Foc TR4
  - B-genome-specific QA/QC SNPs
- QA/QC needs to be streamlined in the breeding process

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