



Kirkhouse Trust Annual Meeting 2023

Joint meeting of the African Bean Consortium (ABC) and
the African Cowpea Program (ACP)

27 February – March 2, 2023
Livingstone, Zambia

Legumes have long served as a food staple for humans, a source of feed and fodder for livestock, and nourishment for the soil. In Africa, as in many other parts of the world, they serve as the major source of dietary protein energy.



The Evolution of an Idea

The Kirkhouse Trust Initiates Agreement to Sequence Important Crop Genome

Oxfordshire, UK - May 9th, 2006 - The Kirkhouse Trust, a UK-based charity committed to improving agricultural crop research for the developing world, announced today the initiation of an agreement to sequence the cowpea genome, a major food source for the majority of the people in West Africa. Under the agreement, researchers at University of Virginia will use proprietary GeneThresher® technology, contracted nonexclusively from Orion Genomics, to reveal DNA sequence tags for greater than 90 percent of cowpea genes in the 1X sequence of the 'gene space'. The project, curated by Michael P. Timko, Ph.D., Professor of Biology at the University of Virginia, is expected to advance the development of disease- and pest-resistant cowpea plants.

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The Cowpea Genomics Initiative (CGI)

30,877	Candidate Simple Sequence Repeats (SSRs)
3,717	SSRs in BLAST annotated unigenes
> 2000	SSRs in disease and pest resistance genes
908	Disease and pest resistance genes (mapped)

>1000 SSR markers for trait mapping and diversity analysis

Timko et al (2008) Sequencing and analysis of the gene-rich space of cowpea. BMC Genomics 9:103

Kirkhouse Trust Cowpea Marker-assisted Breeding Project - Annual Meeting

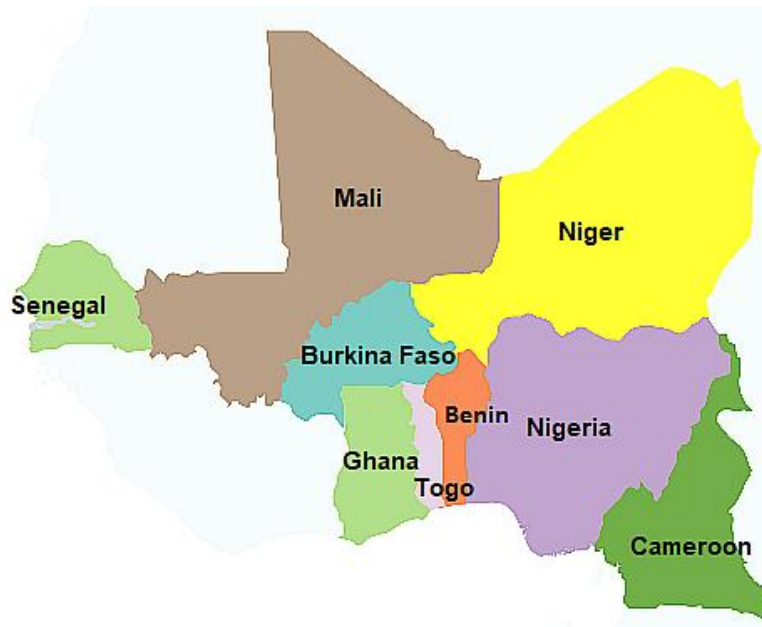
19-20 March 2007

Golden Tulip Hotel,

Accra, Ghana

West African Cowpea Consortium (WACC)

For ~16 years, has supported the genetic improvement of cowpea for West Africa through capacity building in the use of marker-assisted breeding and selection.



Partners (Past and Present)

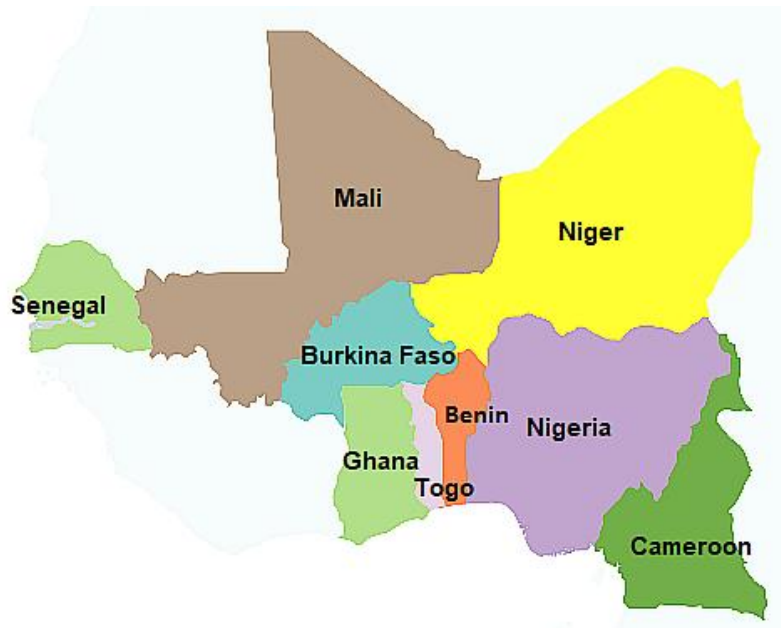
Nigeria
Burkina Faso
Cameroon

Senegal
Mali
Niger
Ghana
Togo
Benin

Helping local breeders create better cowpea for local farmers

In 2023, the WACC transitioned to the African Cowpea Program (ACP)

engaging researchers in Malawi, Zambia, and Zimbabwe and other locations in the future with the hope of transferring molecular tools, breeding materials, and technical know-how from the WACC to speed the development of improved genotypes for the region



Helping local breeders create better cowpea for local farmers

Kirkhouse Model

Focus on a small set of major constraints under simple genetic control to accelerate the improvement of local germplasm and train local breeders in the use of MAS

Local Cultivars



Identify Key Constraints
Identify Donor Germplasm
Determine Heritability
Development Segregating
Populations

Trait Mapping &
Marker Development
Marker-assisted Selection
(MAS)

Improved
Varieties

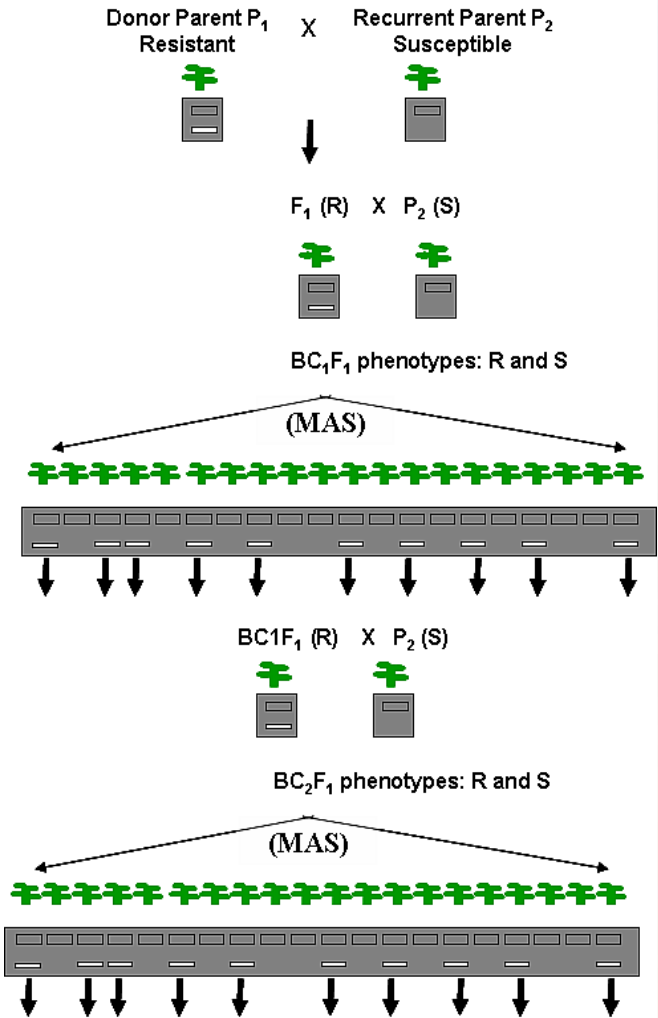
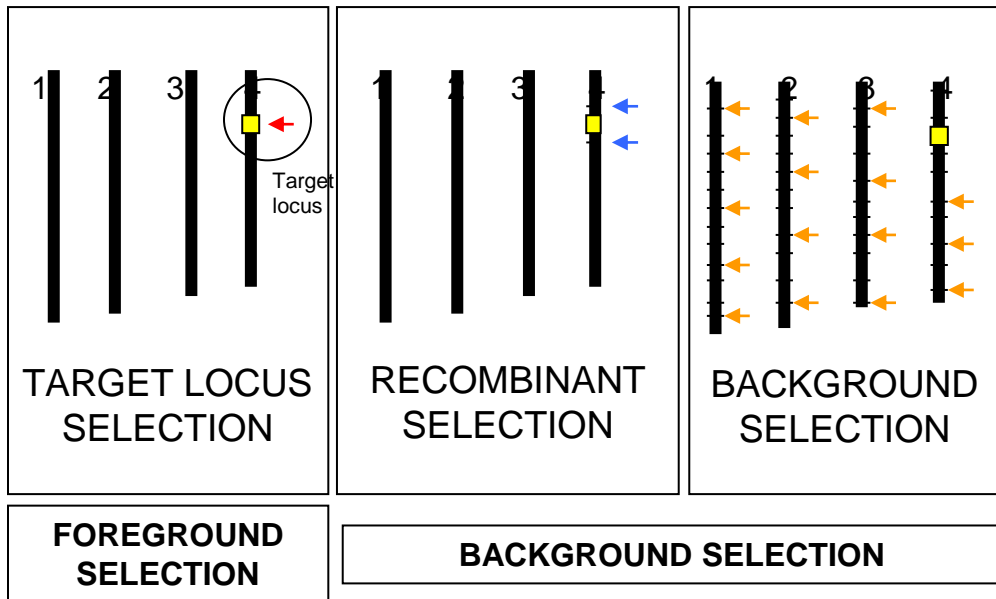


Technology Development
Capacity Building
Infrastructure Improvement

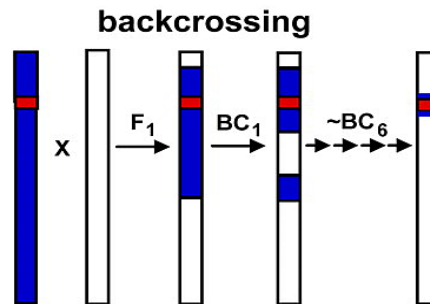
Field Testing
Farmer Engagement
Seed Dissemination



Development of tools and techniques for MAS that are affordable, readily available, easily applicable in African laboratory settings



Effective selection of target loci
 Accelerated recovery of recurrent parent
 Minimize linkage drag



Understanding the race structure of cowpea witchweed (*S. gesnerioides*) in West Africa and its implications for *Striga* resistance breeding of cowpea

Historical and proposed race designation for *S. gesnerioides* in West Africa based on differential host parasitism

Parker and Polniaszek (1990)		Lane et al. (1996)		Botanga and Timko (2007)		Proposed designation	
Country ^a	Race	Country ^a	Race	Country ^a	Race	Country ^a	Race
BF	SG1	BJ, BF, ML, NG, TG	SG1/SG4/SG6	BF, BJ, SN	SG1	BJ, BF, CM, GH, NG, TG	SG1
ML	SG2	ML	SG2	ML	SG2	ML, SN, TG	SG2
NE, NG	SG3	NE, NG	SG3	NE, NG	SG3	CM, NE, NG, TG	SG3
	SG4	BJ	SG4z	BJ	SG4	BJ	SG4
CM	SG5	BJ, BF, CM, NG	SG5	CM	SG5	BF, CM, GH, NG, TG	SG5
					SG6	NG	SG6

^aAbbreviations: BF, Burkina Faso; BJ, Benin; CM, Cameroon; GH, Ghana; ML, Mali; NE, Niger; NG, Nigeria; SN, Senegal; TG, Togo.



Effective genetic markers for *Striga* resistance in cowpea lines across West Africa

Race	524B	58-57	B301	IT82D-849	IT97K-499-35	IT81D-994	Suvita-2
1	S	R	R	R	R	R	R
2	S	S	R	R	R	R	R
3	S	S	R	R	R	S	S
4	S	R	S	S	S	R	R
5	S	R	R	R	R	S	S
6	S	S	R	S	R	S	S

^aAbbreviations: S, susceptible; R, resistant.

1. SSR Markers **SSR-1** (race RSG3), **C42B** (race RSG 5) and **61R/Mahse2** (RSG 1 & 2) colocalize on chromosome **Vu11**. SSR1 is an effective selection for the entire locus.
2. Breakdown of resistance in this locus has given rise to race **RSG4z** (Zakpota, Benin)
3. A QTL for resistance to **RSG 1,4** from Suvita-2 and IT81D-994 that maps in the interval from **SNP 2_11800** on the top of chromosome **Vu10** (~ 783 kb). This resistance is effective against **RSG4z**.
4. Landrace 58-57 presents a unique resistance fingerprint and likely contains unique resistance genes /alleles; **the genomic location of the *Striga* resistance in 58-57 is unknown.**

Release of improved local germplasm - a WACC reality

FUAMPEA 1 and FUAMPEA 2

(developed by Lucky Omoigui and his team at UAM Nigeria.)

'Zaayura Pali', 'Soo-Sima', 'Diffeelee', 'Wang Kae' and 'Kirkhouse Benga'

(developed by Francis Kusi and his colleagues at SARI, Ghana)

Komcalle, Tiligre, Nafi, and Gourgou

(developed by Benoit Batiemo and his colleagues at INERA, Burkina Faso)

IR15-MA-02 and IR15-MA33

(developed by Sobde Gonne at IRAD, Cameroon)

CZ06-3-1 (Acar 1), CZ06-2-17 (Simbo), CZ06-1-12, CZ06-4-16, and CZ06-1-05

(developed by Sory Diallo and his coworkers at IER, Mali)



Lucky Omoigui and his team at AVISA trials Benue



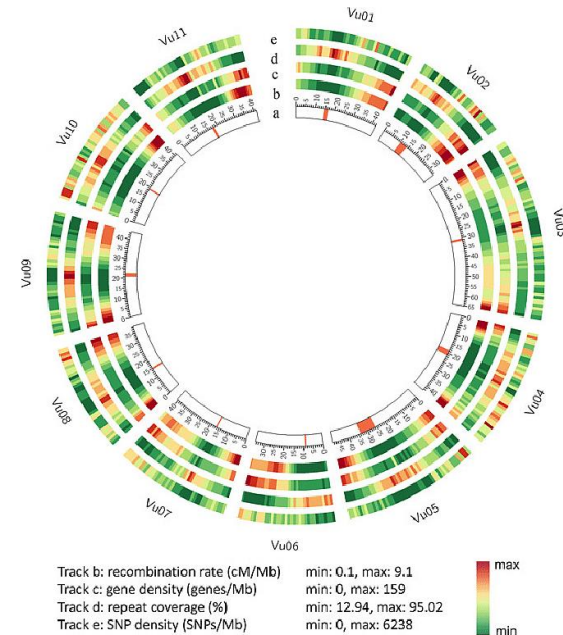
Francis Kusi - Best Agricultural Research Scientist in Ghana

Integration with Cutting Edge Genomic Tools

Refined Cowpea Genome Assemblies

A highly refined genome assembly using single-molecule real-time sequencing combined with optical and genetic mapping; a revised numbering system for cowpea chromosomes based on synteny with common bean (*Phaseolus vulgaris*).

(Lonardi S, Muñoz-Amatriaín, et al (2019). *Plant J.* 98, 10.1111/tpj.14349).



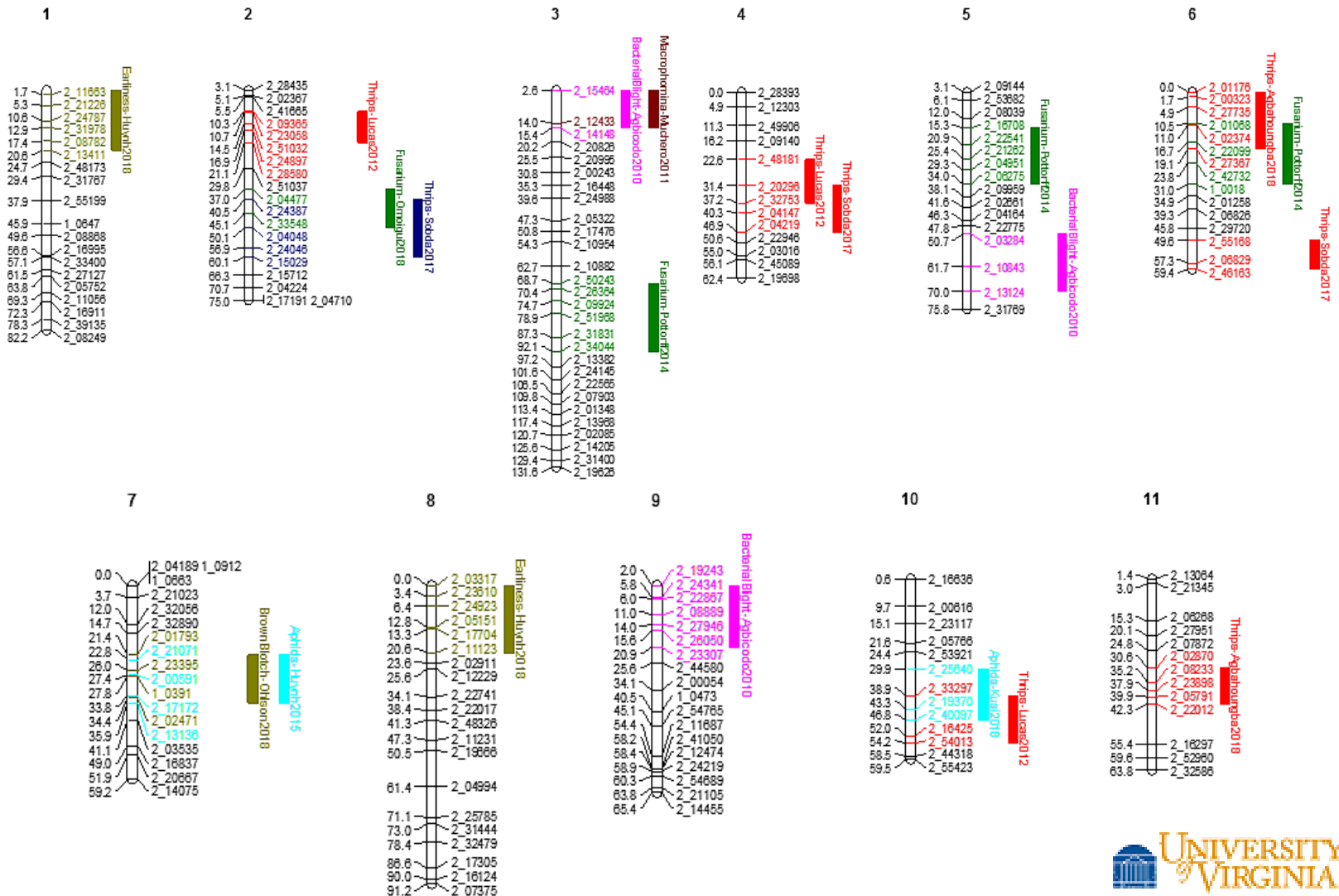
Development single nucleotide polymorphism (SNP) platforms for linkage mapping, genetic diversity analysis and cultivar fingerprinting

Illumina Cowpea iSelect Consortium Array (Muñoz-Amatriaín et al., 2017)

Kompetitive Allele Specific PCR (KASP) (Wu et al., 2021 *Legume Science*)

Single gene and QTL mapping

Development and application of 200 well-space, linkage group-associated allele-specific (AS) PCR primers for foreground and background analysis



Location of key trait associated and allele-specific SNPs on cowpea genetic map



New sources of aphids [*Aphis craccivora* (Koch)] resistance in cowpea germplasm using phenotypic and molecular marker approaches

L. O. Omoigui · G. C. Ekeuro · A. Y. Kamara · L. L. Bello · M. P. Timko · G. O. Ogunwolu



ORIGINAL ARTICLE

Genetic analysis of Fusarium wilt resistance in cowpea (*Vigna unguiculata* Walp.)

Lucky O. Omoigui^{1,2} | Catherine C. Danmaigona¹ | Alpha Y. Kamara² | Ebenezer J. Ekefan¹ | Michael P. Timko³



Quantitative trait loci analysis of brown blotch resistance in cowpea variety KN1

Erik W. Ohlson · Gilles I. Thio · Mahamadou Sawadogo · Paco Sérémé · Michael P. Timko

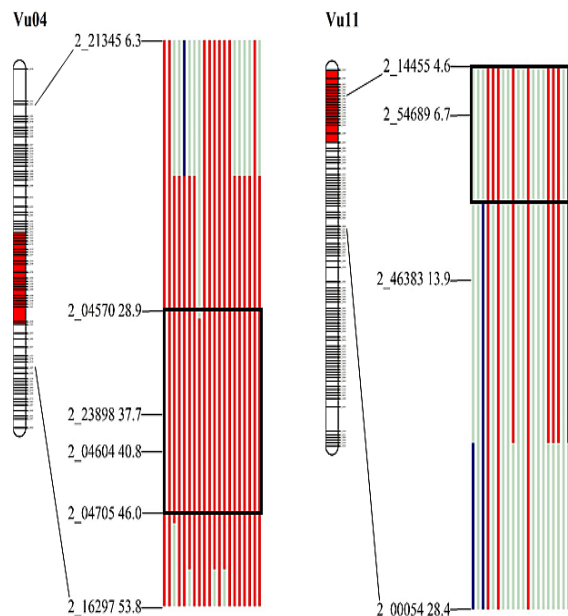


Article

Mapping and Validation of *Alectra vogelii* Resistance in the Cowpea Landrace B301

Erik W. Ohlson ^{1,2} and Michael P. Timko ^{1,*} 

Phenotyping and genotyping of segregating BC3F2 and BC3S1 families developed from a cross of **524B** (*Alectra* susceptible) and **B301** (*Alectra* resistant) identified 2 resistance loci against the parasite found in the Kikomba- Dodoma regions of Tanzania.



Rav1 located on a 10 cM interval between SNP markers 2_07872 and 2_04604 on chromosome Vu04. Resistance at this loci approximated a 3:1 segregation indicative of a single dominant gene (X2 test $p < 0.05$).

Rav2 mapped near the top of chromosome Vu11, in a 6.7 cM interval between SNP markers 2_41050 and 2_46383. Resistance appears to be consistent with homozygous recessive trait.

The Rav1 (Vu4) and Rav2 (Vu11) resistance intervals are R-gene hotspots
9 BLAST hits mapping to Vu04; 27 BLAST hits mapping to Vu11

Rav1 interval

Vigun04g080050 encodes a RPP13 like NBS-LRR (ARC) domain-containing disease resistance protein that is a potential paralog of the *S. gesnerioides* resistance gene RSG3-301 (e-value < 4 e 10⁻⁷)

Rav2 interval

Contains 27 annotated genes. Tightly linked to the RSG3-301 gene (conferring resistance to *Striga gesnerioides*),

Surprisingly, no RSG3-301 paralogs were detected with the interval.

Phosphorus Use Efficiency (PUE) QTL in the Cowpea MAGIC population

Rationale: Variation in phosphorus uptake efficiency among cowpea genotypes is due to differences in root architecture, root hair length and rhizosphere induced processes.

MAGIC parental lines: IT89KD-288, IT845-2246, IT845-2049, CB27, IT93K-503-1, SUVITA-2, IT00K-1263, IT82E-18, TVU-14676, 524B. **IT89KD-288** has been reported to be superior to other cultivars in terms of efficiency in the utilization of phosphorus.

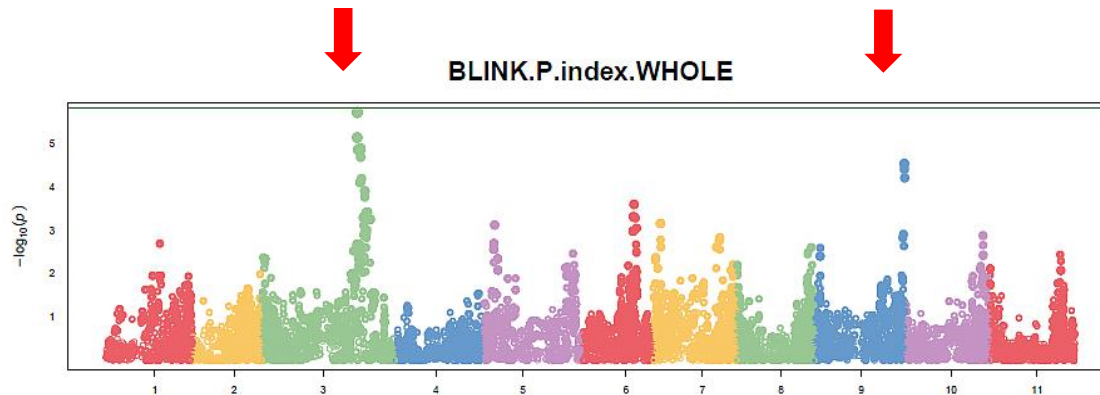
Experimental Approach: A drain and flood hydroponic system was constructed in order to examine differential growth of cowpea under high (136 g/L Monopotassium phosphate - KH_2PO_4 (pH 6)) and no phosphorous. Replicates were grown for 6 wk, and shoot and root tissue were collected, dried at 100°C and shoot (SDW), root (RDW) and total dry weight (TDW) were determined.

- (i) As a first approximation, we generated 14 groups and assigned each phenotype a number (1-14) and conducted an ANOVA based on differences in whole plant weight under high PO_4 and low / no added PO_4 .
- (ii) We then generated a phenotypic index for stem, root, and whole plant determined as weight under low P / weight under high P * 100 considering each as separate phenotype.

We used three models (BLINK, MLM, and FarmCPU) for analysis and compared the predicted QTL in each model.

When then performed genotype (0.05) and phenotype filtering (at 2 SD and 3 SD, respectively) and examined the FDR adjusted p values to improve on the ability to identify significant QTLs. .

We found two QTL one on chromosomes Vu03 and another on Vu09 that accounted for the majority of the variation in phosphate use efficiency (PUE)



Approximately 28 candidates and 12 candidates, respectively, are found at these QTL.

One gene encoding **carbamoyl phosphate synthetase**, *CPSase* was previously reported to increase 2-10-fold in response to P starvation in shoots and is part of a coordinated transcriptionally regulated pathway that salvages and utilizes phosphates for UMP and pyrimidine nucleotide formation,

Possible Research Directions in Cowpea

Breeding varieties for agronomic characteristics:

- dual purpose (grain and leaves for human consumption; fodder)
- spreading versus erect (better adapted for intercropping)
- perennialism

- Super-nodulation phenotypes or improved biological nitrogen fixation potential
- Phosphate utilization efficiency
- Water utilization efficiency

- stay-green meristem (drought/heat stress)
- root architecture (deep or tap rooted)

- micronutrients (e.g., iron, magnesium, zinc, potassium, B vitamins)
- removal of anti-nutritional components in weaning foods
- cooking / processing time

We also likely need a better understanding of the genetic X environmental factors that affect yield and how to select for genotypes that best fit agronomic practices and associated factors.



Domestication, stem/root architecture, and perenniality potential

We examined an F7 RIL population developed by single-seed descent from a cross between 524B, a California black-eyed cowpea, and 219, a wild cowpea from subsp. *unguiculata* var. *spontanea* (Schweinf.) Domestication was accompanied by changes in root/stem architecture, floral structure and scent, flowering, stress tolerance and perenniality and were able to map 10 domestication-related traits (DRTs) of cowpea.

(2014) Andargie, M, Pasquet RM, Gowda BS, Muluvi GM, Timko MP (2014) Molecular mapping of QTLs for domestication-related traits in cowpea (*V. unguiculata* (L.) Walp.) *Euphytica* 200:401-412

Sassoum Lo and her colleagues examined a RIL population derived from a wild parent (TVNu-1158) with a perennial growth habit and a cultivated parent (IT99K-573-1-1) with an annual life cycle and identified 16 QTL for these nine domestication related traits and in a follow up study identified two QTL for floral scent and one QTL for longevity.

Lo, S., Muñoz-Amatriaín, M., Boukar, O. et al. Identification of QTL controlling domestication-related traits in cowpea (*Vigna unguiculata* L. Walp). *Sci Rep* 8, 6261 (2018).
<https://doi.org/10.1038/s41598-018-24349-4>

Lo S, Fatokun C, Boukar O, Gepts P, Close TJ, Muñoz-Amatriaín M. Identification of QTL for perenniality and floral scent in cowpea (*Vigna unguiculata* [L.] Walp.). *PLoS One*. 2020 Apr 28;15(4):e0229167

Analysis of stem/root architecture and perenniality potential

We believe there is a correlation between stem/root architecture (i.e., larger thicker stems), drought and stress tolerance, and perennial growth potential (that is the ability to die back and re-shoot or ratoon).

Using our 524B (a blackeye type) x 219-01 (perennial wild cowpea) as well as additional domesticated X perennial crosses we are interested in testing:

- (i) Heritability of differences in root/stem architecture [stem diameter (SD) (mm) at soil level, tap root diameter (TRD) (mm) at 5, 10, 15 and 20 cm below the soil surface, basal root number (BRN), and adventitious root number (ARN)] and their effects on WUE, PUE, and photosynthetic partitioning.
- (ii) Potential for perennialism following water [drought] and nutrient stress.
- (iii) Potential for regrowth/ratooning/perennialism following grazing or herbivory mimicry.

Screening cowpea mutant populations for drought tolerance at the seedling stage using mutant population

We plan to screen a Mutant Diversity Panel of ~650 individuals developed by Kelvin Kamfwa for important physiological and morphological traits that may contribute to drought adaptation. Parental lines, mutants, and several cultivars previously reported to have contrasting responses to drought (e.g., tolerant lines -Danila, IT96D-602 and TVu 11986 and highly susceptible lines - TVu 7778) as controls

(i) **Delayed Leaf senescence (DLS)** will be scored on a scale of 1-5:

1= Green and fresh leaves; 2= Green and slightly wilted leaves; 3= Green-yellow and moderately wilted leaves;
4= Yellow-green and severely wilted leaves; 5= Yellow to brown leaves.

(ii) **Stay-Green Meristem (SGM)** will be rated 0 or 1, where 0= not green and 1= green.

(iii) **Leaf chlorophyll content and photosynthetic capacity** - reported to decrease sharply with drought stress and is a good indicator of plant health.

(iv) **Plant recovery** will be scored at 16 days after resumption of watering.

(v) **Structural characteristics** - root and shoot weight (RWT,SWT), stem diameter (SD) Diameter of stem (mm) at soil level, tap root diameter (TRD) (mm) at 5, 10, 15 and 20 cm below the soil surface, and adventitious root number (ARN) to determine if correlations exist with stem/root architecture and seedling drought tolerance.

Goals of this meeting: Current status of work within the ACP

1. What are our most important accomplishments this past year and what have we learned since our last meeting?
2. What new sources of germplasm have been identified that can be used as donors for alleviating key biotic/abiotic constraints and improving important agronomic characters; what do we know about the heritability of the genes controlling these constraints?
3. What are the best strategies for integrating foreground and background selection to achieve breeding success? How do we ensure the integrity and performance of our improved lines?
4. How should we prioritize our activities in order to best address the most important needs in each country's cowpea improvement program? What constraints and agronomic characteristics best reflect local farmers' needs.
5. How many local varieties do we need to improve (and for how many traits) to meet the needs of the broadest number of farmers?
6. Is the Kirkhouse Trust "model" working and what changes, if any, do we need to make to ensure the future productivity of the ACP?

Thank you

Kirkhouse Trust SCIO