

Usage of cowpea low and mid-density panels

Patrick O. Ongom: IITA, Kano Nigeria
Ousmane Boukar: IITA, Kano, Nigeria
Christian Fatokun: IITA, Ibadan, Nigeria
Timothy Close: UCR Riverside, USA
Stefano Lonardi: UCR Riverside, USA
Ana Luisa Garcia-Oliveira: CIMMYT, Nairobi, Kenya
About Togola: IITA, Kano, Nigeria

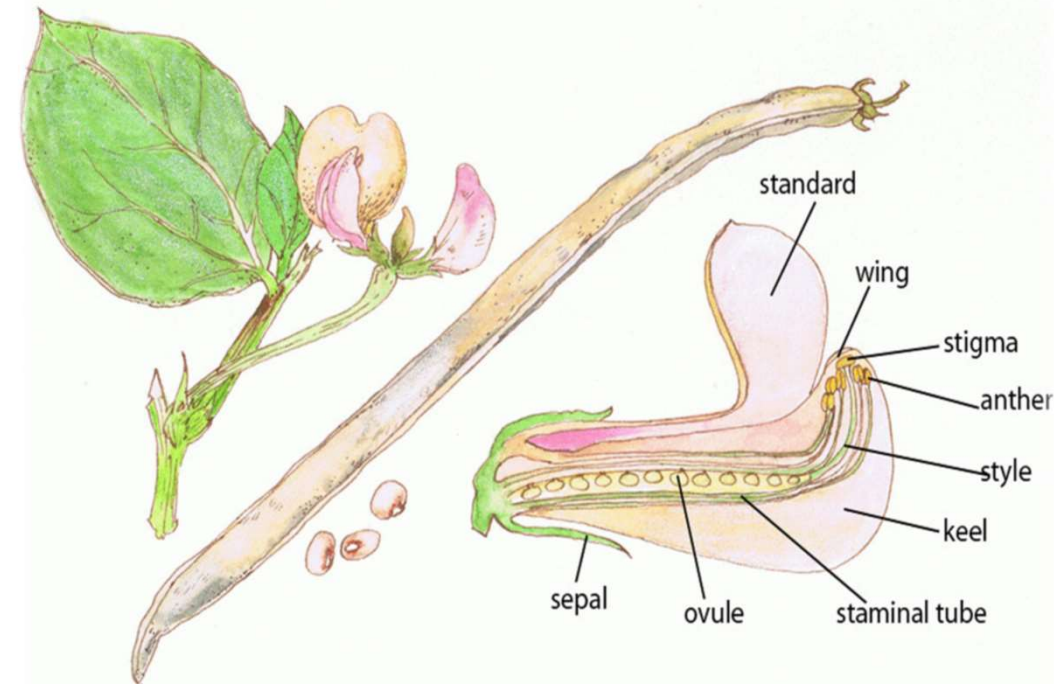
Crops to End Hunger Case Studies in Africa and Beyond: Supporting CGIAR Partners through Genotyping Services. October 19th 2023.

Outline

- Background
- Breeding objectives
- Cowpea genomic resources
- Genomic integration strategy
- Low-density panels: Development and applications
- Mid-density panels: Development and applications
- Summary

Cowpea

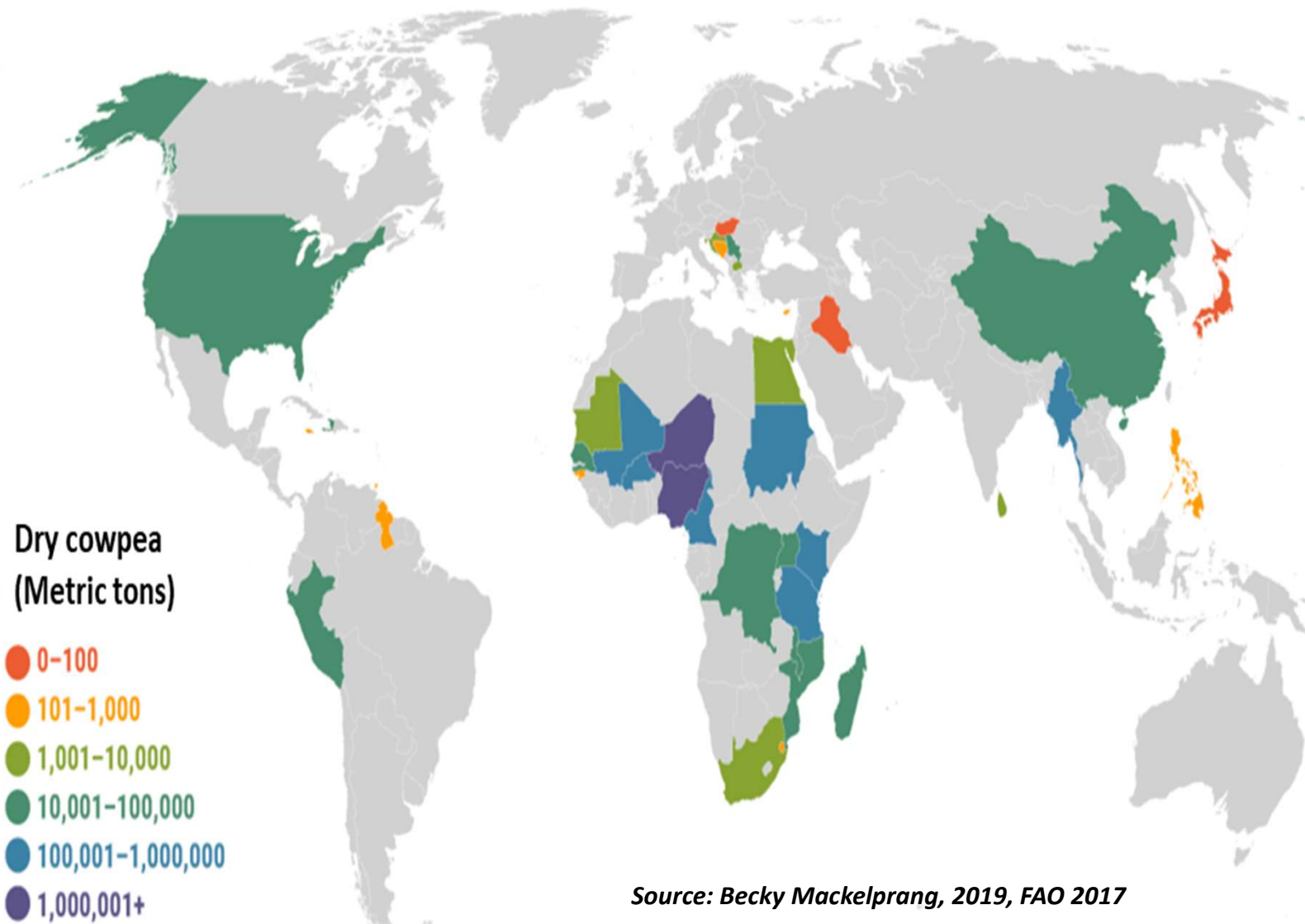
- **Name:** *Vigna unguiculata* (L.) Walp.
- **Genome:** Diploid ($2n = 2x = 22$), genome size of **640.6 Mbp**
- **Reproduction:** Self-pollinated, **1-2% outcrossing**
- **Origin:** Recent evidence shows **West Africa as the centre of domestication**



Flowering shoot, seed pod, seeds, and longitudinal section of flower of *Vigna unguiculata*, cowpea

Cowpea statistics

- Area: 14.9 m ha
- Production: 8.9 m tons
- SSA: 7.8 m tons
- SSA Yield: 602.6 Kg/ha
- Cultivated >88 countries
- Nigeria is the largest producer in Africa (46% of world production)



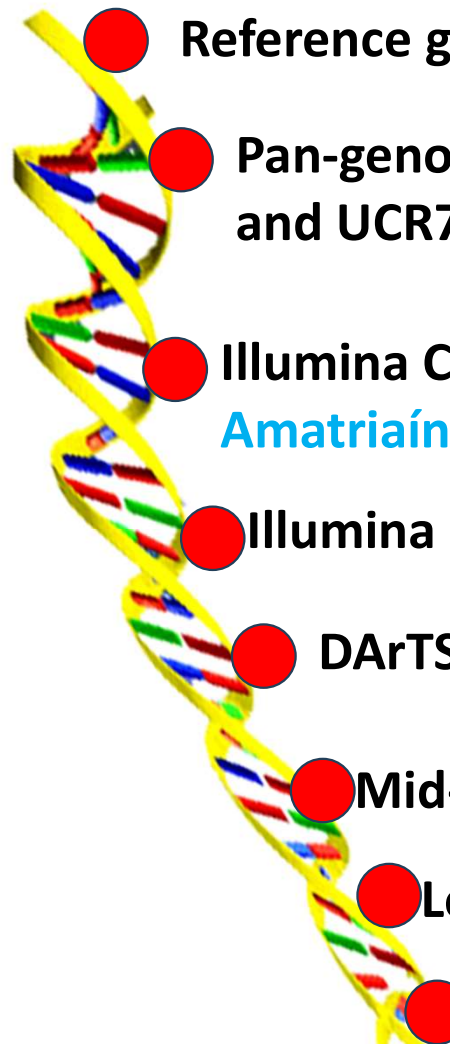
Source: Becky Mackelprang, 2019, FAO 2017

Constraints and Breeding Focus

Objectives: Develop Short, Medium, and Late duration cowpea varieties with enhanced resistance to Striga, Insects, and Diseases, and with agronomic attributes of high yield, and well adapted to the dry and hot zones in the tropics, targeting medium to large, white and brown seeded cowpea for food and feed.



Cowpea genomic resources

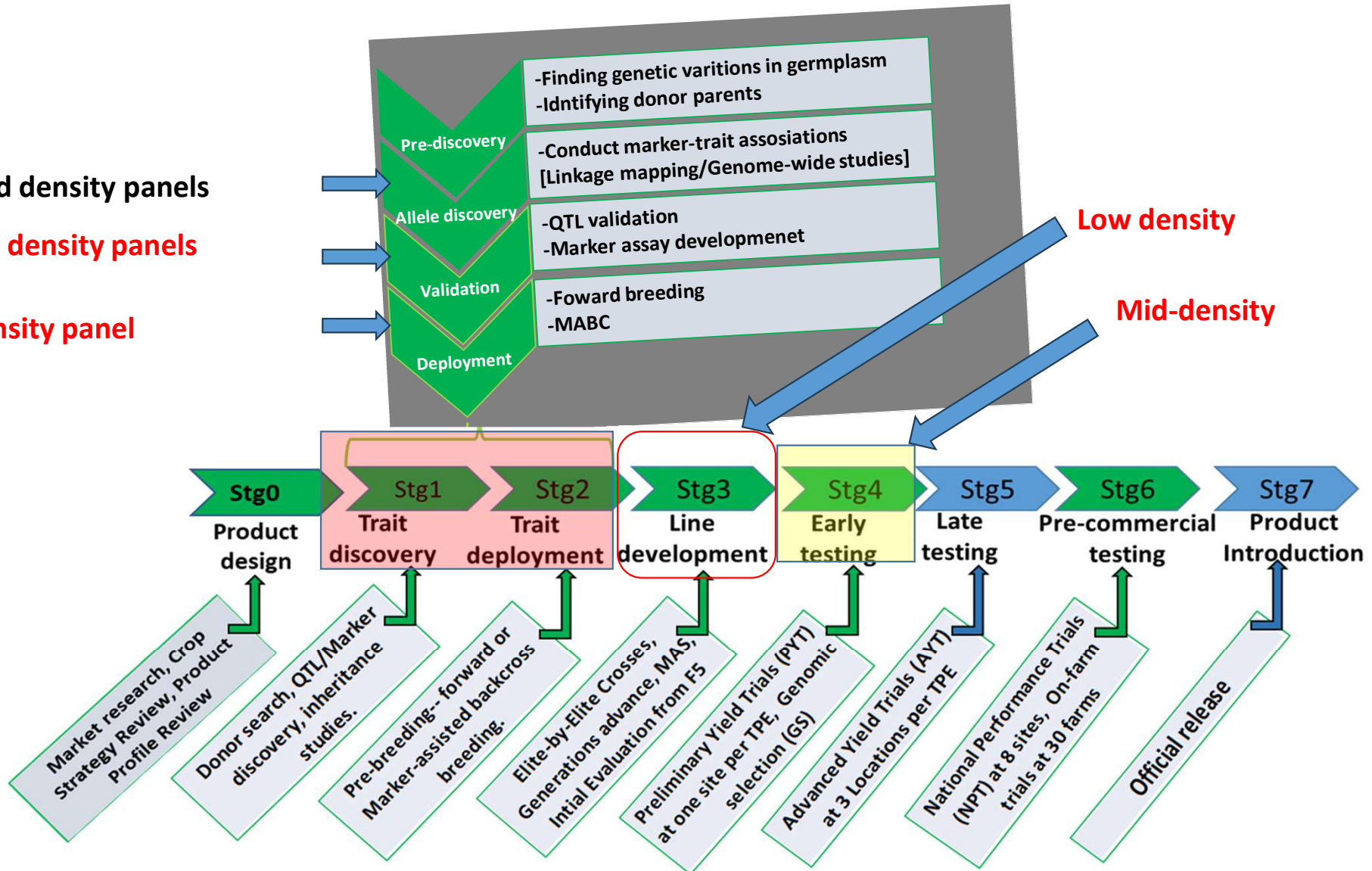
- 
- Reference genome of IITA variety IT97K-499-35 [[Lonardi et al 2019](#)].
 - Pan-genome: de novo assemblies of CB5-2, TZ30, ZN016, Suvita-2, Sanzi, and UCR779 [[Liang, Muñoz-Amatriaí...Close...et al 2023](#)]
 - Illumina Cowpea iSelect Consortium Array: 51 128 SNPs [[Muñoz-Amatriaín ..Close..et al 2016](#)]
 - Illumina 1,536-SNP GoldenGate genotyping array [[Muchero et al 2009](#)]
 - DArTSeq platform
 - Mid-density DArTag platform: ~ 2K SNPs [[EiB, Ongom et al.pre-print](#)]
 - Low-density QC/QA KASP panel: 17SNPs [[EiB, Ongom et al 2021](#)]
 - Low-density Traits-based KASP panels [[EiB](#)]

How genomics is being integrated

High/mid density panels

Mid/low density panels

Low density panel



Low-density marker panels

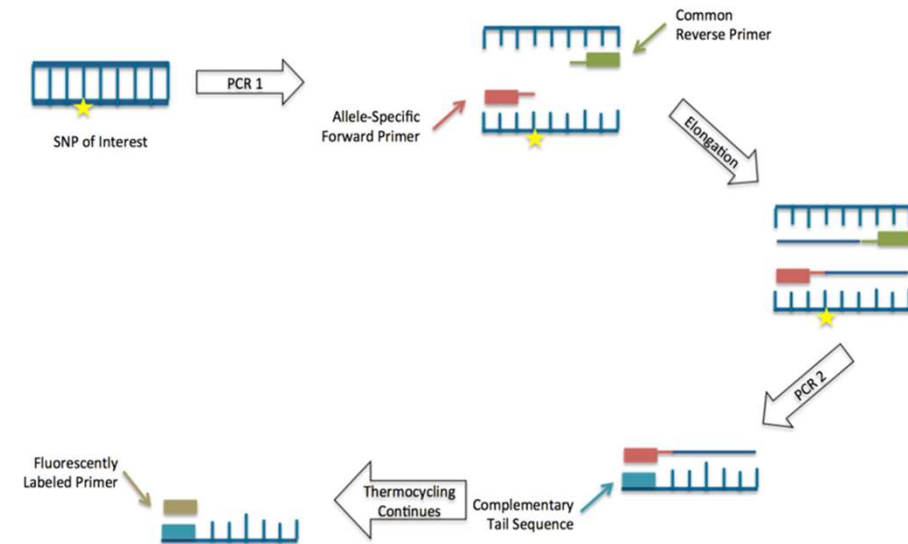
Two Types of low-density markers

1. QC/QA markers
2. Traits-specific markers

All based on KASP (Kompetitive allele-specific PCR)

KASP assay reaction contains:

- 2 Allele specific forward primers with fluorescent labels,
- 1 common reverse primer and the sample DNA



Fwrd Primer 1: TCTAGCACCAGACCAGAGCCACGGA---FAM-tail
 Fwrd Primer 2: TCTAGCACCAGACCAGAGCCACGGT---HEX-tail
 Reverse Primer: TCGCATCATCAAACCTTGTAATAGG

A = ● Homozygous for A allele
 T = ● Homozygous for T allele
 AT = ● Heterozygous for T allele

Majeed et al 2019; and LCG genomics

Cowpea QC/QA panel: SNP selection

Initially, 177 diverse cowpea lines were genotyped with **Illumina 1,536-SNP GoldenGate assay**

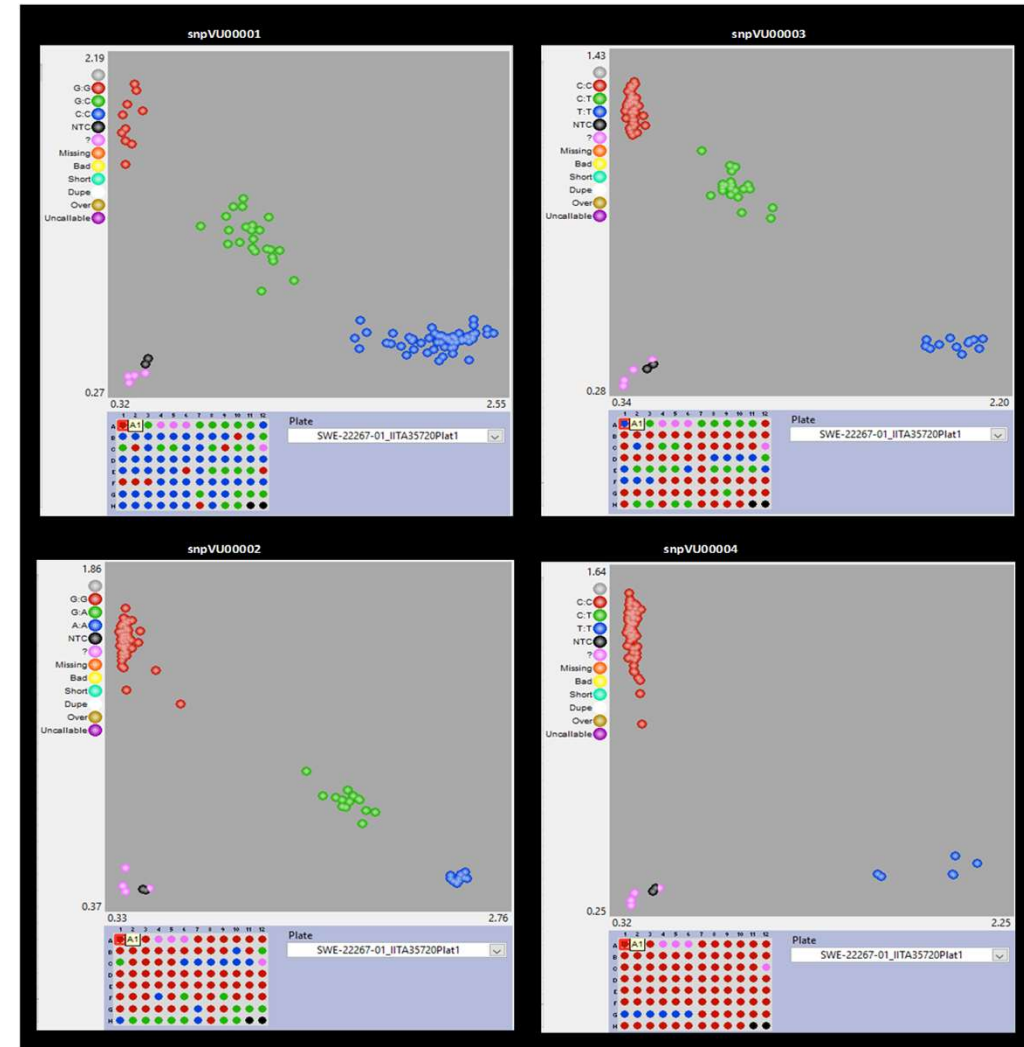
SNPs for QC were selected based on:

- High polymorphism
- SNPs call neutrality
- Low locus heterozygosity (<4%)
- Linkage group coverage (2 SNPs/ LG)

SNP ID	Chr	SNP	Design sequence
snpVU0001	1	c/g	ATGGAGGATTATATCCTCCATTGAGCAGAAGGAGGAGAGCAGAGGCAATGAGGACCACGT[C/G]GCTATTATAAAGGAGTACAGGGGAAAGATTGAGACCCGAACTCAGCAAGATCTGTGATGGG
snpVU0002	1	a/g	GCTCGTAGCAGCCTTATCTCCACTGTTCCATCCAAGCTGCAACAATCATTTCATTCTGG[A/G]ACATGGGAAGATCGAACTAGAGTCAGGTCCTCTCCGAGCATTGATCGCTCCAGTAATA
snpVU0003	2	a/g	GGGAGTTGAATGAAAACCTCTGCTCACTGAKGTAGAGATGGAAGATTCAGAAGAAGCGAC[A/G]AGTTTTGCGGCGGATCTGAGTGC GTTGTGAAGGCCATTGGTAGCGCCGATTGGATTGCGA
snpVU0004	2	a/g	AGAAGCATCAGTCAACTCTTCACAGCATCCAATGTCCAGTGCCTCAAGGTTTCTGCATTG[A/G]CTTAAAACACAGCTCAATGAGGAATCAGAAATGTTTAGACCAATCCATCCTTAAGTTT
snpVU0007	4	a/g	AAAAGACTAGAACCAACAAAACATACTCCTCGTAACTACTTTTGTAGACAGATTTTCAG[A/G]CACCATCAAACATTCATCAAAAATACTCATTTCTGGATTTCTTTGGCGCTTGTGCAGGT
snpVU0008	5	a/g	GGCAGTGATGCTGTCATCTACCCTGCTCCAGAATTGTTGGAGAAAGAAGCAGAGGATAAA[A/G]TCCAGCTCTACCCAAAATTTGTTCGAGGACTACATGAAGCTCTATGCTAAGCTCAAAT
snpVU0009	6	a/c	AGAGGTTCCGCAATCGCTCAGAAATGATGATTCTTCTTTCTCTCGGGTYTTGGTTCTCAG[A/C]TTCAATCTGGATTAAGGTTTTACTAGTGGTTTTAGTTGTTTCTATTTTCTTTGGTAAG
snpVU0010	6	a/c	ACCAAAAACAAAACAGCTAAGAACTCACTATAACAGGCAAAAAATAGAAAACCAACTCTTA[A/C]CAAAAGGCGCAACAAAATCATAGGTTTTGTAAGACTACGCAATAATTTTCACTTGAAGC
snpVU0011	7	a/g	AGTGAATATGATCATTCCAGTCTCATCAACCAACCAACATTGAGCAATTCGCATTTGGCG[A/G]GACTGAGGACCATCAGAACGACCTTTCTGCATCCATCTTAGCATTGACCACCTTCAACA
snpVU0012	8	a/c	TGGCAATGTGGTGGAGGCCATCAAGAAATGGTACCTTGGTGGCTCAAGGGCAGTGTCTTC[A/C]ACAACCATTCAATCTCTGGCTGCCACTTTTTCTCAGGAGCTCTCCRAGTGAACATAACTT
snpVU0013	8	a/t	ATTATTATCAGGTGTCCGGACCTTCTTGAAAGTGTAATCCACAATCTCCYTTGCAAACT[A/T]GCGAGTTACATGGAAGTCGATGAACCTACCTTAAGGACTATCTTGATGACATAACAAGCAC
snpVU0014	9	a/g	TAWGTTTTCTGCCATATTTCACTGGTGTATTTATATAAATGGTAAATTTTCTTCTTTT[A/G]GTTTCGTTAGCCAGTAGGCAATAGCTCACATAAGTCATTGCCTTAATGTATGTTCTGGAG
snpVU0015	9	a/g	AGAGGCTTCTGGAGAACTGAGAGAAGTGAATTGAAATGGAATGGGAAGAGGAAGTGA[A/G]GGAAAGGTTCTTCATTTGGGAAGGAAGGGTAAGCCATGAGCACGAAACAGCGGTTGCCAT
snpVU0016	10	a/g	AATTCCTGCCAACAAAGGGGAAAACACAGCATTGGATGTCTCTTTGGCTTCTTCCAG[A/G]ATGGTGTGCAAAATGAGGGGTACCATTCTGCTGGCTMAAATGGGATGTTATGGTGGAC
snpVU0017	10	c/g	GTCTTCGCCGGAAAATGGCTTCTGTGTTCTCACAACCGCTACAACCGCCACAACCCCAA[C/G]CTCTCTTCTTCACTCATCCTCCATTTTCTCTTCTTCAACCAATCCACATTCACAA
snpVU0018	11	a/g	GTCCTAACAGAGGGCAGTATGTAATTTGGGATGCCTTCCATCCATCTGAAAAGGCCAATA[A/G]ACTTATTGTGGAGGAAATCATGTGAGGTTCTAAAGCTTACATGAACCTTATGAACCTTAG
snpVU0019	11	a/c	TGGGTCTTCTCCATGCTCCTTTGGTGTCCACAAAGGAAATAGCAAGGAATTCAATGAC[A/C]AGAATCGTGGGTAGTGTGCCCATGGAAGTGGGTTGCTAGGTAGGTAGCTTGGCCCCA

QC/QA panel: KASP development

- Initially, 19 QC SNPs were selected and tested with sample including both fixed lines and F1s
- 17 SNPs passed KASP assay technical verification
 - SNP alleles that were able to differentiate between homozygotes and heterozygote*
- The 17 SNPs constitute a cowpea QC panel



QC/QA panel: Deployment

We tested reliability of 17 SNP-based KASP markers for routine QC/QA in cowpea

220 parents crossed to IT99K-573-1-1 and IT99K-573-2-1

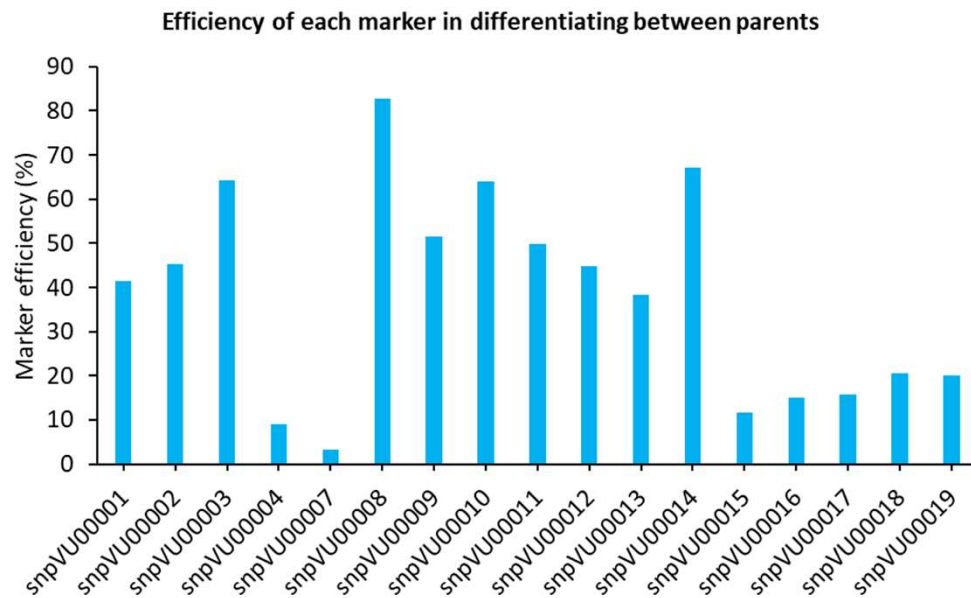
Created 225 unique cross combinations

This gave rise to a total of 1,436 F1s

SNP ID	Chr.	Position (ref. v1.1.)	Allele 1	Allele 2	Source
snpVU0007	1	36773526-36773649	T	C	IITA
snpVU0011	2	22941996-22942128	T	C	IITA
snpVU0018	4	16415787-16415919	A	G	IITA
snpVU0019	4	24230438-24230570	T	G	IITA
snpVU0001	5	399824-399956	C	G	IITA
snpVU0002	5	43326556-43327417	A	G	IITA
snpVU0009	6	30511313-30511445	A	C	IITA
snpVU0010	6	34246871-34247003	T	G	IITA
snpVU0003	7	4914544-491665	T	C	IITA
snpVU0004	7	39680298-39680430	T	C	IITA
snpVU0008	8	34271840-34271972	A	G	IITA
snpVU0012	9	29111205-29111337	A	C	IITA
snpVU0013	9	37010557-37010817	A	T	IITA
snpVU0016	10	37900312-37900440	A	G	IITA
snpVU0017	10	967432-967564	C	G	IITA
snpVU0014	11	34083600-34083732	A	G	IITA
snpVU0015	11	12936036-12936168	T	C	IITA

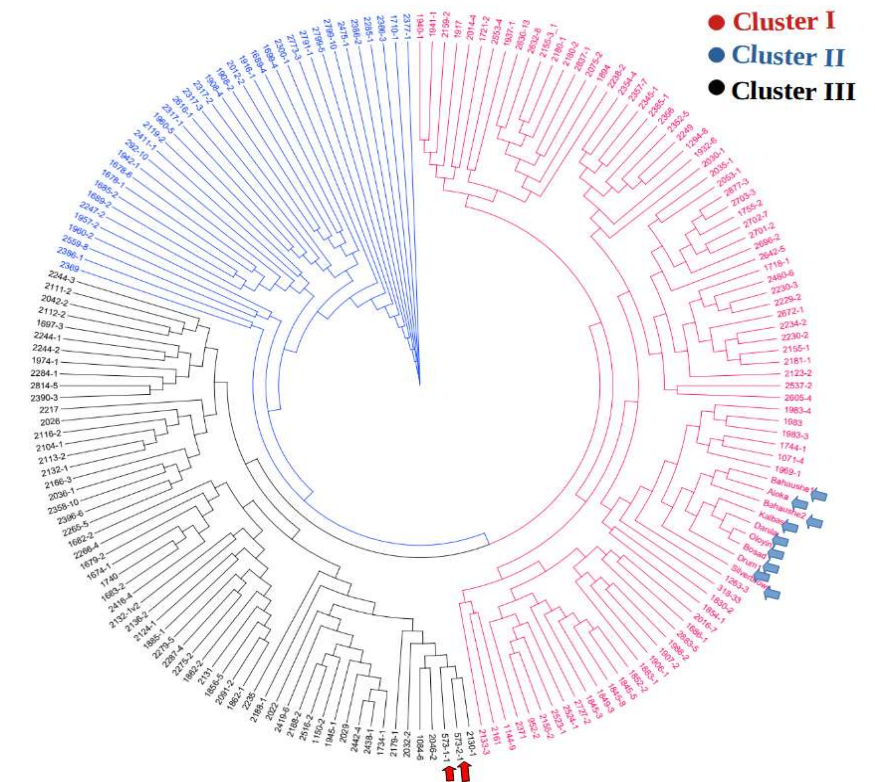
QC/QA panel: Performance

Efficiency of QC markers



Efficiency = ability to differentiate between the parents of a cross

Detecting Parental relatedness

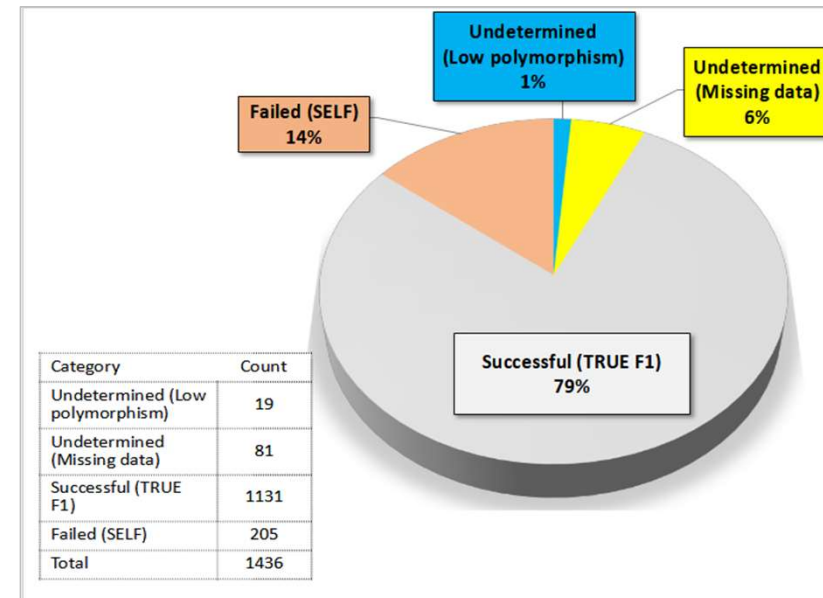
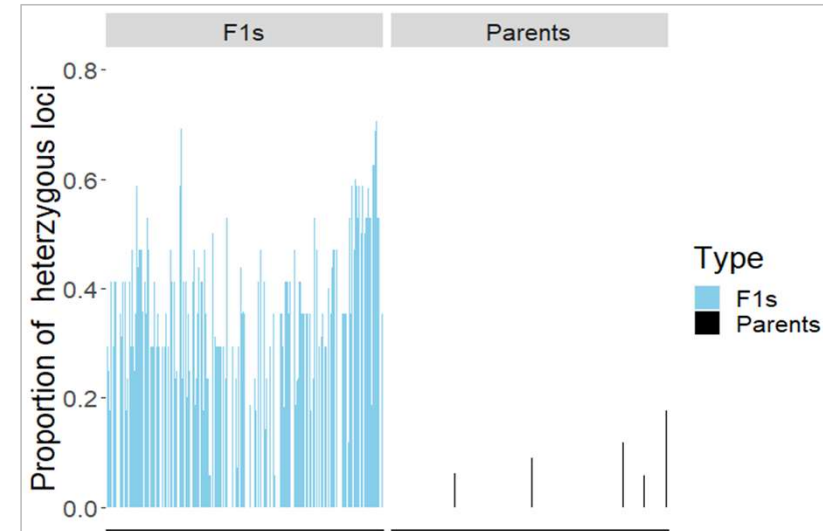


QC markers able to detect closely related parental lines

QC/QA panel: Performance

Hybridity and purity authentication

- QC markers able to identify **heterozygous or mixed lines**
- QC markers distinguished between **self vs product of successful crossing**



SNP	IT97K-573-1-1	Danila	F1-1	F1-2	F1-3	F1-4	F1-5
SNP1	C:C	T:T	C:T	C:T	C:T	C:C	C:T
SNP2	G:G	A:A	A:G	A:G	A:G	G:G	A:G
SNP3	A:A	G:G	A:G	A:G	A:G	A:A	A:G

Routine usage: F1 verification

support to our NARs under CtEH Genotyping project

Customer Sample Name	#PolySNPs	#hetLoci	missingLoci	%hbridity	Comment	snpVU000	snpVU000	snpVU000	snpVU000	snpVU000	snpVU000
Parent 1	11					C:C	A:A	T:T	C:C	C:C	A:A
Parent 2	11					C:C	G:G	C:C	C:C	T:T	G:G
F1-1	11	7	0	63.6	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-2	11	7	1	70.0	TRUE	C:C	G:A	C:T	C:C	Uncallable	A:A
F1-3	11	7	0	63.6	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-4	11	8	0	72.7	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-5	11	7	0	63.6	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-6	11	8	1	80.0	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-7	11	8	0	72.7	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-8	11	8	0	72.7	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-9	11	8	0	72.7	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-10	11	7	0	63.6	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-11	11	8	0	72.7	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-12	11	8	0	72.7	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-13	11	8	0	72.7	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-14	11	8	0	72.7	TRUE	C:C	G:A	C:T	C:C	C:T	A:A
F1-15	11	0	0	0.0	FALSE	C:C	G:G	C:C	C:C	T:T	G:G
F1-16	11	9	0	81.8	TRUE	C:C	G:A	C:T	C:C	C:T	G:A
F1-17	11	9	0	81.8	TRUE	C:C	G:A	C:T	C:C	C:T	G:A
F1-18	11	0	0	0.0	FALSE	C:C	G:G	C:C	C:C	T:T	G:G
F1-19	11	0	1	0.0	FALSE	C:C	G:G	C:C	C:C	T:T	G:G
F1-20	11	0	0	0.0	FALSE	C:C	G:G	C:C	C:C	T:T	G:G

Ghana:

- Verified 72 putative F1s + 6 parents
- 46 lines purification

Hybridity

$$= \left(\frac{\#Het}{\#pol.SNPS - \#mis} \right) * 100$$

Line purity assessment

support to our NARs under CtEH Genotyping project

Customer Sample Name	#loci	#homLoci	missingLoci	%homo	DisimilarityIndex	comment	npVU0000	npVU0000	npVU0000	npVU0000	npVU0000	npVU0000
A-12-1	20	20	0	100	0.00	all can be bulked	C:C	G:G	C:C	C:C	C:C	G:G
A-12-2	20	20	0	100	0.00		C:C	G:G	C:C	C:C	C:C	G:G
A-12-3	20	20	0	100	0.00		C:C	G:G	C:C	C:C	C:C	G:G
A-12-4	20	20	0	100	0.00		C:C	G:G	C:C	C:C	C:C	G:G
A-12-5	20	20	0	100	0.00		C:C	G:G	C:C	C:C	C:C	G:G
A-12-6	20	20	0	100	0.00		C:C	G:G	C:C	C:C	C:C	G:G
A-12-7	20	20	0	100	0.00		C:C	G:G	C:C	C:C	C:C	G:G
A-12-8	20	20	0	100	0.00		C:C	G:G	C:C	C:C	C:C	G:G
A-12-9	20	20	0	100	0.00		C:C	G:G	C:C	C:C	C:C	G:G
A-12-10	20	20	0	100	0.00		C:C	G:G	C:C	C:C	C:C	G:G
A-11-1	20	19	0	95	0.20	Needs purification, do plant to row	C:C	G:G	C:C	C:C	C:C	G:A
A-11-2	20	20	0	100	0.07		C:C	G:G	T:T	T:T	C:C	A:A
A-11-3	20	20	0	100	0.07		C:C	G:G	T:T	T:T	C:C	A:A
A-11-4	20	20	0	100	0.07		C:C	G:G	T:T	T:T	C:C	A:A
A-11-5	20	20	0	100	0.05		C:C	A:A	T:T	T:T	C:C	A:A
A-11-6	20	8	12	100	0.18		C:C	A:A	?	?	?	?
A-11-7	20	19	1	100	0.05		C:C	A:A	T:T	Uncallable	C:C	A:A
A-11-8	20	18	2	100	0.06		C:C	A:A	T:T	T:T	C:C	A:A
A-11-9	20	18	1	94.73684	0.06		C:C	G:A	T:T	T:T	C:C	A:A
A-11-10	20	19	1	100	0.05		C:C	A:A	T:T	T:T	C:C	A:A

$$\text{Homozygosity} = \left(\frac{\#Hom}{\#SNPs - \#mis} \right) * 100$$

Dissimilarity matrix

	A-12-1	A-12-2	A-12-3	A-12-4	A-12-5	A-12-6	A-12-7	A-12-8	A-12-9	A-12-10	index
A-12-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A-12-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A-12-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A-12-4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A-12-5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A-12-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A-12-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A-12-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A-12-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A-12-10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

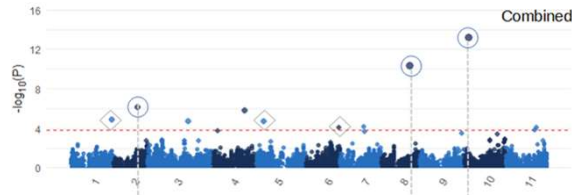
QC panel was very useful in identifying breeding lines that are mixed up and should then be purified.



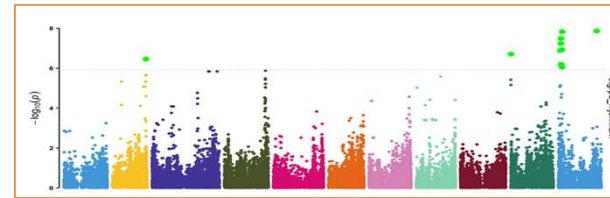
Trait specific markers

Assembly of trait-linked SNPs

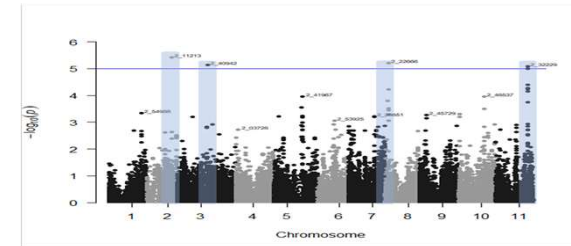
In-house mapping



Aphid: Ongom et al 2022

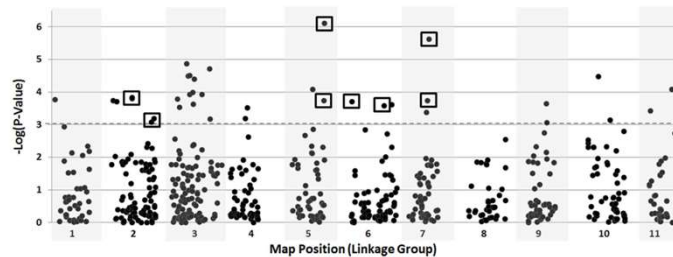


Striga resistance

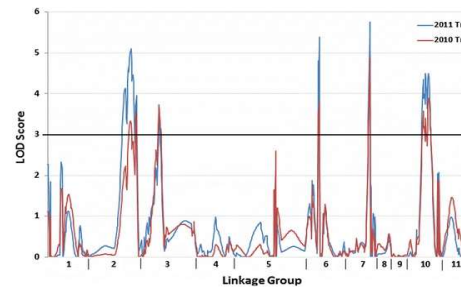


Flower thrips

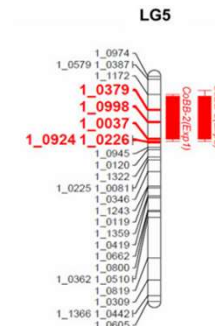
Previous mapping



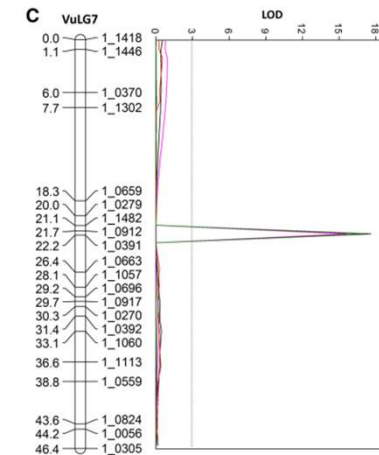
Seed size: Lucas et al 2013



Heat: Lucas et al 2013



Bacterial blight:
Agbicodo et al 2010



Aphid: Huynh et al 2015

Trait markers: KASP assay development

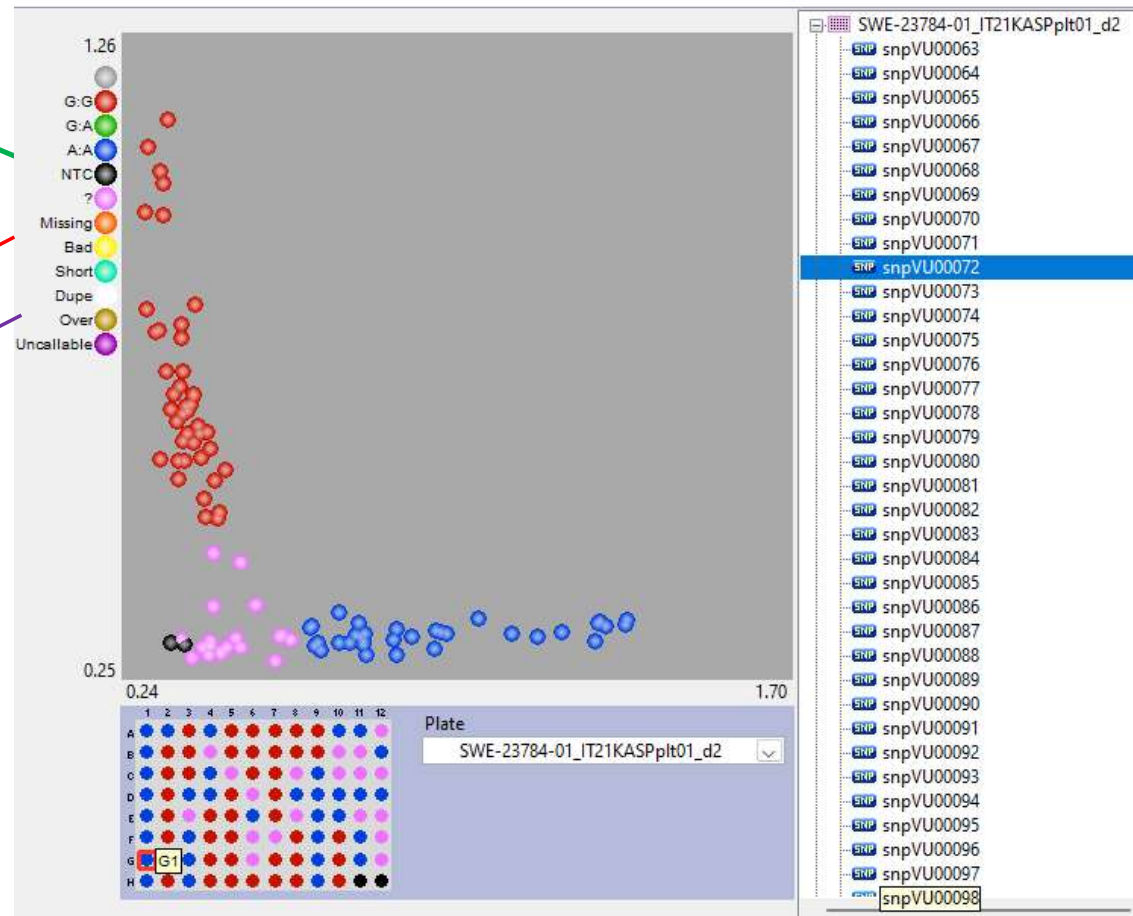
- Developed KASP assays for the **75 trait-linked SNP markers**
- Used diverse genotypes for KASP Technical verification :
 - **17 diverse cowpea lines**
 - **Included 3 technical replicates per line**
 - **43 F1 progenies**
- KASP Assay development done at INTERTEK lab, Sweden

Supported by HTPG

S/no	SNP Name*	Alternative ID	SNP*	Chr	Sequence*
1	snpVU00063	2_05789	A/G	2	TGTCTAAATGGTGAACAAAGATGCGACAGGGATAATTTAAAGTTTCTGGTGGTCATTTG[A/G]CAGCTTTGGATGACGATTTGACATTTAAGTACAAATGATGAAACCTTTGTCCAAAATGTT
2	snpVU00064	2_18924	T/C	2	CCCAACCCGTTCAATATCRCTCTGTGGAGAAAGAAAGTACTCATCMTCTACATTGTGGAC[T/C]AAGTGGTTTCCCGTTTGAGATTCTAGGTAATTAGTGGTGATCCTCTGCACTGTATCCACG
3	snpVU00065	2_18925	A/C	2	CTTCTTCCTTGCCTTTCCAAAATACATCCTGTTAAAGGGAGAGATAAGAGTTAAGAGTTAA[A/C]GAACAGAATCCACTTATCAATGTATCCATAAAAATCATATTTATCATTCTAGAGAGGGATC
4	snpVU00066	2_22778	A/G	2	CAGCTCCATGTTTCATGGTT-CAGGGGCAAAAATGATGAAAATCTGAATAGTCACATGACC[A/G]AAGAAAATCAACCGTAGGTGATCCGGAATCATAATTGGTTTCTTTAACCATGAAAAAC
5	snpVU00067	2_48732	T/G	2	CACAATGTGGGTCGGCATGTTTCATCATGCATTTTAACTGTAGGTTGAAAAAGTCTGGTCC[T/G]TCTCACATAAATGCGAGTTTGCGAACCTGTGGGCTAGTCCACTTTTTTAATTTATTTTA
6	snpVU00068	2_00674	A/G	7	TGCGCTACTGCCGAAGACCATATATTGTACTCAATACAATCAAAATGTTGCTCACATTGA[A/G]TTATGTTCTGTATGAAATGTTTTCGCTATGACCATTGATTACAAAATTACACAAGTATAC
7	snpVU00069	2_14573	A/G	7	AAAGTTGTTTATTGAGTGTGTATAAECTGAAACTATAAGTTTTTGTGAGTTTGTGGAA[A/G]CTGTTAATACCGTTGAATTTATTTGATATAATTTGTGGTTGTAGTTCTGCCTTTTTTGGAA
8	snpVU00070	2_20936	T/G	7	CTTTCTTTCTGTTTTTGTCTACTTCCATGAAAGAYGAAAAACGCAAAAACGYCATTC[T/G]TTCTTCTGTTCTGATGTCCCTCGATACTGGGCTGGGCCTGGCCCTCTGGCCCATCAAATG
9	snpVU00071	2_24995	A/C	7	ATRTAATTCAGAAACACTATTTTAGAATAAGTTATATTCTACAACATTTTTTGTTAATA[A/C]AAAAATTTAAAAGATTTTCTTATAAATTTTTTCTCCTTATAGCATAACGAAATTTAAGA
10	snpVU00072	2_27836	A/G	7	GAATCAGAAACACTGGTAAGGTTGGTTTATTGTGGTTGAAAATAATTTGCCATTATTAC[A/G]TATAATTAATCAAGGACTCTGGACAAGTGAATAAAAACCTCACCTTTTGGTTTTGGACAAG
11	snpVU00073	2_32524	C/G	10	ATAATCGGAGAGGAAAGCTTATTGGCATCCCAGTTTGCACCCAACACTTTACTTTACATTG[C/G]TAGAGCAGAAGCTCTAGAAAAGTAAAACCTTGAAGACACATTGGGCGAAAGGCGCAATCT
12	snpVU00074	2_46726	A/G	10	ACCACTGGACCAAGTTCTAACATGCCAACCTGAAACTGAACAAATAGAAAGAAATGTCACTT[A/G]ACAGAGAAAAGAACCATGAAAAGAAATTAGCCAACCTGATATTGGATACCAATCTCTATGCG
13	snpVU00075	2_07557	T/C	11	AGTGAATAGACCAAAATGTCTCTCARATTTATAACCTYGCTTGTATTTCATCAACAT[T/C]GCAAAACAAGTAAGTCTCTATAGGCCACCTGGTCTCTTCGGAGTTCCTCCACTCCACTCTTT
14	snpVU00076	2_15481	T/G	11	GAAATTAATTGCTTTGCTGATTGAGTAATGCCCTTCCATGTCTTCTCATAGAAAAGTAAA[T/G]TCCCCTGTTTCAAAGTATTTGATTTGGTAAGCAAGAACATTATAGAAGTGTCACTTGAYG
15	snpVU00077	2_42259	C/G	11	GTTCTTATAGTTGTTACAAGATTGACATTAATTTCTATTTTCTGTTCTTTGTGGCATTAT[C/G]TGTTTCTTAACTTACATGTTATACCTTTCAATTTCTATTATATGTCTTCTACA-TACA
16	snpVU00078	2_49024	A/G	11	TTTAGTGCTCTTCTATCAACACACTTACCACCCTTTGTGTTTTTACCACCTTTACATCA[A/G]GGTTCTATCCAAAAACYAATAGAAACACAAACAYAAACTTGTATGCACAAGATTCTCATT
17	snpVU00079	2_50655	T/C	11	GTCATCATTATAAAAGYCATGTTGTTAAGTGATGATGGAGAAGAACTTCATGACGAAGAC[T/C]CACTTTGGAGCATTGACTACTTATTTGGTTCCTCATGATGGGTAATAAGACGTATAC

Trait markers: KASP assay verification

Intertek SNP ID	Customer SNP ID	SNP quality assessment	Number of clusters
snpVU00063	2_05789	Very good	3
snpVU00064	2_18924	Very good	3
snpVU00065	2_18925	Very good	3
snpVU00066	2_22778	Very good	3
snpVU00067	2_48732	Very good	3
snpVU00068	2_00674	Very good	3
snpVU00069	2_14573	Very good	3
snpVU00070	2_20936	Very good	3
snpVU00071	2_24995	Bad	0
snpVU00072	2_27836	Inconclusive	2
snpVU00073	2_32524	Inconclusive	1
snpVU00074	2_46726	Good	3
snpVU00075	2_07557	Medium	3
snpVU00076	2_15481	Very good	3
snpVU00077	2_42259	Good	3
snpVU00078	2_49024	Very good	3
snpVU00079	2_50655	Medium	3



Trait markers: Validation

The 75 trait-linked markers being validated in different genetic backgrounds

Traits	No. of Markers	Pop size	Validation population
Striga resistance	17	300	1. DANILA♀ X IT97K-499-35-1♂ 2. DANILA♀ X B301♂
Aphid resistance	15	300	1. Tvx3236♀ X Tvu6464♂ 2. Tvu-1727♀ X Tvu-801♂
Thrips resistance	16	300	1. VITA7♀ X SANZI♂ 2. VITA7♀ X Tvu-8671♂
Seed size	14	300	1. ACHISHURU♀ X IT107-318-33♂ 2. ACHISHURU♀ X IT89KD-288♂
Heat tolerance	13	300	1. IT86-D-1010♀ X CB27♂ 2. IT98K-589-2♀ X IT98K-1111-1♂
Total	75		

- DANILA ♀ X IT97K-499-35-1 ♂
- DANILA ♀ X B301 ♂

Susceptible

Resistant



F1



F2

1 2 3 4300

.

.

F5

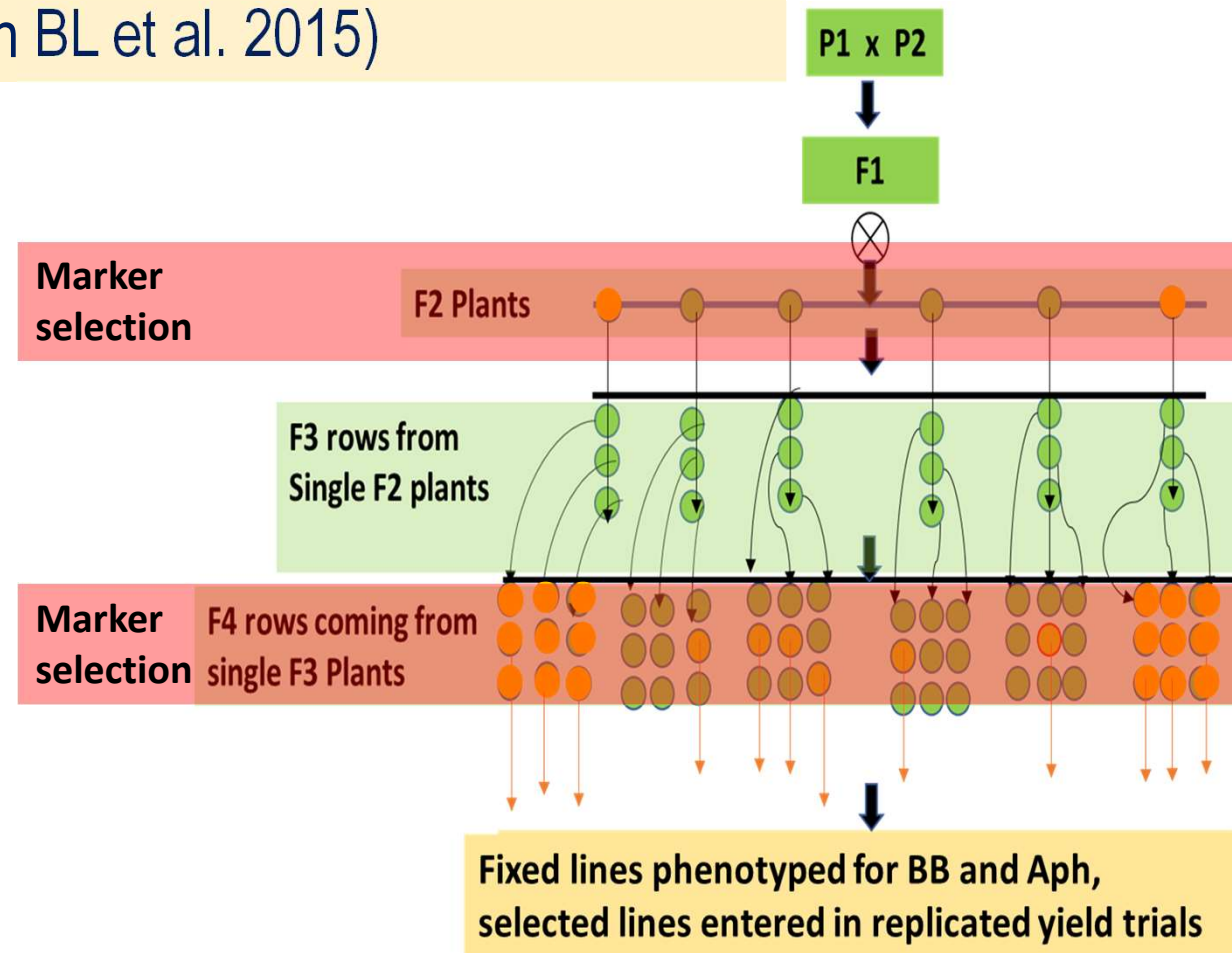
1 2 3 4300

Trait markers: Deployment

Forward breeding using KASP markers for **Bacterial blight** (Agbicodo et al. 2010) and **Aphid** resistance (Huynh BL et al. 2015)

	snpVU000041	snpVU000042	snpVU000046	snpVU000047	snpVU000049	snpVU000060	snpVU000061	snpVU000062	Bacterial blight markers
IT99K-573-2-1	T:T	T:T	A:A	T:T	T:T	C:C	T:T	T:T	
DANIILA	C:C	T:T	A:A	T:T	T:T	C:C	T:T	T:T	

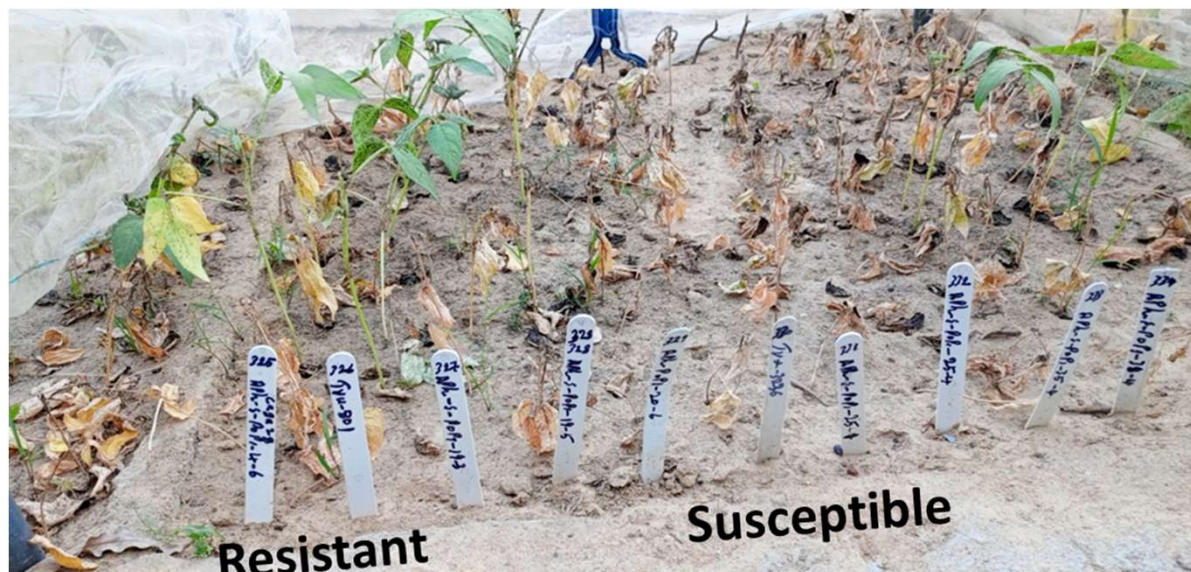
	snpVU000024	snpVU000025	snpVU000031	snpVU000032	Aphid markers
IT90K-76	T:T	C:C	T:T	C:C	
IT97K-556-6	T:T	G:G	T:T	C:C	
IT97K-556-6	T:T	G:G	T:T	C:C	
TVu1158	A:A	G:G	C:C	C:C	
IT90K-277-2	T:T	C:C	C:C	T:T	
IT97K-556-6	T:T	G:G	T:T	C:C	



- 400 BB and 1000 Aph lines developed
- F6 Lines undergoing phenotypic validation

Trait markers: phenotypic validation

The genotyped lines are being phenotyped for confirmation.



Aphid populations phenotyping in special insect-proof cages in Kano, Nigeria



Bacterial blight populations being phenotyped in 3 hot-spot locations in Nigeria.

Discovered lines to be used as parents and/ or handed over for advanced yield testing

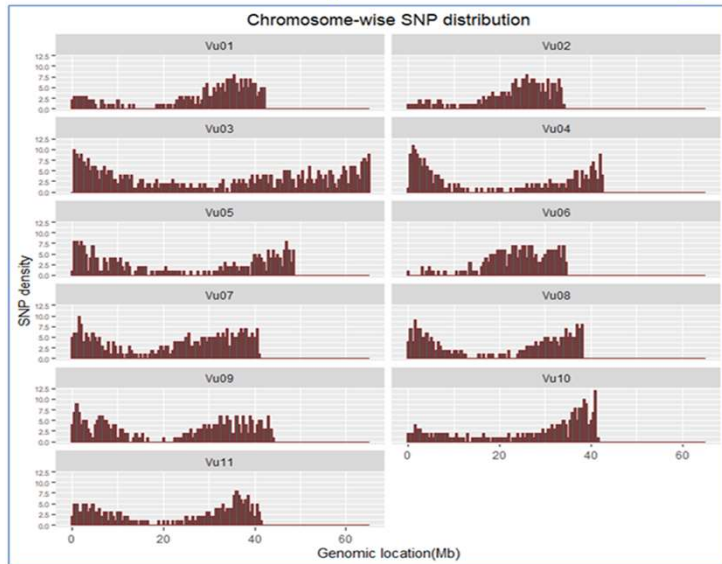
Mid-density marker panel

Medium density genotyping panel for cowpea

Recently developed a mid-density genotyping panel based on diversity array technology (DArT)

The panel has 2,602 DArTag SNPs, derived from a 51K cowpea iSelect array

Modest density and even coverage



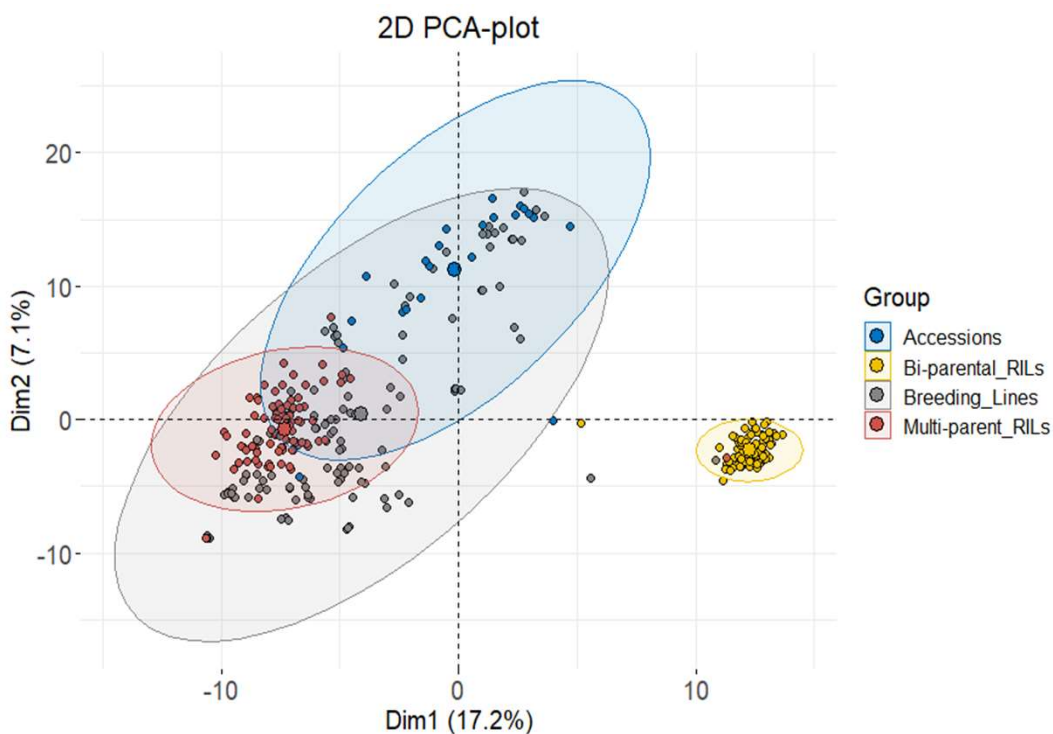
SNP selection

- Missing data rate < 5%
- MAF > 0.2
- Even spacing

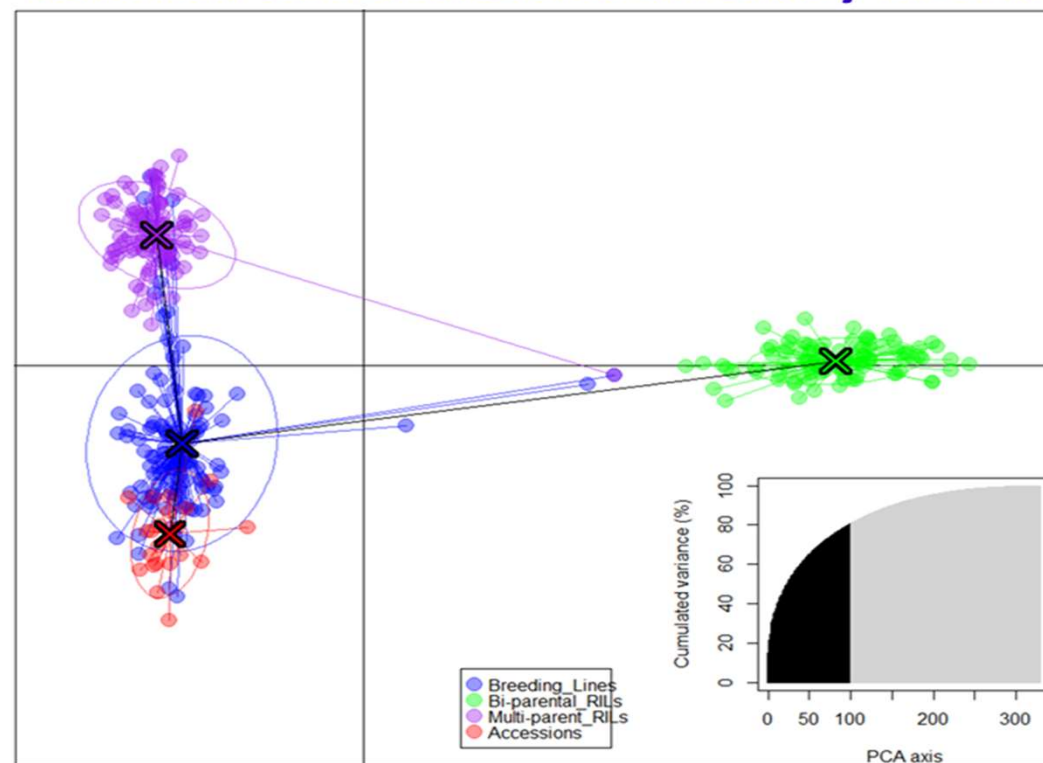
Snapshot of mid-density SNPs

Marker Name	Reference Genome plus Version Used	Chrom	Chrom Pos	Variant Alleles Def	QC
2_24641	Cowpea_IT97K-499-35_v1.0	Vu01	78762	[T/C]	PASS
2_19235	Cowpea_IT97K-499-35_v1.0	Vu01	224200	[G/A]	PASS
2_27984	Cowpea_IT97K-499-35_v1.0	Vu01	385444	[A/C]	PASS
2_16623	Cowpea_IT97K-499-35_v1.0	Vu01	528696	[C/T]	PASS
2_12556	Cowpea_IT97K-499-35_v1.0	Vu01	674116	[A/G]	PASS
2_54157	Cowpea_IT97K-499-35_v1.0	Vu01	840388	[T/C]	PASS
2_19327	Cowpea_IT97K-499-35_v1.0	Vu01	991646	[C/T]	PASS
2_55445	Cowpea_IT97K-499-35_v1.0	Vu01	1128064	[C/T]	PASS
2_28825	Cowpea_IT97K-499-35_v1.0	Vu01	1269293	[T/C]	PASS
2_28136	Cowpea_IT97K-499-35_v1.0	Vu01	1468530	[A/C]	PASS
2_25371	Cowpea_IT97K-499-35_v1.0	Vu01	1715942	[C/T]	PASS
2_29496	Cowpea_IT97K-499-35_v1.0	Vu01	1867588	[A/G]	PASS
2_49900	Cowpea_IT97K-499-35_v1.0	Vu01	2056510	[A/C]	PASS
2_40451	Cowpea_IT97K-499-35_v1.0	Vu01	2206327	[C/T]	PASS
2_09060	Cowpea_IT97K-499-35_v1.0	Vu01	2356889	[C/T]	PASS
2_29605	Cowpea_IT97K-499-35_v1.0	Vu01	2542990	[A/G]	PASS
2_30402	Cowpea_IT97K-499-35_v1.0	Vu01	2705447	[G/A]	PASS

The panel can discern stratification within a population



Valid for structure and diversity studies

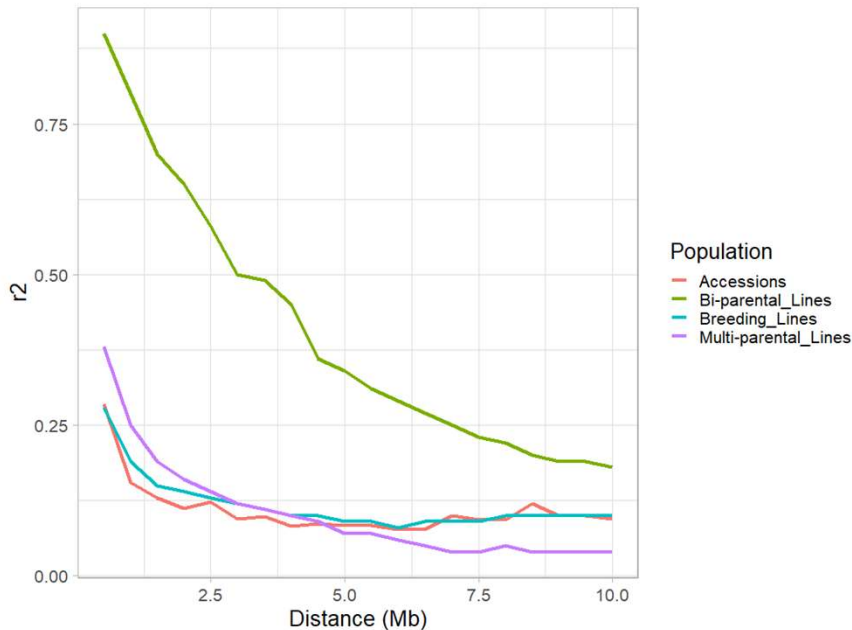


PCA

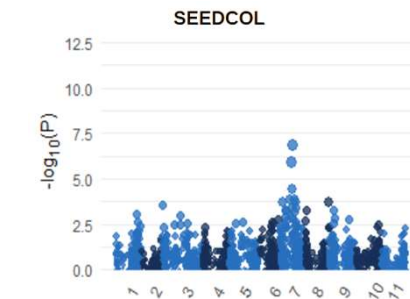
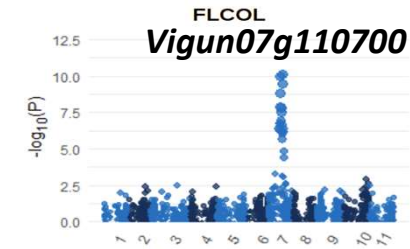
DAPC

The panel can assess LD and genomic regions associated with traits of interest

Correct assessment of LD decay

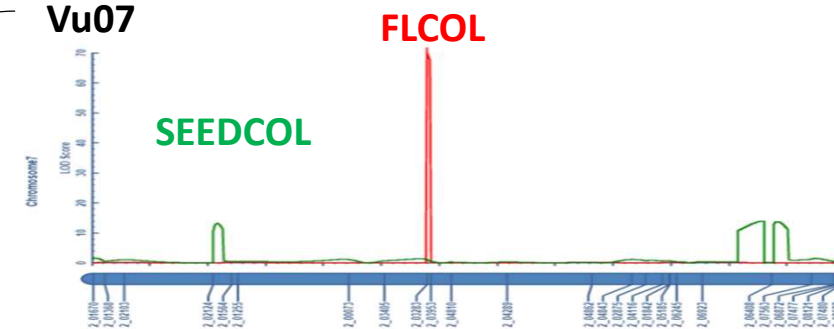


GWAS



Bi-parental mapping

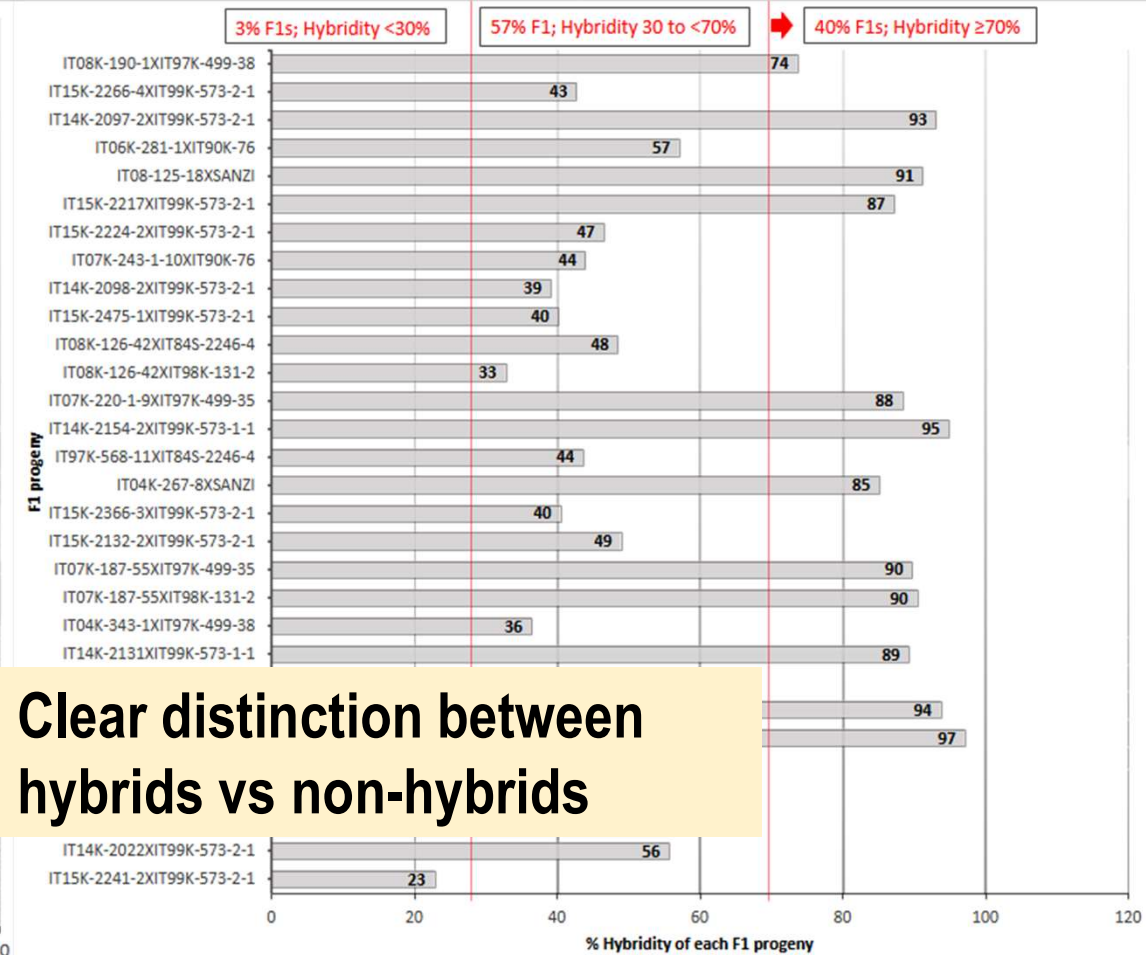
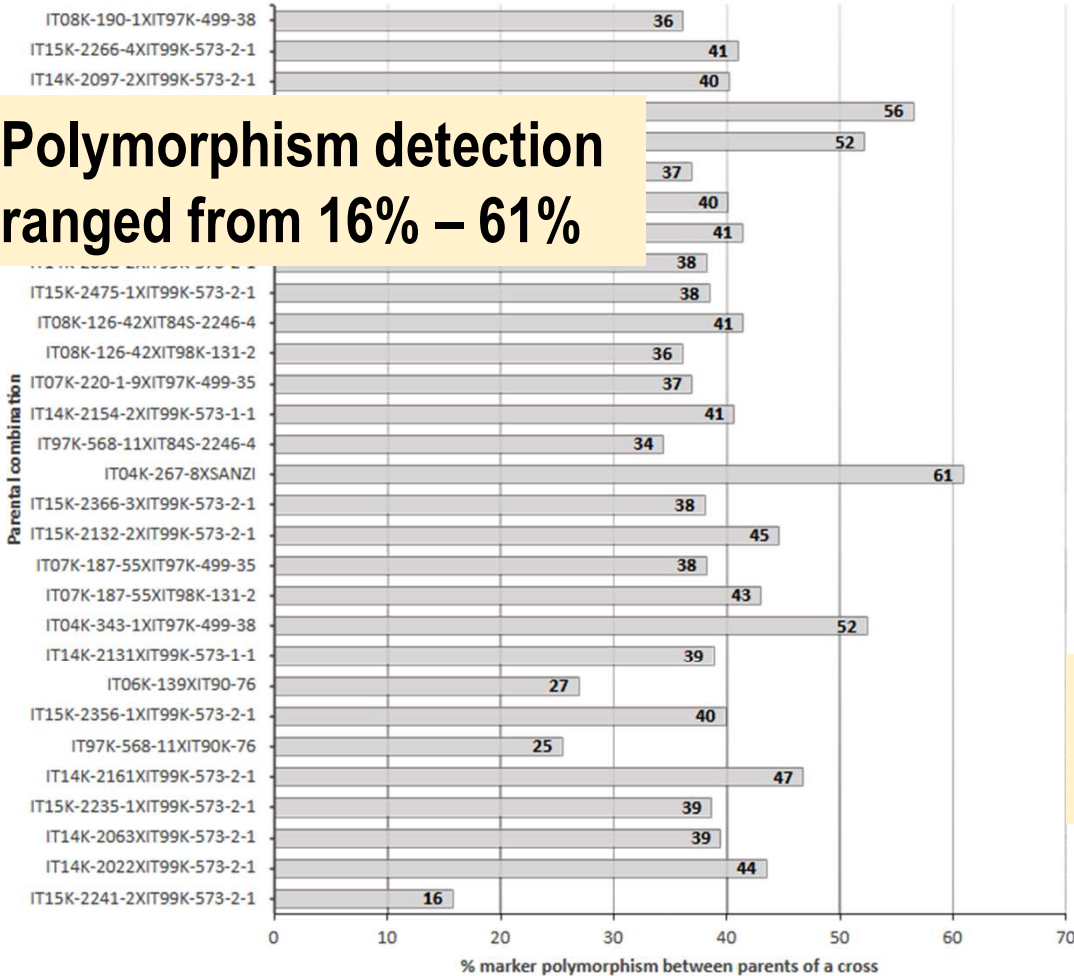
Vu07



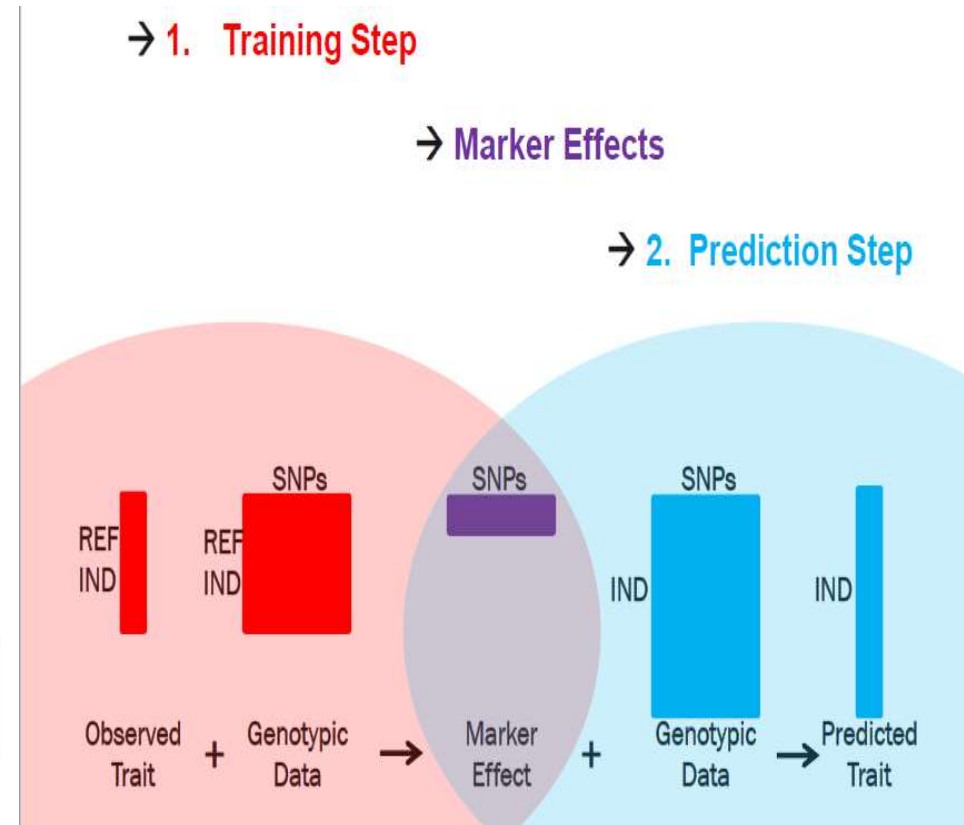
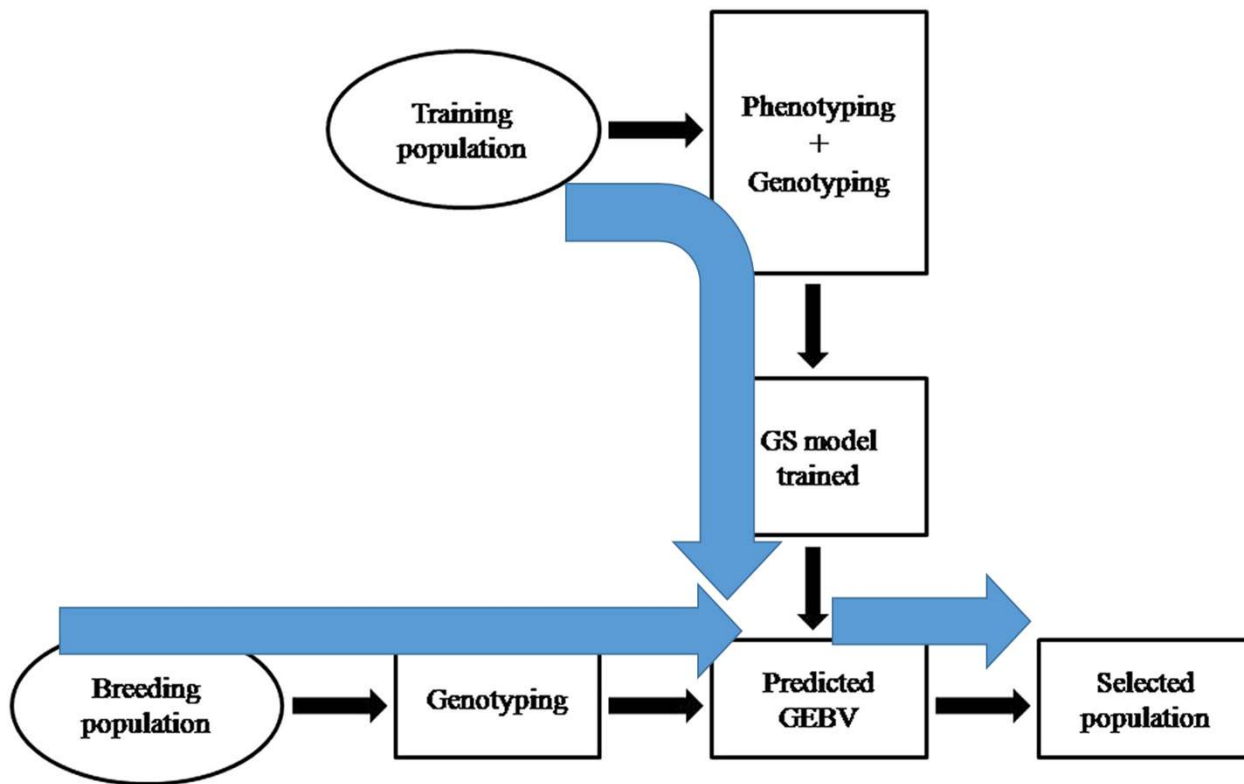
Able to differentiate parents of a cross

Able to discern true hybrids

Polymorphism detection ranged from 16% – 61%

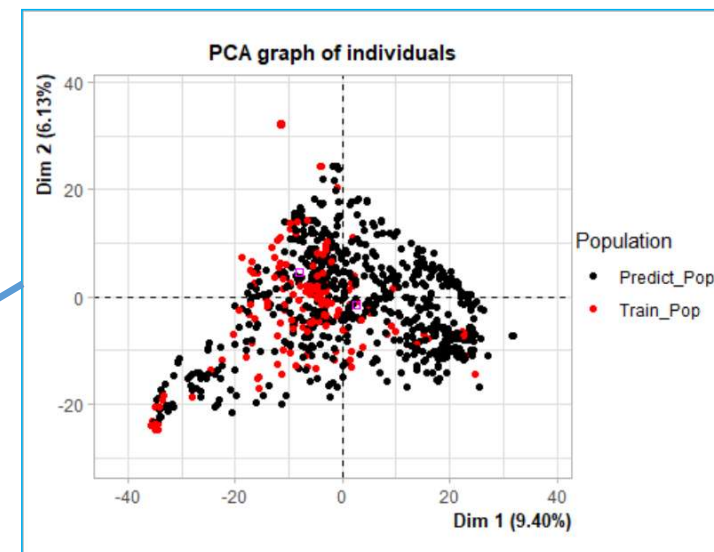
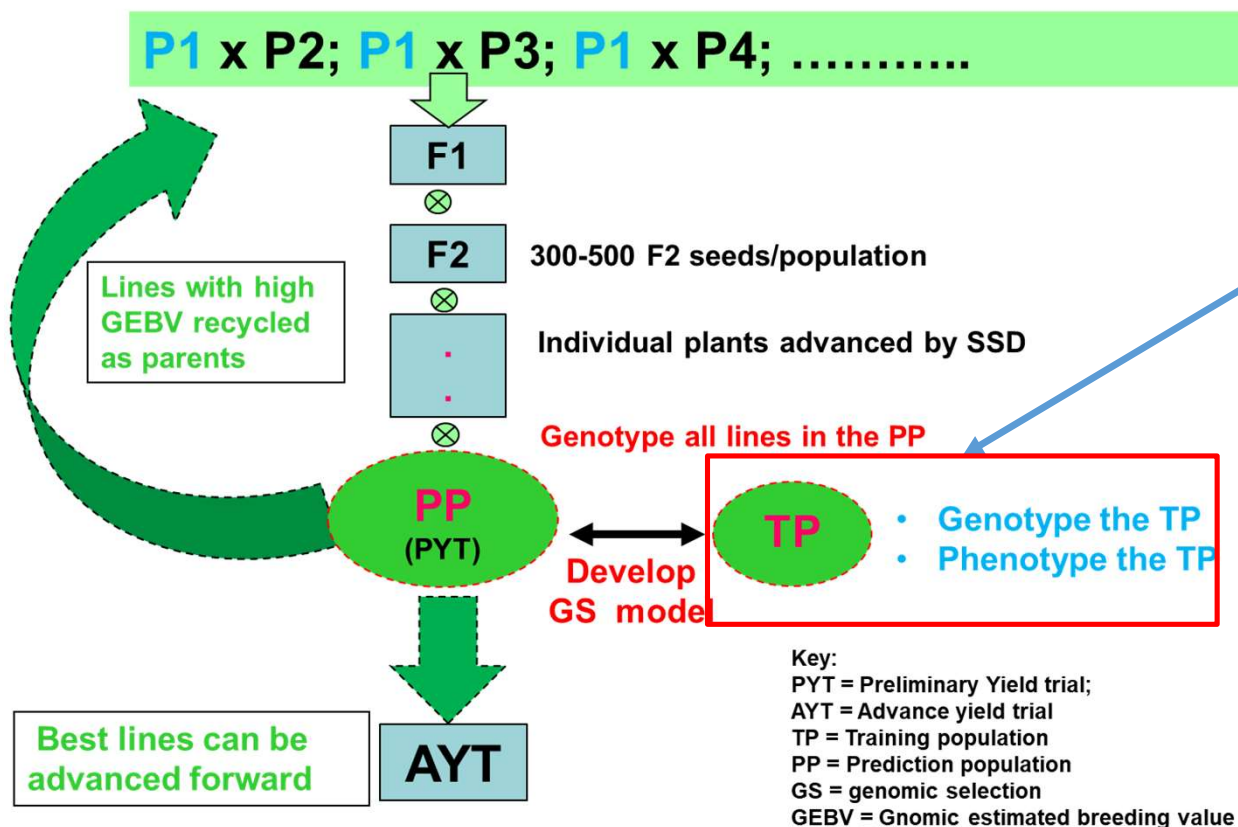


Clear distinction between hybrids vs non-hybrids



Bhat et al., 2016

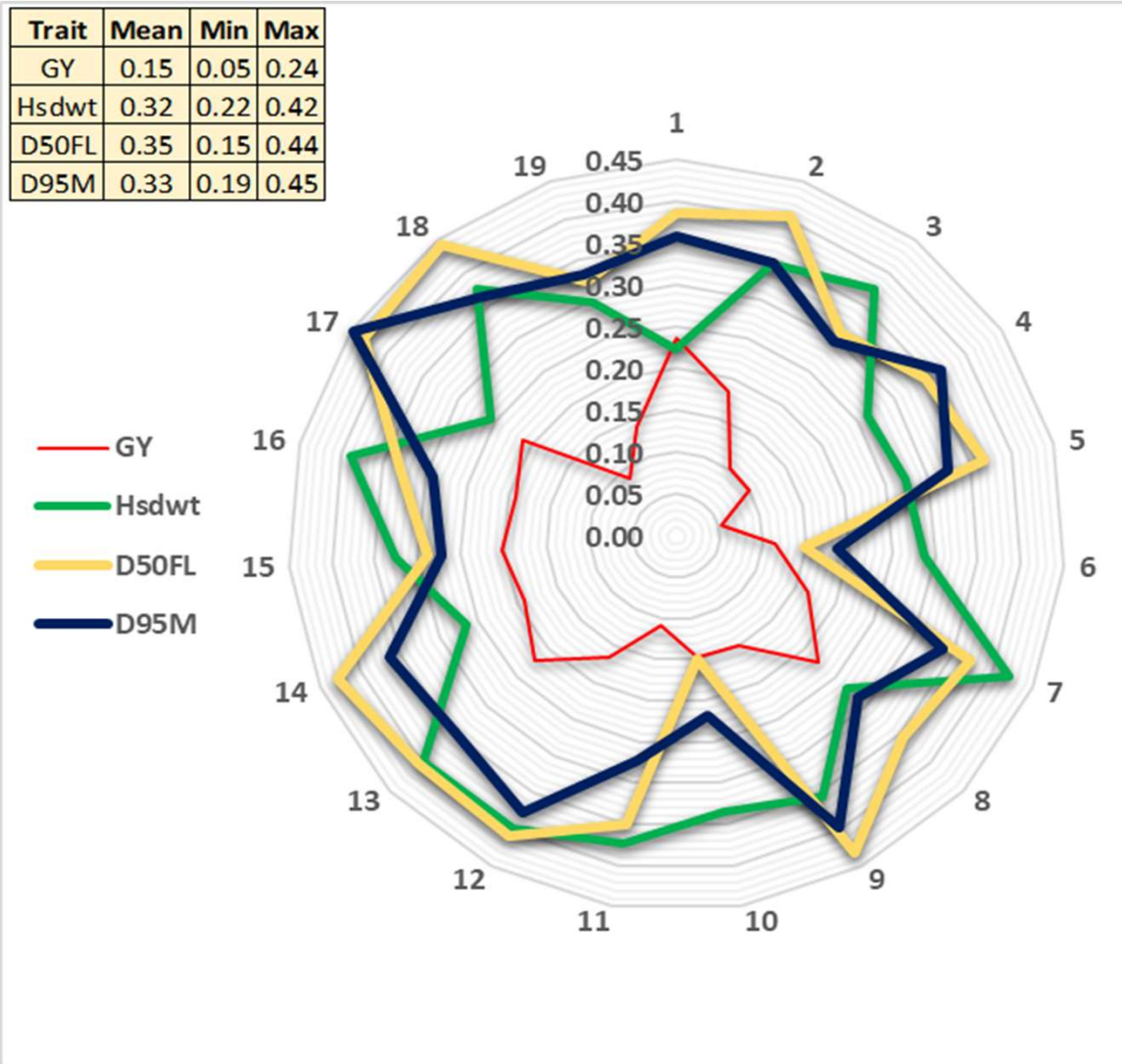
Proposed GS implementation in cowpea



We genotyped 840 breeding using the mid-density panel.

Created a sub-set of 210 lines being used as a training set

The training set is undergoing phenotyping.



Preliminary analysis:

- Used G-BLUP model
- K-fold cross-validation with k= 5, 20 iterations
- Used one year of data on all 841 lines
- Phenotyped across 4 locations
- Max GY prediction accuracy obtained was 0.24
- DT50 had the highest prediction accuracy (max

- **Significant progress made in developing, validating, and deployment of Low and mid-density marker panels in cowpea**
- **Markers are now used for:**
 - **Parental selection**
 - **Routine F1 verification**
 - **Line purification**
 - **Genetic diversity studies**
 - **Trait mapping**
 - **MAS—Forward breeding**
 - **GS**

Acknowledgement

BILL & MELINDA
GATES foundation

AVISA
Healthier and
Prosperous Africa

UNIVERSITY OF CALIFORNIA
UC RIVERSIDE

IITA
Transforming African Agriculture



CGIAR



FEED THE FUTURE
The U.S. Government's Global Hunger & Food Security Initiative

CRP-GLDC

(CGIAR Research Program on Grain Legumes and Dryland Cereals)



USAID
FROM THE AMERICAN PEOPLE



Excellence in
Breeding
Platform



illumina®



IITA is a member of the CGIAR System Organization.

www.iita.org | www.cgiar.org