





Harnessing the potential of highly informative KASP and middle density SNP markers panel for yam breeding

Crops to End Hunger webinar August, 2023





Outlines

Introduction

Low density SNP markers

- QC/QA marker development, validation and application
- Trait marker validation

Middle density SNP markers

 Panel SNP markers: Middle density of 3092 SNP for GS/GPCP

Development of reference genome

Ongoing molecular activities

Take home message









Introduction

- Mis-labelling error frequently occurred in germplasm management
- Genetic purity and variety tracking
- Pedigree verification (intra and inter specific)
- Trait performance prediction at early generation through MAS
- Optimizing the breeding program
- Parental selection & heterotic group
- Implementation of genomic
 prediction and cross performance

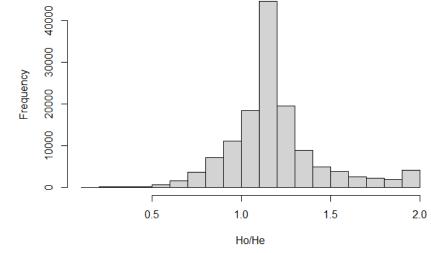


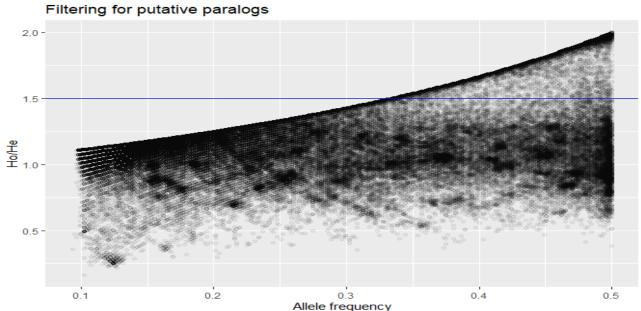


QC/QA Marker: Selection procedure

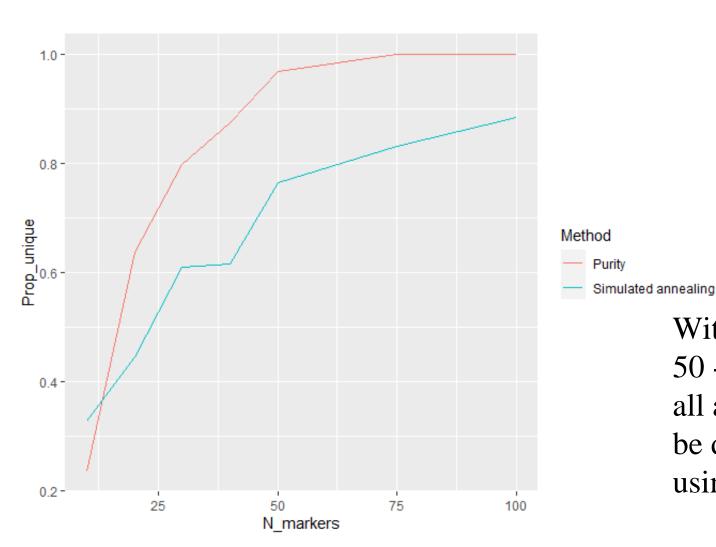
Histogram of Ho/He

- o PIC
- Ho/He
- MAF
- **Putative Paralogs**
- GC content
- Duplicate
- Missing %
- **INDELS**





QC/QA Marker: Selection procedure

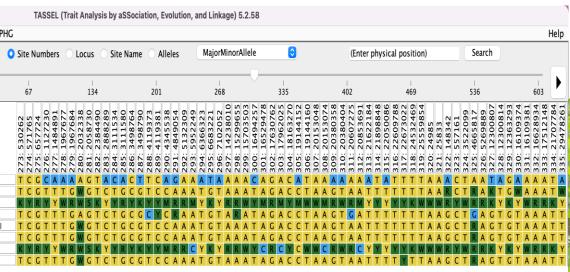


With a range from 50 - 100 markers, all accessions could be distinguished using Purity method

https://github.com/HPCBio/eib-marker-design/blob/main/Pedigree_verification.md



Low density SNP markers: QC/QA QC/QA Marker: selection procedure

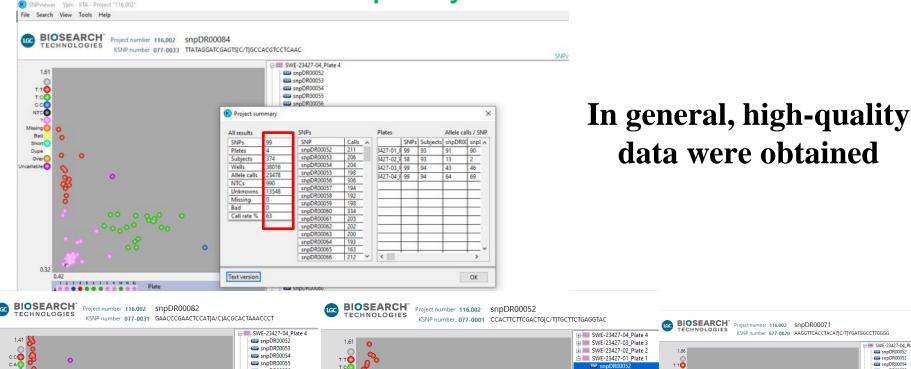


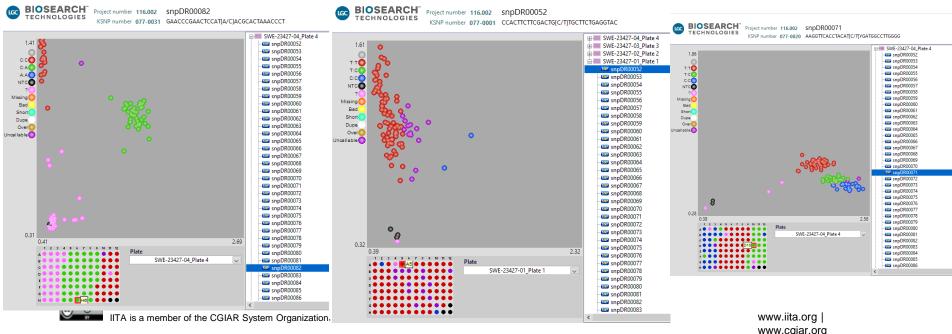


- 99 SNP markers well distributed across the genome were selected and successfully converted to KASP
- 374 diverse clones representing D. alata, D. rotundata, D. praehensilis, D. esculenta D. cayenensis sent to INTERTEK for validation

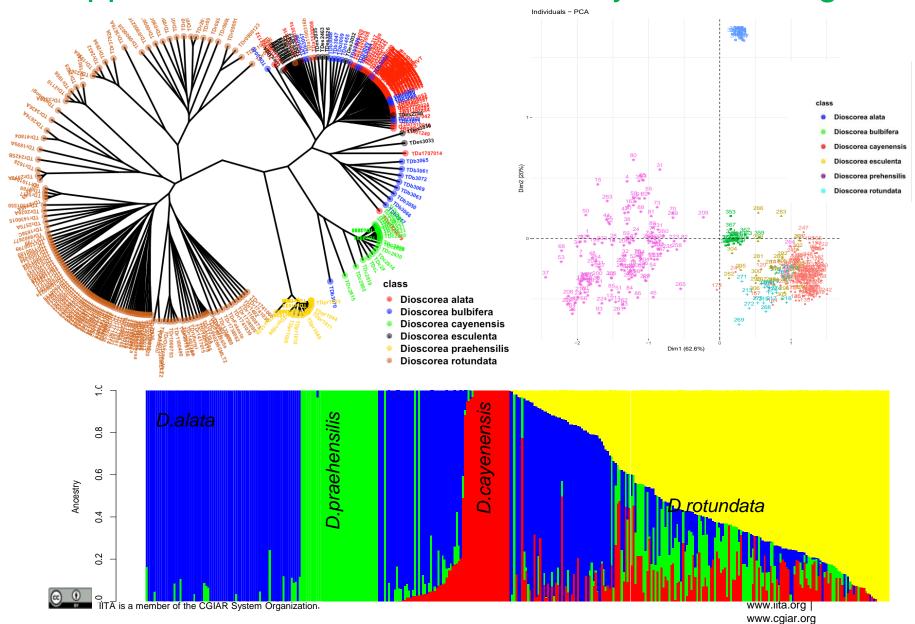


SNP markers quality view

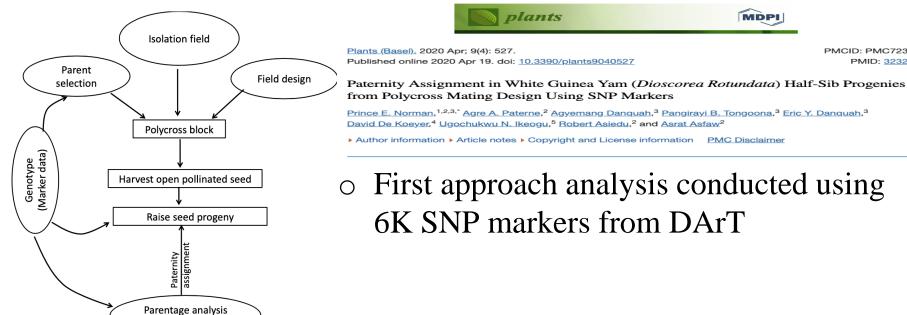




Application of QC/QA markers in yam breeding

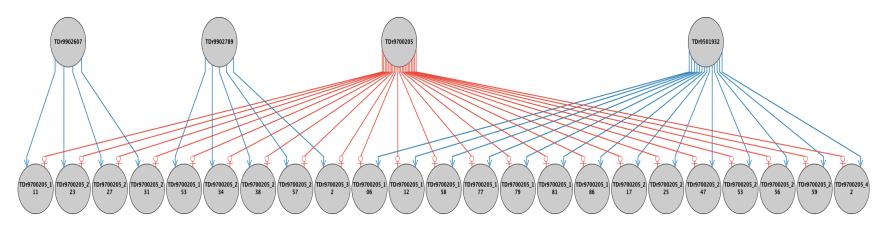


Application of QC/QA markers: Pedigree reconstruction



First approach analysis conducted using

Replicated work using only 50 QC markers



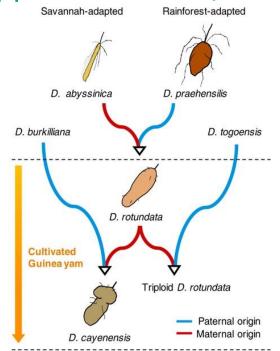
PMCID: PMC7238154

PMID: 32325826

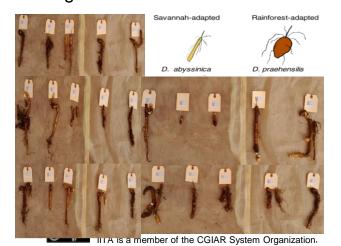
(Molecular data+model)



Application of QC/QA markers: Fidelity of crosses/hybridity test



Sugihara et al. 2020



GG (100%G) TT (100%T) TT (100%T) TT (100%T) TC(505T & 50%C) TC(505T & 50%C) Hybrid INTERSPCE-D09 INTERSPCE-G09 INTERSPCE-C10 INTERSPCE-A12 INTERSPCE-B12 INTERSPCE-C02 INTERSPCE-D02 INTERSPCE-F02 INTERSPCE-G02 INTERSPCE-H02 INTERSPCE-BO3 INTERSPCE-CO3 INTERSPCE-D03 INTERSPCE-E03 INTERSPCE-E03 INTERSPCE-G03 INTERSPCE-H03 INTERSPCE-A04 Hybrid

Marker segregation among the inter-specific progenies

Genetic purity

- Fourteen SNP selected for varietal tracking, verification
- Genotypes were selected from trials: APT, NPT, RVT & VVT
- Six plants selected per plot and rep across various locations

TDrRVT2022IBN_106	Oju-iyawo	TC	TA	GG	TC	GA	TC	GC	TC	TC	GA	TC	TC	TC
TDrRVT2022IBN_127	Oju-iyawo	TC	TA	GG	TC	GA	TC	GC	TC	TC	GA	TC	TC	TC
TDrNPT1 2022IBN 11	Ojuiyawo	TC	TT	GG	TC	GA	TC	GG	TC	TC	GA	TC	TC	TC
TDrNPT1 2022IBN 21	Ojuiyawo	TC	TT	GG	TC	GA	TC	GG	TC	TC	GA	TC	TC	TC
TDrNPT1 2022IGO 11	• •	TC	TT	GG	TC	GA	TC	GG	TC	TC	GA	TC	TC	TC
	Ojuiyawo													
TDrNPT1_2022IGO_21	Ojuiyawo	TC	TT	GG	TC	GA	TC	NN	TC	NN	GA	TC	TC	TC
TDrNPT1_UB111	Ojuiyawo	CC	NN	NN	CC	NN	NN	NN	CC	TT	NN	NN	NN	CC
TDrNPT1_UB121	Ojuiyawo	CC	NN	GG	NN	NN	NN	GG	TC	NN	NN	CC	CC	CC
TDrRVT2022Ubiaja_103	Ojuiyawo	TC	TT	GG	TC	GA	TC	GG	TC	TC	GA	TC	TC	TC
TDrAPT_2022IBN3	TDr1000048	TC	TT	GG	TC	GA	TC	GC	TC	TT	GA	TC	TC	TC
TDrAPT_2022IBN12	TDr1000048	TC	TT	GG	TC	GA	TC	GC	TC	TT	GA	TC	TC	TC
TDrAPT_2022IBN23	TDr1000048	TC	TT	GG	TC	GA	CC	GC	TC	TT	GA	TC	TC	TC
TDrAPT_2022IBN25	TDr1000048	TC	TT	GG	TC	GA	CC	GC	TC	TT	GA	TC	TC	TC
TDrAPT_2022IBN37	TDr1000048	TC	TT	GG	TC	GA	TC	GC	TC	TT	GA	TC	TC	TC
TDrNPT2_2022IBN_2	TDr8902665	TC	TT	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrNPT2_2022IBN_27	TDr8902665	TC	TT	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrNPT2_2022_IGO_6	TDr8902665	TC	TT	GC	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrNPT2_2022_IGO_28	TDr8902665	TC	TT	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrRVT2022IBN_112	TDr8902665	TC	TT	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrRVT2022IBN_132	TDr8902665	TC	TA	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrAPT_2022IBN6	TDr8902665	TC	TT	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrAPT_2022IBN13	TDr8902665	TC	TT	GG	TC	GA	TC	GC	TC	TT	GA	TC	TC	TC
TDrAPT_2022IBN24	TDr8902665	TC	TT	GG	TC	GA	TC	GC	TC	TT	AA	TC	TC	TC
TDrAPT_2022IBN26	TDr8902665	TC	TT	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrAPT_2022IBN38	TDr8902665	TC	TT	GG	TC	GA	TC	GC	TC	TT	GA	TC	TC	TC
2022VVT_IWO_104	TDr8902665	TC	TT	GC	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
2022VVT_EDE_4	TDr8902665	TC	TT	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrNPT2_2022Ubiaja_6	TDr8902665	TC	TT	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC	TC
TDrNPT2_2022Ubiaja_28	TDr8902665	TC	TT	GG	TC	GA	TC	GG	TC	TT	GA	TC	TC CC	TC
TDrRVT2022Ubiaja 116	TDr8902665	TC	TT	GG	TC	GG	TC	GG	TC	TT	AA	TC	CC	CC

www.cgiar.org



Selection of promising markers

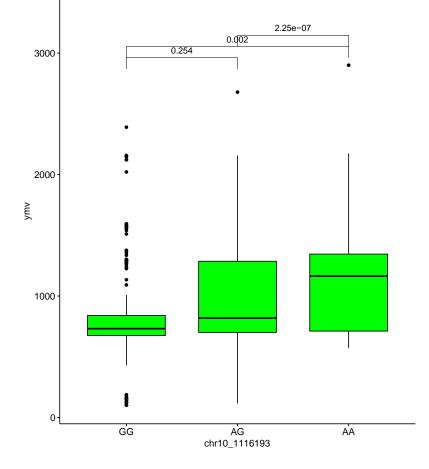
SNP markers already identified using GWAS and QTL mapping

Traits	Species	Sources	Number of QTL	Promising QTL	Validated QTL		
YMV	D.rotundata	Association mapping	15	4	2		
Yield per plant	D.rotundata	Association mapping	18	3	2		
Plant sex	D.rotundata	Association mapping	14	2	2		
Flowering Intensity	D.rotundata	Association mapping	7	4	2		
Plant vigor	D.rotundata	Association mapping	3	1	1		
Tuber appearance	D.rotundata	Association mapping	4	2	1		
YAD	D.alata	Linkage mapping	5	2	NA		
Dry matter	D.alata	Association mapping	2	2	NA		
Oxidation	D.alata	Association mapping	4	2	NA		
Plant sex	D.alata	Association mapping	57	-	-		
ACR	D.alata	Association mapping	12	-	-		
PHC	D.alata	Association mapping	6	-	-		
PHC	D.rotundata	Association mapping	2	-			
ACR	D.rotundata	Association mapping	2	-			



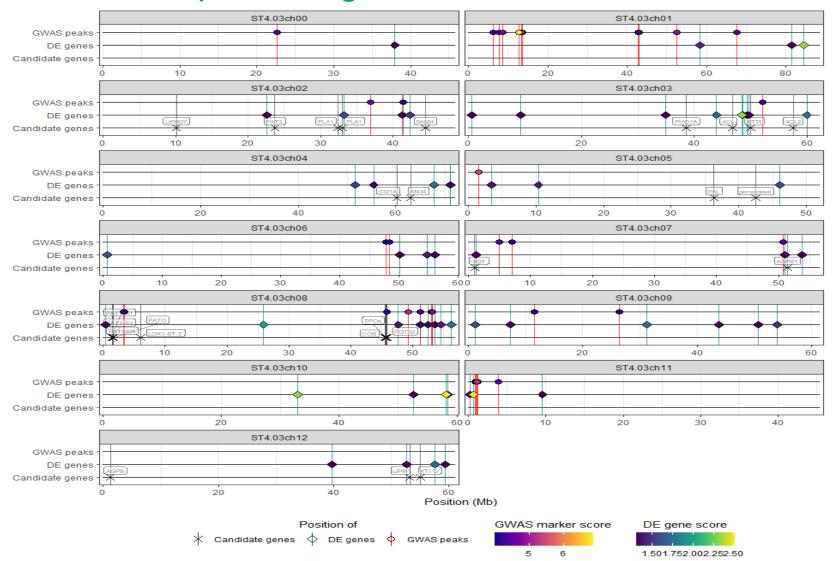
Selection of promising markers

	_					
YMV	chr03_6338751	Нар1	GGGT	0.427	1.000	ns
		Нар2	GGTT	0.320	1.000	ns
		Нар3	GTTT	0.466	1.000	ns
	chr10_1116193	Нар1	AAAG	0.226	0.254	ns
		Hap2	AAGG	0.309	0.003	**
		Нар3	AGGG	0.465	6.75 e ⁻⁰⁷	***
	chr15_3906069	Hap1	AAAC	0.214	0.882	ns
		Hap2	AACC	0.281	0.882	ns
		Нар3	ACCC	0.412	0.882	ns
	chr16_1482029	Нар1	AAAT	0.307	0.096	ns
		Hap2	AATT	0.424	2.01 e ⁻⁰⁴	***
		Hap3	ATTT	0.576	0.006	**
	chr05_30671001	Нар1	AAAG	0.365	1.000	ns
		Нар2	AAGG	0.265	1.000	ns
		Нар3	AGGG	0.369	1.000	ns





Selection of promising markers



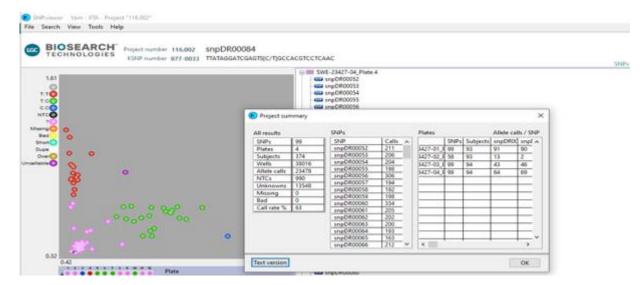


Seven traits were considered for the marker validation in *D. rotundata*

- Tuber yield per plant
- Yam mosaic virus
- Plant sex,
- Flowering intensity
- Plant vigor
- Tuber appearance
- Tuber shape

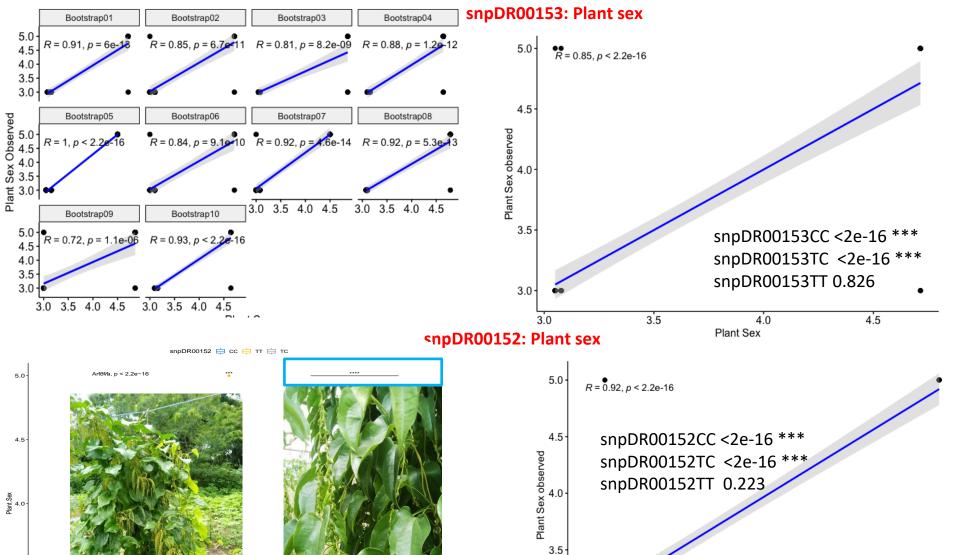
Flanking sequencing around the target region associated with each trait was developed and sent to INTERTEK for conversion into KASP-PCR

- For traits like sex, clones with well known sex information and those with unknow sex status were selected for the validation
- For disease, clones susceptible or tolerant to disease were used for the validation
- We then developed haplotype variant to estimate the marker prediction accuracy of each marker and for each trait



snpDR00152 IIIA IS A IIIEIIDEI OI LIE OOMAN SYSTEIII OIYAIIZALIOII-

Low density SNP markers: Trait markers



3.0

3.0

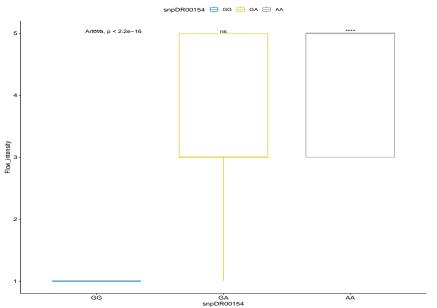
3.5

4.0

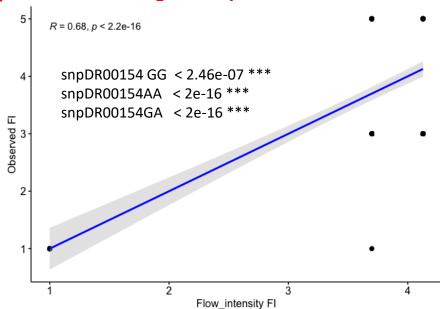
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Plant Sex

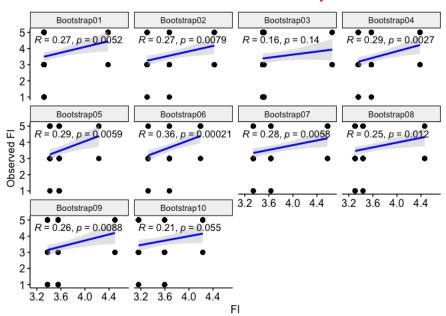
4.5

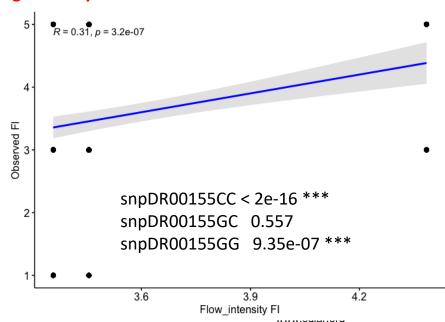


snpDR00154: Flowering intensity

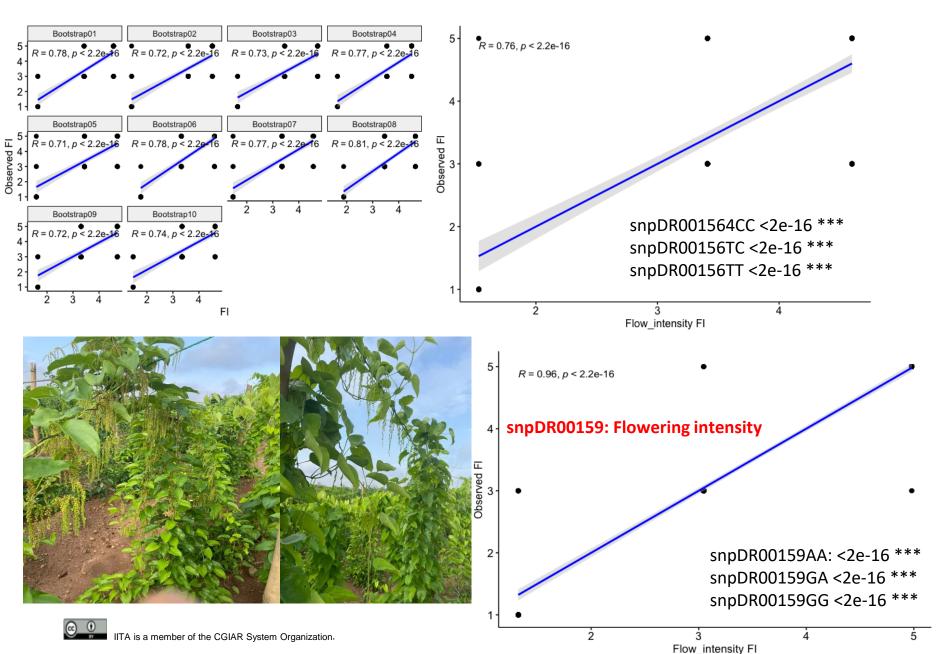


snpDR00155: Flowering intensity

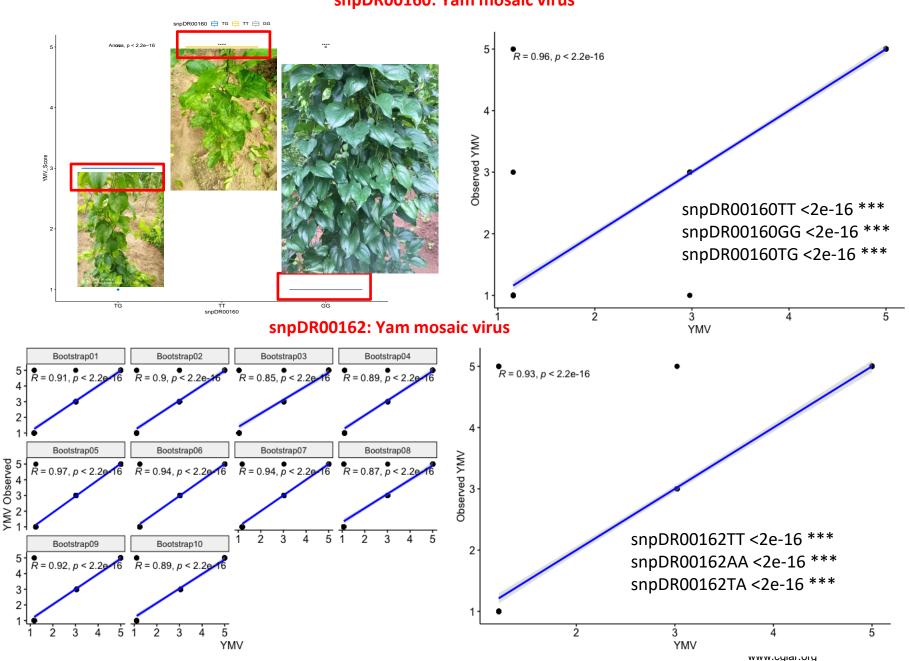


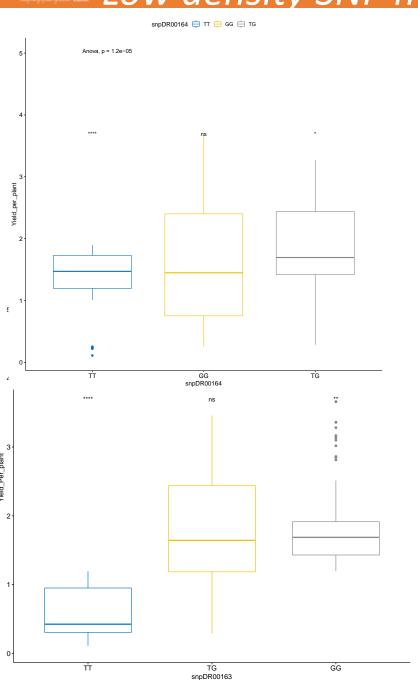


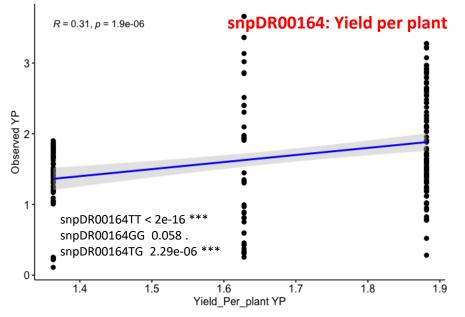
snpDR00156: Flowering intensity



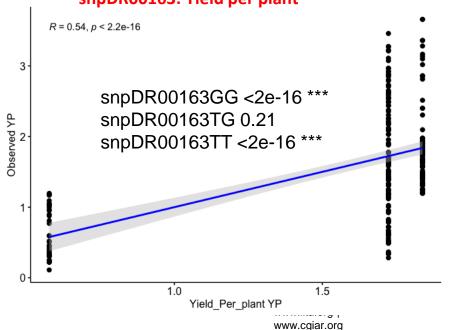








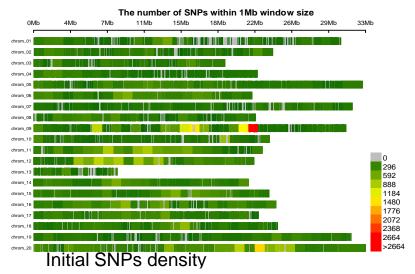


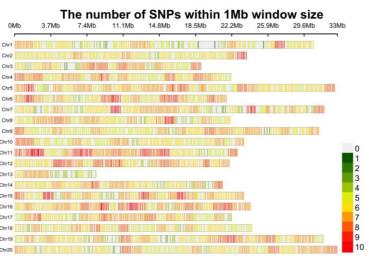


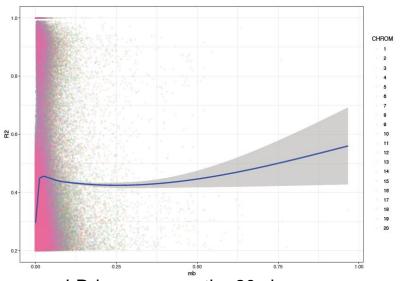


Middle density SNP markers

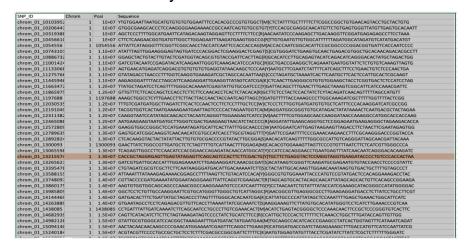
Highly Informative SNP Marker Panels in Yam







LDdecay across the 20 chromosomes



A total of 3092 SNP markers selected across the yam genome including the QC/QA the traits associated markers



www.iita.org | www.cgiar.org

*Additional yam reference genome



https://data.cimmyt.org/dataset.xhtml?persistentId=hdl%3A11529%2F10548882





Ongoing molecular activities

- Deployment of MAS in the breeding program
- Annotation and gene model prediction
- Gene expression analysis for semi-dwarf genotypes
- Validation of middle density SNP markers and deployment for product advancement and for GPCP
- Identification of tolerant/resistant inter-specific genotypebased marker coupled with quantitative method
- Variety tracking and monitoring adoption of improved yam varieties
- Implementation of GS and prediction
- Effect of heterozygosity level on trait association



Take home message



QC/QA markers were successfully developed and deployed in yam breeding for routine activities



Trait linked markers developed and deployed for MAS for key traits; ongoing work on validating additional markers



Middle density SNP markers developed and in the process of validation for GS as well as for GPCP



Additional reference genome developed to accelerate discovery in yam with the support of EiB/CtEH



Continuing application of low, middle and high-density SNP markers in the breeding program for breeding optimization.





Acknowledgements



Excellence in Breeding

















Total Quality. Assured.







