

Feed-the-Future Workshop, PAG XX, Jan. 17, 2012

Modern Cowpea Breeding to Overcome Critical Production Constraints in Africa

**CRSP and GCP Teams
Presented by Jeff Ehlers
*University of California, Riverside***



USAID
FROM THE AMERICAN PEOPLE



PULSE CRSP

Dry Grain Pulses Collaborative
Research Support Program

**MICHIGAN STATE
UNIVERSITY**

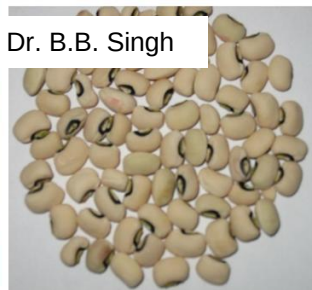


Cowpea - *Vigna unguiculata*

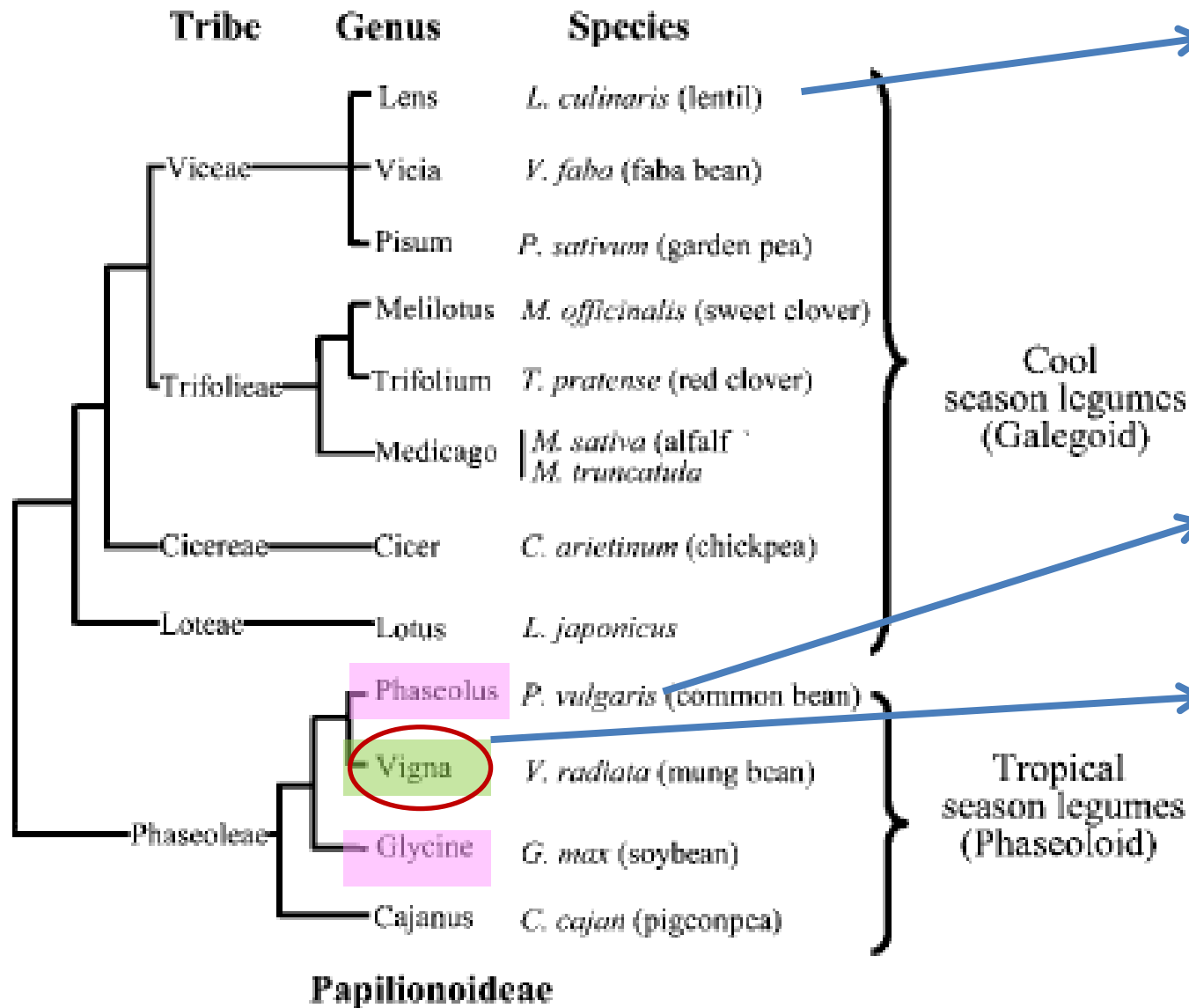
- Most widely grown grain legume in Africa
 - >10 M ha (vs <4M ha for common bean)
 - Semi-arid regions (Sudanian-Sahelian belt)
 - Crop of Feed-the-Future Target Agro-ecology
 - Dry grain, fodder, leaves and immature pods eaten
- African domesticate
 - with millets and sorghum
- Off to India and other parts of Asia
- Southern Europe from Roman times - still grown in Italy, Greece
- Brought to the Americas with slave trade- 17th century (blackeyed pea, cream peas, etc)
- Main grain legume in Northeastern Brazil



Photo courtesy Dr. B.B. Singh



Relatives in the Fabaceae Family



Importance

- Nutritious, high protein food
 - High protein (similar to common bean)
 - Complements cereal-based protein
- Cropping system health
 - Nitrogen fixation for subsequent crops
- Heat and drought tolerant
 - Stable production in face of erratic rainfall
- Adapted to poor soils
 - Especially good at mining Phosphorus
- Income production and stability
 - First crop to sell (green pods, leaves, fodder)
 - Contributes to diverse rural income streams



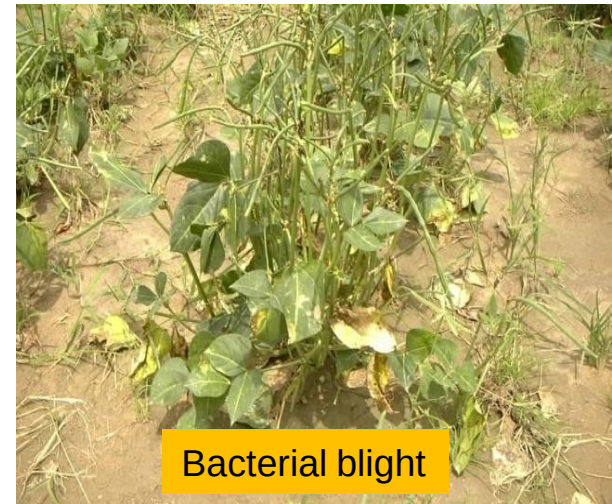
Yield Constraints addressable by breeding



Drought



Flower thrips



Bacterial blight



Viruses



Root-knot nematodes



Striga



Macrophomina

Why Breeding *Resistant* Cowpea Varieties Important

- ✓ **Most farmers women**
 - Low access to inputs
 - sprays, fertilizer
 - Fields with poor soils
- ✓ **Pesticides (Insecticides) hazardous**
 - Lack of safety precautions
 - Choice of product
 - Mixing, application
 - Container re-use
- ✓ ***Put the resistance in the seed!***



CRSP Breeding and training partnerships in semi-arid cowpea zones:

ISRA, Senegal/UCR (1980-Present)

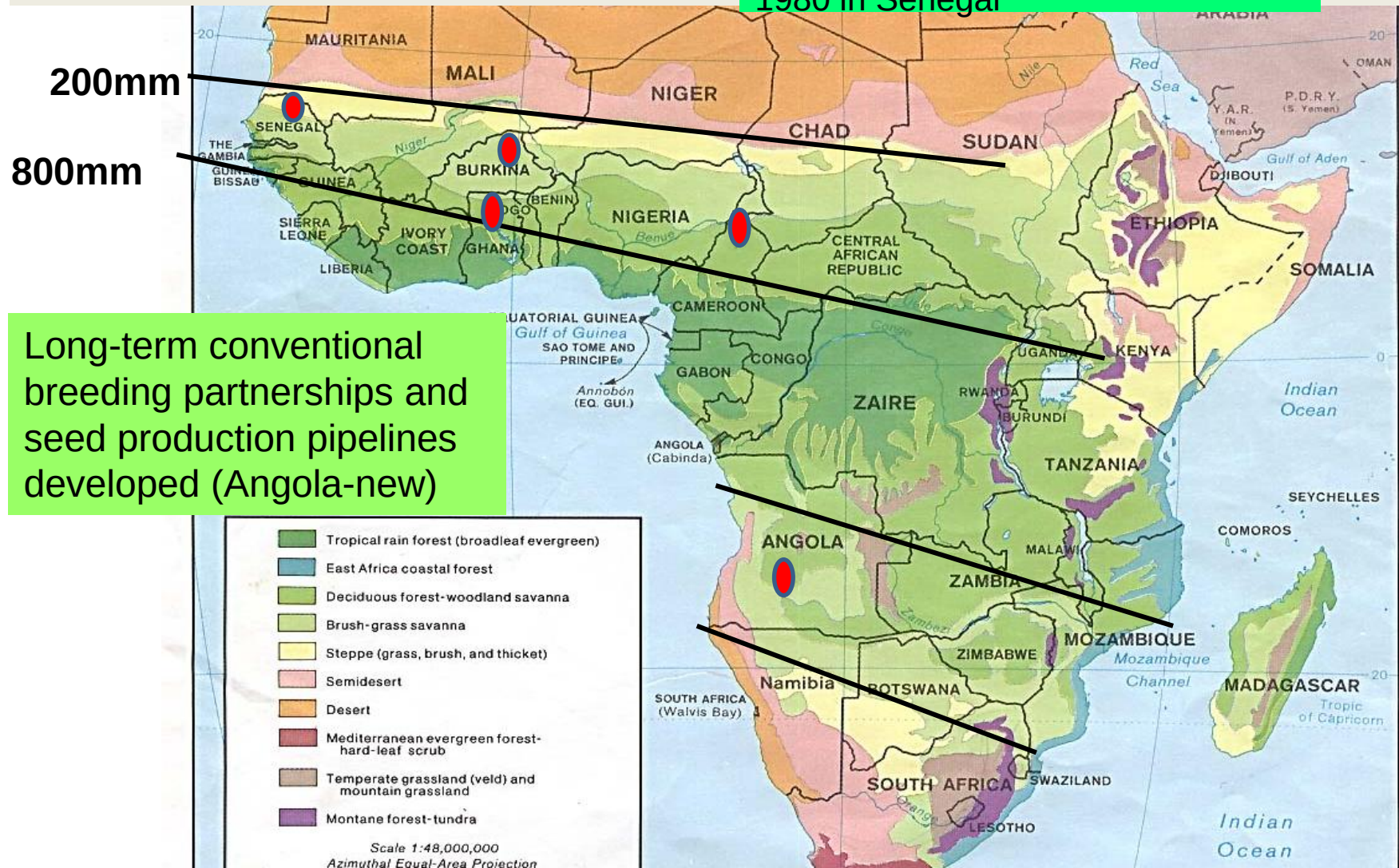
IRAD Cameroon/Purdue 1993-2003

SARI, Ghana/UCR&USDA (1997-2002)

INERA, Burkina Faso/UCR (2003-Present)

IIA – Angola/UCR 2009-Present

Emeriti Prof. Anthony Hall pioneered CRSP cowpea breeding work in 1980 in Senegal



Important CRSP varieties developed

<u>Cultivar</u>	<u>Developer</u>	<u>Pedigree</u>	<u>Released</u>	<u>Key Tolerance & Res Traits</u>
Mouride	ISRA-Senegal	IT81D-1137/ISRA 58-77	1996	Mid-season Drought Tolerance, weevil, CAbMV
Melakh	ISRA-Senegal	IT83S-742-13/IS82-292	1997	Aphid, Bacterial blight, Terminal Drought
CRSP Niebe	IRAD-Camroon	Vya/IT81D-985	1999	Cowpea weevil, foliar diseases, CAbMV
Lori Niebe	IRAD-Camroon	IT86D-364/IT81D-1138	1999	Cowpea weevil, foliar diseases, CAbMV
Apagbaala	UCR-SARI Ghana	UCR 148-1/IT81D-1137	2004	Aphid, Bacterial blight, Striga
Marfo-Tuya	UCR-SARI Ghana	UCR 518-2/Sumbrisogla	2006	Aphid, Bacterial blight, Striga
Yacine	ISRA-Senegal	Melakh/Ndiaga Aw	2005	Aphid, Bacterial blight, CABMV, Drought
KVx 421-2J	INERA-Burkina	KVx396-4-2/KVx30G-467	2008	Striga, Bacterial blight, drought
KVx442-3-25	INERA-Burkina	KVx 396//Sakoula/IT85D-3577	2008	Striga, bacterial blight

CRSP Training & Impacts



Trainee	Country	MSc	PhD	Post Doc	Present Position
Joesph Batieno	Burkina Faso		WACCI		PhD student
Ousmane Boukar	Cameroon		Purdue		Senior Cowpea Breeder, IITA
K.O. Marfo	Ghana		UC Riverside		Senior Cowpea Breeder, deceased
Arsenio Ndeve	Mozambique		UC Riverside		PhD student
Ndeye-Ndack Diop	Senegal			UC Riverside	Program Leader, GCP
Ndiaga Cisse	Senegal	UC Davis	Purdue		Senior Cowpea Breeder, ISRA
Samba Thiaw	Senegal	UC Riverside	UC Riverside		Senior Agronomist, ISRA
Abdel Ismail	Sudan		UC Riverside	UC Riverside	Principle Rice Scientist, IRRI
Wellington Muchero	Zimbabwe		UC Riverside	UC Riverside	Scientist, Oak Ridge Ntl Lab



Marker-assisted/Modern Breeding

- *Traditional breeding –hampered by low trait heritability, sequential evaluation of traits, specialized screening skills/protocols for each trait*
- MAB advantages
 - Near perfect heritability
 - Can be done any time of year
 - Can select simultaneously for several traits
 - Can select individuals for crossing prior to flowering
 - Specialized techniques/environments not needed for every trait during all selection cycles



5 Key Tools for Modern Cowpea Breeding Now Available

- 1. high-throughput genotyping platform
 - Based on Illumina 1536 GoldenGate Assay
- 2. high quality consensus genetic map
 - 13 RILs, 1,293, 1,107 markers
- 3. QTL and trait-linked markers
 - Drought tolerance
 - Resistance to flower and foliar thrips, ash-stem blight, bacterial blight, root-knot nematodes
- 4. fingerprints of >600 parental lines
 - 370 IITA/GCP mini-core
 - 230 IITA, West African lines/cultivars
- 5. outsourced genotyping platform



Key Tool No. 1 - *High-throughput genotyping platform*

- SNPs from ESTs (gene based)
- Illumina 1536 SNP 'fixed array' GoldenGate Assay
- 1375 technically successful SNPs
- Applied to mapping populations and germplasm
 - Map construction (Key Tool 2); marker-trait associations (Key Tool 3)
- Applied to germplasm
 - Parental information (Key Tool 4) for breeding, association and diversity analysis



<http://www.illumina.com>

SNP	IT84S-2246	IT93K-503-1	524-B	IT84S-2049
1_0635	GG	GG	GG	AA
1_1408	GG	GG	AA	GG
1_1383	TT	TT	--	TT
1_0136	TT	TT	CC	CC
1_0766	AA	AA	AA	AA
1_0465	AA	AA	AA	AA
1_0132	--	--	AA	AA
1_0617	GG	GG	GG	GG
1_1437	GG	GG	GG	GG
1_0342	TT	TT	TT	TT
1_0655	AA	AA	GG	AA
1_0963	CC	CC	CC	CC
1_0134	TT	TT	TT	TT
1_0541	TT	AA	TT	TT
1_1146	TT	CC	TT	TT
1_1157	TT	CC	TT	CC
1_0142	AA	AA	TT	AA
1_1339	AA	AA	AA	AA
1_0656	TT	TT	CC	TT
1_0982	GG	GG	AA	GG

Use Platform to Genotype Mapping Populations

Population	Origin	Type	Individuals	Mapped SNPs
CB27 x UCR 779	UCR	RIL	56	560
CB27 x IT97K-566-6	UCR	RIL	92	438
524B x IT84S-2049	UCR	RIL	85	438
Yacine x 58-77	ISRA	RIL	97	435
CB27 x IT82E-18	UCR	RIL	160	430
Sanzi x Vita 7	IITA	RIL	122	413
CB46 x IT93K-503-1	UCR	RIL	114	374
TVu14676 x IT84S-2246-4	IITA	RIL	136	345
CB27 x 24-125B-1	UCR	RIL	87	329
Dan Ila x TVu-7778	IITA	RIL	79	288
LB30#1 x LB1162 #7	ZAAS-China	RIL	90	180
IT84S-2246 x Mouride	UCR	F4	87	347
IT84S-2246 x IT93K-503	UCR	F4	88	155

Mapping population data summarization

- Make maps for each RIL - *JoinMap* map making software
- MergeMap software to get Consensus Map

1293 individuals from 13 Mapping populations

SNP	05066-001	05066-002	05066-003	05066-004	05066-005	05066-008	05066-010	05066-011	05066-013
1_0489	TA	AA	AA	AA	AA	AA	AA	AA	AA
1_0496	AA	--	GG	AA	AA	--	AG	AG	AG
1_0502	GG	AA	GG	AA	AA	GG	GG	AG	AG
1_0504	TT	CC	TT	CC	CC	TT	TT	TT	TC
1_0514	CC	TT	CC	TT	TC	TT	CC	TT	CC
1_0519	CG	CC	CC	GG	CC	GG	CC	CG	CC
1_0524	CC	CC	CC	CC	CC	CC	CC	CC	CC
1_0531	CC	GG	GC	GG	GC	GC	CC	CC	GG
1_0539	TT	TC	CC	TT	TT	TT	CC	TC	TC
1_0541	TT	TT	TT	TT	TT	TT	TT	TT	TT
1_0544	TT	CC	CC	TT	TT	TC	TC	CC	TT
1_0546	AT	AA	AT	TT	TT	AA	AA	TT	AA
1_0547	AG	AA	AA	AA	GG	AA	AA	AG	GG
1_0557	GG	GG	GG	GG	GG	GG	GG	GG	GG
1_0559	TT	TT	TT	TT	TT	TT	TT	TT	TT
1_0563	TT	--	TC	TT	CC	TC	TT	CC	CC
1_0566	TT	CC	CC	CC	CC	TT	CC	TC	TC
1_0586	AA	AT	TT	TT	TT	AA	TT	AT	AT
1_0587	CC	CC	CC	CC	GG	CG	CG	CC	CG
1_0588	AG	AG	AG	AG	AG	GG	AA	AA	GG
1_0595	CC	CC	CC	CC	CC	CC	CC	CC	CC
1_0604	AA	TT	TA	AA	AA	TT	TT	TA	AA
1_0611	AA	AC	AA	AC	AA	AA	AA	AA	AA

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Key Tool No. 2 – *High quality consensus map*

- Muchero et al., 2009, *PNAS* map very good, based on 6 RILs, 741 individuals
- New map based on 11 RILs, 2 F4 breeding populations, 1,293 individuals
- 1107 mapped SNPs, 856 bins
- 33% more bins
- 19% more markers
- improved order

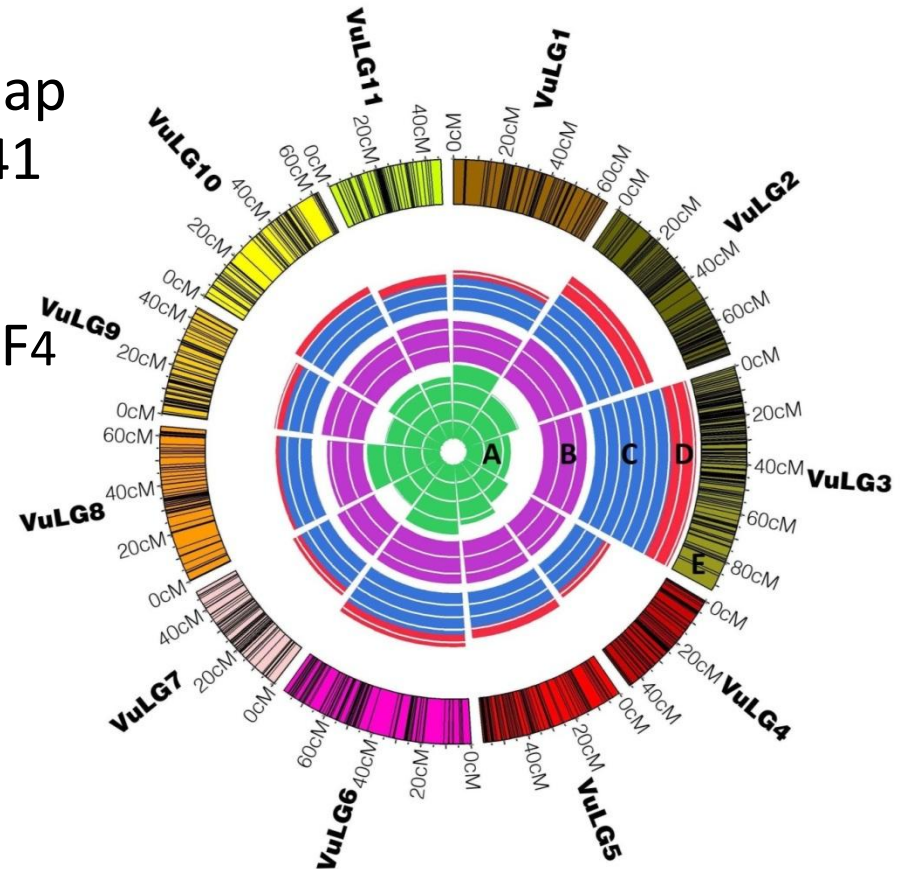


Figure 1 Consensus genetic map of cowpea and parameters depicting map characteristics. (A) Average distance between bins (0.25cM). (B) Average number of markers per bin (0.5units). (C) Number of bins (25units). (D) Number of markers (25units). (E) Bin locations. (C and D) begin at the same radial position.

Available on www.HarvEST:cowpea;
Lucas et al 2011. The Genome 4:1-11

Key Tool No. 3 – *Markers for traits*

Biotic stress markers on the map-Example: Root-knot nematode resistance

1. RIL mapping population:

- ✓ CB27 (Res) x 24-125B-1(Sus)

2. Near isogenic/closely related lines:

- ✓ CB46 – Res
- ✓ **CB46Null - Sus** (BC6 line)
- ✓ Kiawah – Res
- ✓ **Speckled Purple Hull - Sus**

3. Phenotyping

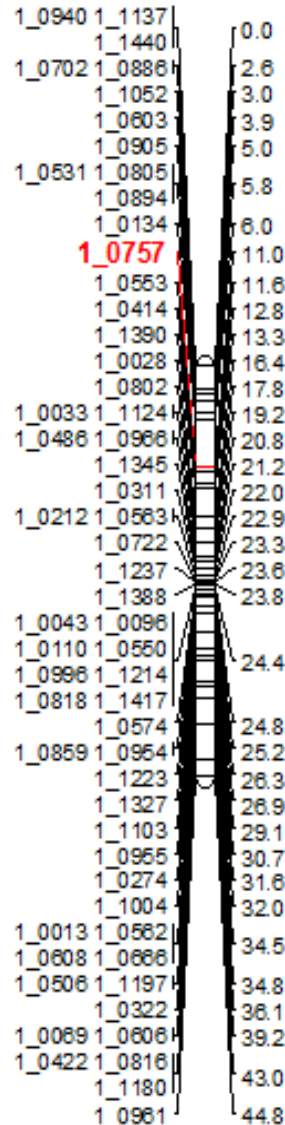
- ✓ Growth Pouch-lab
 - Egg mass count
- ✓ Field
 - Gall score



Nematode screening in the field

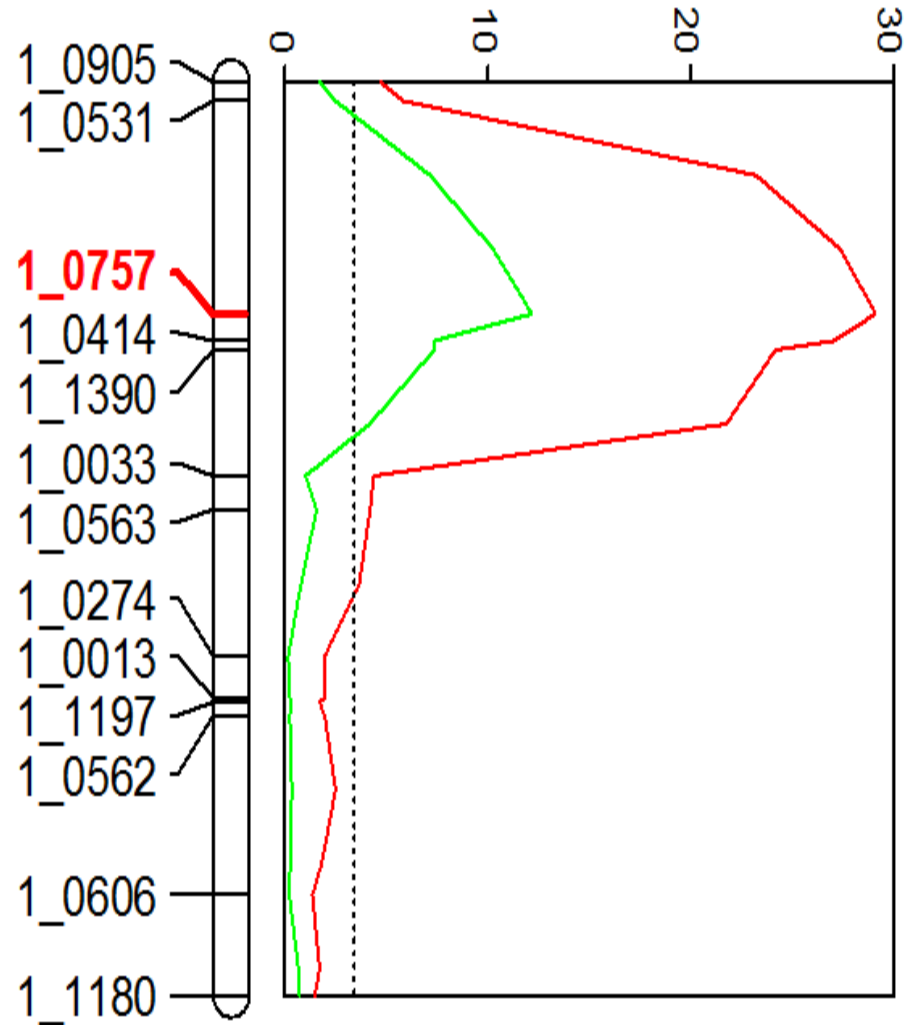


Cowpea LG11



QTL
Analysis
From
RIL
Phenotype
And
Genotype

LOD Score Traces for *Rk* QTL

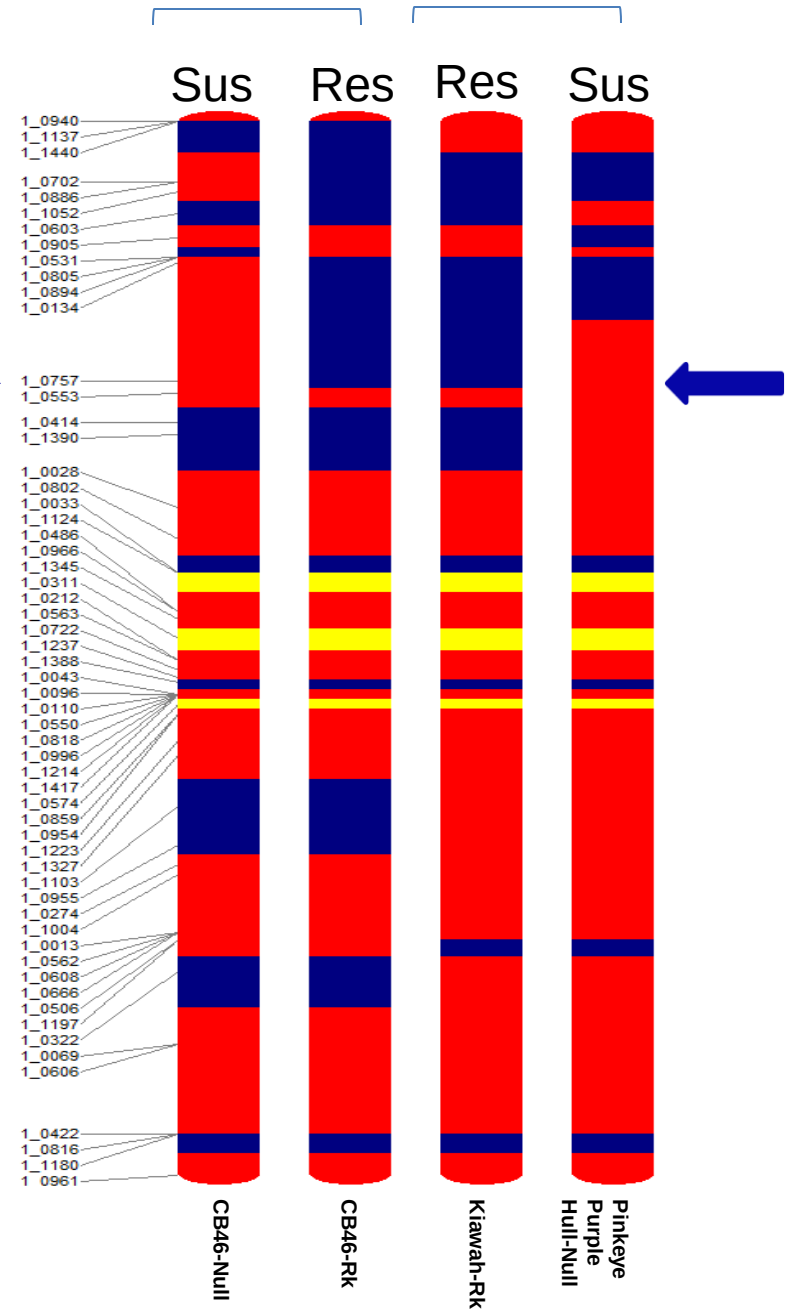


Green = Field California – summer 08

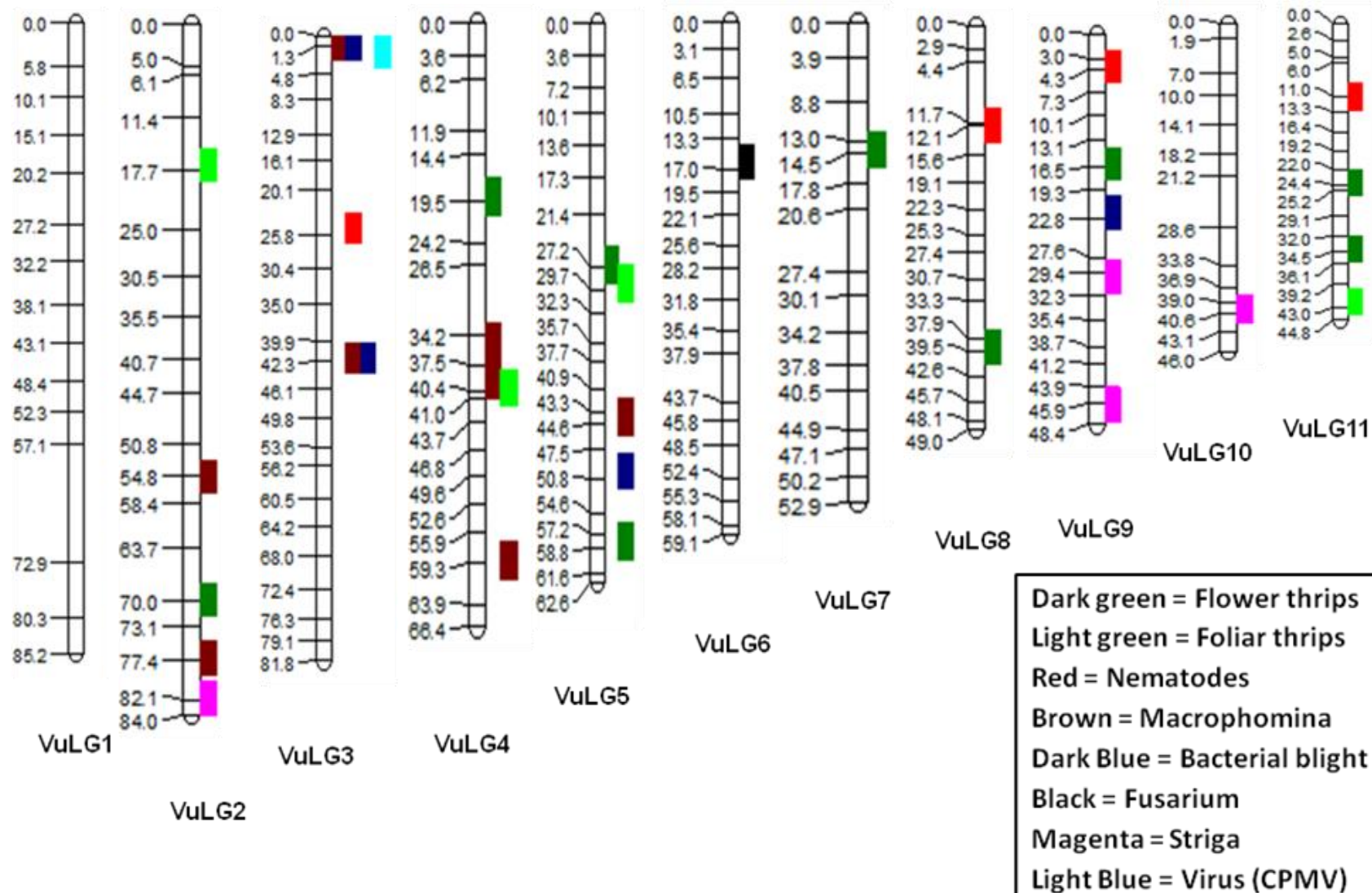
Red = Growth chamber pouch test Feb. 09

Confirmation from Near Isogenic Lines

- Resistant lines have same haplotype at nematode locus identified in RIL analysis
- Confirms *Rk* gene region near marker 1_0757



Key Tool No. 3 – Markers for biotic res traits



Map linked to 600 lines genotyped

Map File (1107 mapped SNPs)   Genotype File (600 lines)

SNP	Linkage Group	Distance cM
1_0972	1	0.000
1_0950	1	4.569
1_0129	1	4.844
1_0055	1	5.119
1_0100	1	5.265
1_0647	1	12.759
1_0876	1	14.092
1_0034	1	14.248
1_1278	1	15.057
1_1088	1	17.417
1_0449	1	20.648
1_1526	1	20.648
1_0072	1	20.809
1_1275	1	21.048
1_0782	1	21.821
1_0085	1	22.355
1_1376	1	22.848
1_0749	1	22.858
1_0308	1	23.631
1_0540	1	24.015
1_1357	1	24.072

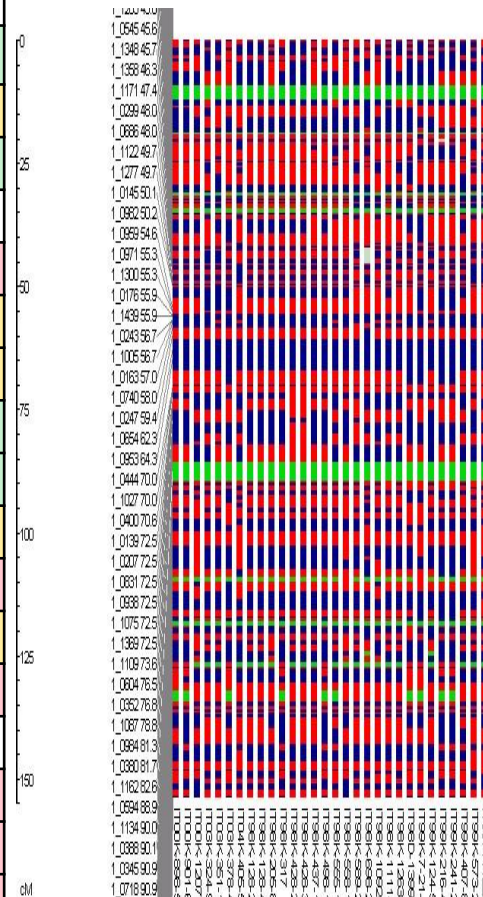
SNP	IT84S-2246	IT93K-503-1	524-B	IT84S-2049
1_0635	GG	GG	GG	AA
1_1408	GG	GG	AA	GG
1_1383	TT	TT	--	TT
1_0136	TT	TT	CC	CC
1_0766	AA	AA	AA	AA
1_0465	AA	AA	AA	AA
1_0132	--	--	AA	AA
1_0617	GG	GG	GG	GG
1_1437	GG	GG	GG	GG
1_0342	TT	TT	TT	TT
1_0655	AA	AA	GG	AA
1_0963	CC	CC	CC	CC
1_0134	TT	TT	TT	TT
1_0541	TT	AA	TT	TT
1_1146	TT	CC	TT	TT
1_1157	TT	CC	TT	CC
1_0142	AA	AA	TT	AA
1_1339	AA	AA	AA	AA
1_0656	TT	TT	CC	TT
1_0982	GG	GG	AA	GG

Key Tool No. 4 – Map-linked Genotypes

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SNP	Linkage Group	cM	IT84S-2246	IT93K-503-1	524-B
1_0972	1	0.00	GG	GG	GG
1_0950	1	4.57	CC	CC	TT
1_0129	1	4.84	TT	TT	AA
1_0055	1	5.12	CC	CC	TT
1_0100	1	5.26	AA	AA	GG
1_0647	1	12.76	CC	CC	TT
1_0876	1	14.09	TT	TT	AA
1_0034	1	14.25	TT	TT	CC
1_1278	1	15.06	CC	CC	GG
1_1088	1	17.42	GG	GG	GG
1_0449	1	20.65	CC	CC	TT
1_1526	1	20.65	CC	CC	TT
1_0072	1	20.81	CC	CC	GG
1_1275	1	21.05	CC	CC	CC
1_0782	1	21.82	GG	GG	GG
1_0085	1	22.35	TT	TT	CC
1_1376	1	22.85	AA	AA	AA
1_0749	1	22.86	CC	CC	CC
1_0308	1	23.63	CC	CC	CC
1_0540	1	24.01	CC	CC	CC
1_1357	1	24.07	CC	CC	CC
1_0775	1	24.14	GG	GG	GG

>600 lines, varieties, germplasm



GGT display of one cowpea LG

Key Tool 5: Lower cost, targeted platform for breeding

- Illumina is fixed array
 - Good for germplasm exploration when polymorphisms unknown
 - Lots of uninformative data points with biparental breeding populations
- Flexible, single-plex platform for breeding
 - KBiosciences KASPAR SNP Genotyping Platform developed
 - By GCP through collaboration of UCR and KBiosciences
 - Can select any number of markers, e.g. subset of those polymorphic in biparental cross
- 1052 Illumina platform ***mapped*** SNPs successfully converted to KBiosciences KASPAR assay
 - Illumina and KASPAR – 99% identical calls
- DNA extraction and genotyping done by KBiosciences
 - Leaf discs sent in 96-well plates by FedEx, DHL, etc
 - Works fine from Africa, data return via email link in weeks

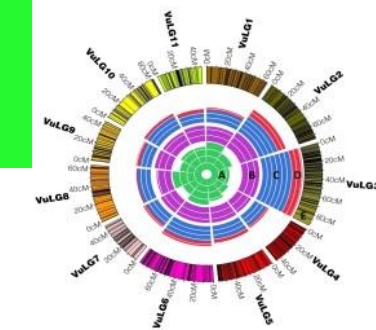
Bringing it together with 'BREEDIT'

- Program to choose a marker set for a given cross
 - **Can quickly compare cost versus marker density**
 - Typically see 200 – 300 polymorphic SNPs/cross
 - Many linked-don't need to pay for all
 - Hand selection laborious
 - Input SNP list (from Key Tool #1)
 - Input marker-trait file (Key tool #3)
 - Input parent genotypes (from Key Tool #2, #4)
 - Program Options
 - Choose any 2 parents from 600 genotyped
 - Choose desired cM interval for background selection
 - Choose cM interval for markers flanking traits
 - Outputs a list of SNPs for KASPAR assay

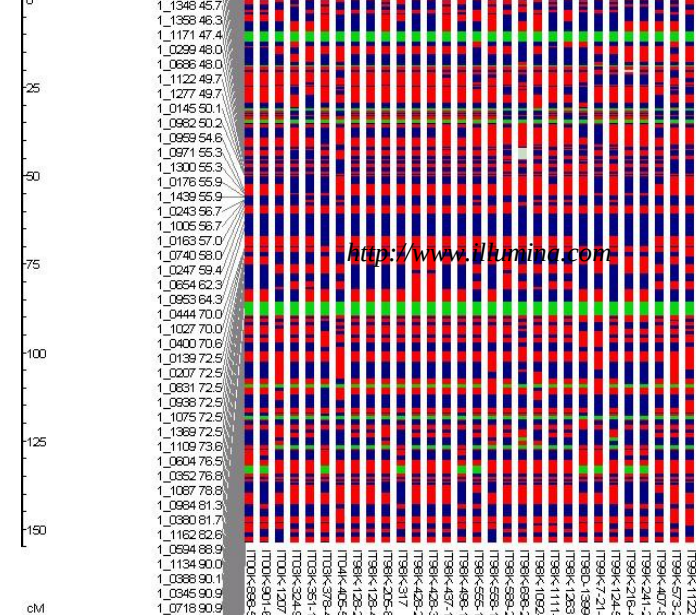
BREEDIT
output file
for marker
selection

SNP	LG	cM	IT93K-503-1	IT84S-2246	Polymorphic	Trait	Selected	Reason		
1_0190	4	6.83	AA	AA	0		0			
1_0502	4	10.27	BB	AA	1		1	Every 5 cM		
1_1148	4	10.36	AA	AA	0		0			
1_0382	4	10.66	AA	AA	0		0			
1_1360	4	12.23	BB	AA	1		0			
1_0504	4	12.76	AA	BB	1		0			
1_1499	4	13.69	AA	BB	1		1	Flanking		
1_1138	4	14.09	BB	AA	1	Flower Thrips	1	Trait		
1_0155	4	15.56	BB	BB	0		0			
1_0082	4	17.97	AA	AA	0		0			
1_0756	4	18.37	AA	AA	0		0			
1_1413	4	18.38	BB	AA	1		1	Flanking		
1_1445	4	19.1	BB	BB	0		0			
1_0847	4	19.48	--	--	0		0			
1_0774	4	20.16	BB	AA	1		0			
1_1221	4	20.16	BB	AA	1		1	Flanking		
1_1242	4	20.72	BB	BB	0	Foliar Thrips	0			
1_0027	4	21.49	BB	AA	1		1	Flanking		
1_0153	4	21.49	BB	AA	1		0			
1_0535	4	22.85	AA	BB	1		0			
1_1261	4	22.92	BB	BB	0		0			
1_1092	4	23.66	BB	AA	1		0			
1_0646	4	24.12	BB	AA	1		0			
1_0874	4	24.4	AA	BB	1		0			
1_1264	4	24.43	--	--	0		0			
1_0826	4	24.55	BB	AA	1		0			
1_0692	4	25	AA	BB	1		0			
1_0403	4	25.31	--	AA	0		0			
1_0106	4	25.57	AA	BB	1		0			
1_0678	4	27.6	BB	AA	1		1	Every 5 cM		

Enabling resources for modern cowpea breeding



High quality consensus SNP map

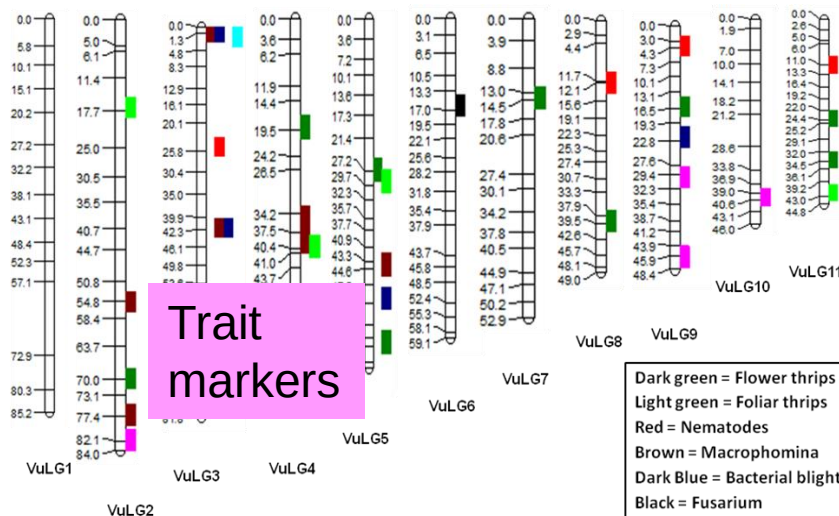


Genotypes of >600 lines

Illumina 1536 and KASPAR high-throughput SNP genotyping platforms

BREEDIT

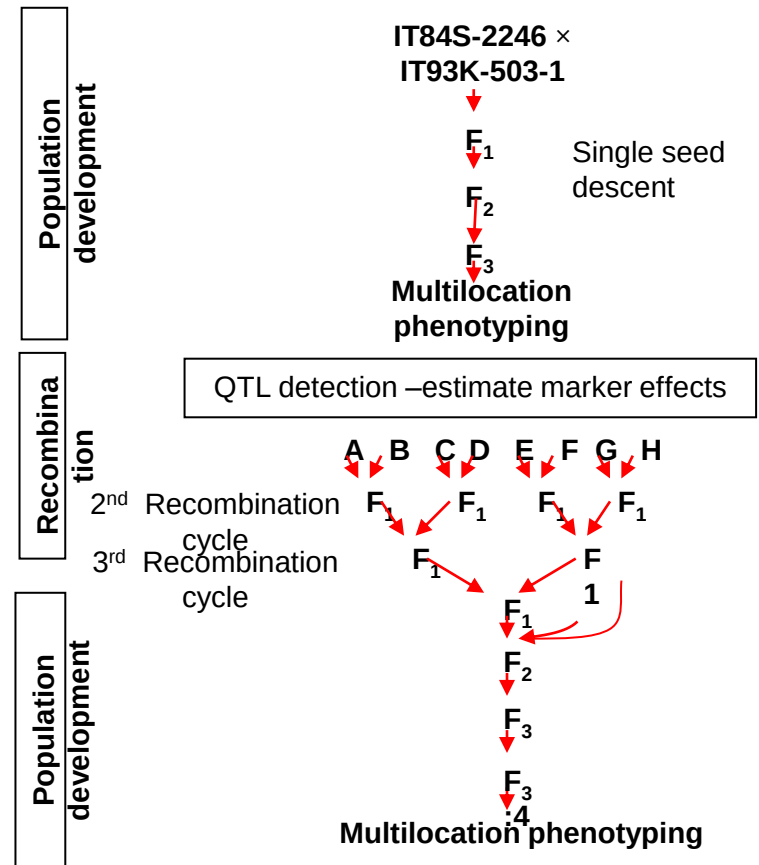
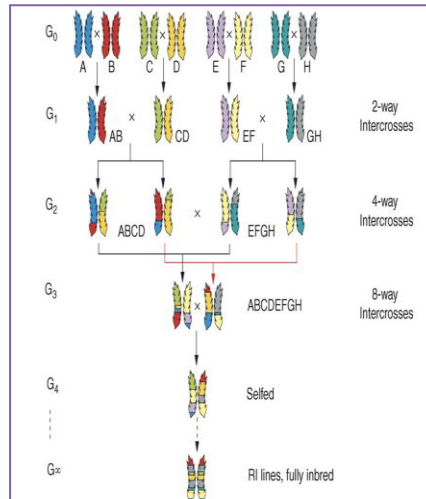
Software for decision support



Molecular Breeding Training underway for African breeders

Current Focus

- Marker-assisted backcrossing
 - Working with NARS partners to improve local cultivars
 - Using markers linked to drought, biotic stresses
- Marker-assisted recurrent selection
- Developing a MAGIC population
 - 8 way, 300 lines developed to F2 at present



**University of California,
Riverside**

Lucas, Mitchell
Muchero, Wellington
Pottorff, Marti
Wanamaker, Steve
Ehlers, Jeffrey D.
Roberts, Philip A.
Close, Timothy J.
Xu, Shizhong
Hu, Zhiqu

**National Agricultural Research
System (NARS)**

Cisse, Ndiaga, ISRA-Senegal
Drabo, Issa, INERA-Burkina Faso
Antonio Chicapa, IIA-Angola
Rogerio Chiulele, Eduardo Mondlane U,
Mozambique

**International Institute
of Tropical Agriculture
(IITA) Nigeria**

Muranaka, Satoru
Boukar, Ousmane
Fatokun, Christian



Thank You



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