Cowpea Improvement Activities at the University of Virginia

Kirkhouse Trust Annual Meeting

African Cowpea Program (ACP), African Bean Consortium (ABC),

and Bambara Breeding Initiative (BBI)

June 16-20, 2024
Arusha,Tanzania

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Research at UVA is focused on the development of molecular markers supporting the breeding efforts of the ACP scientists aimed at creating disease and pest resistant cultivars that are "best fit" for local agronomies and local farmer needs.

Genotype X Environment X Farmer Preference

- Biotic stress resistance (*Striga*, *Alectra*, insects, microbes)
- Phosphate utilization efficiency (PUE)
- Seedling drought tolerance
    - stay-green meristem (drought/heat stress)
    - root architecture (deep or tap rooted)

- Perennialism / Dual purpose types
    - spreading versus erect for intercropping
    - leaves and stover (for human and animal nutrition, respectively)

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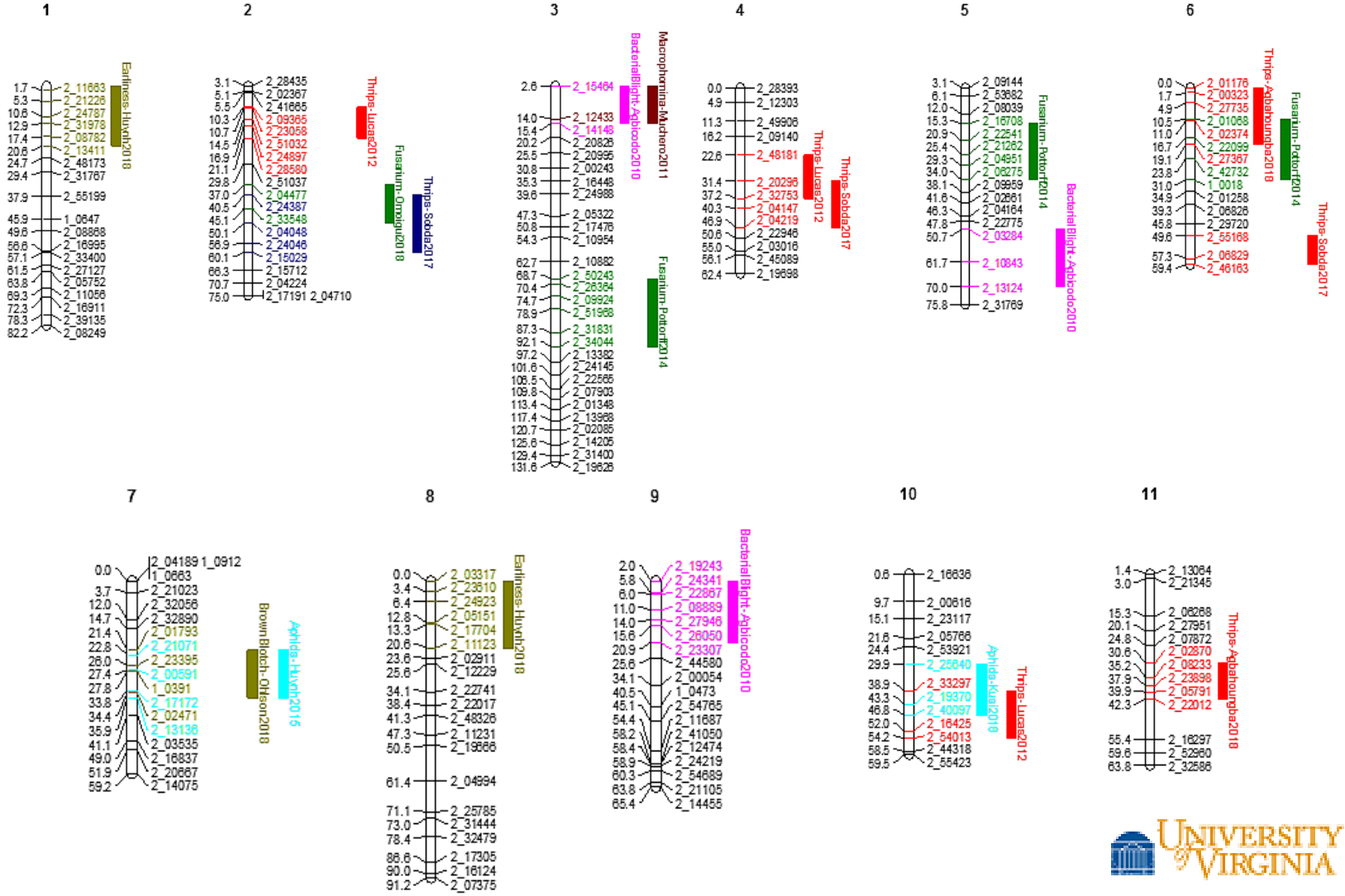
Development of ~200 well-space, linkage group-associated allele-specific (AS) PCR amplifiable markers useful for foreground and background selection



Identification of improved markers for Aphid resistance

CP171/172 is a marker associated with aphid resistance in SARC1-57-2 (a major source of resistance in West Africa). Unfortunately, CP171/172 is about 9-12 cM from gene. To improve utility, Dr.. Pramod Khadka used the existing SNP information and the genome assembly for cowpea to locate SNPs located upstream and downstream of the SSR and converted them to new AS-PCR markers.

Marker Name	Distance from CP171-172	Annealing Temp	Product Size	Sequence	SNP
2_08228-F	1490235	55	160	CGTGTGAGGTGAAATTGGAA	
2_08228-R1		55		ATTACTATTTAGATACAGCACTTTGATTGGAACAAC	G
2_08228-R2		55		TACTGCAGCACTTTGATTGGAACTAT	A
2_33297R	-643624	55	177	GGCAATGAGCCACCATAGAT	
2_33297F1		55		ACCATACATTACATAGAACTAAGACAAACAACCAACA	A
2_33297F2		55		ATCAAGAACTAAGACAAACAACCATCT	T
2_49641-R	544224	60	190	TCCCAACCACATTTTCACTTC	
2_49641-F1		60		TTATAATAACTATACTGTCTGCATGTTGGTGTTGCGT	T
2_49641-F2		60		TTATATCTGCATGTTGGTGTTGGGC	C
2_04879-R	231504	60	153	TGAGGTCCCAAGCGTAACTC	
2_04879-F1		60		TGTATACTATTACATGACCCTAAAGTATGGAATGAACCCTTA	A
2_04879-F2		60		GCTAGCCTAAAGTATGGAATGAACCCATC	C
2_15418-F	-417644	60	150	ACCTCTCACGGAGGTCACTG	
2_15418-R1		60		TAATCAAGTTCATATAATCAGAGCCGCAGAACCA	T
2_15418-R2		60		GCATATCAGAGCCGCAGAAGCG	C
2_15880-R	-1010565	55	200	TCCACAAACCCATCTCCATT	
2_15880-F1		55		TAATACATTAAGAATTCTCATCTTCTTTCCTTGAGATGTAA	A
2_15880-F2		55		CTATCGTTCATCTTCTTTCCTTGAGATGAAG	G
2_19370R	2087046	55	143	TGTTCTCACTGCGGAACAGT	
2_19370F1		55		ACCATACATTACATATGTTTCCATTTTCACACAAG	G
2_19370F2		55		TGCGGTGTTTCCATTTTCACACTAA	A
2_53921R	-5795767	55	198	CTCACACACACACCCGAAAC	
2_53921F1		55		ACCATACATTACATAGAGCACAGGTTTTCACAGACAT	T
2_53921F2		55		ATCAAGAGCACAGGTTTTCACAGAGAC	C
2_19370R	-2087046	55	143	TGTTCTCACTGCGGAACAGT	
2_19370F1		55		ACCATACATTACATATGTTTCCATTTTCACACAAG	G
2_19370F2		55		TGCGGTGTTTCCATTTTCACACTAA	A
2_10563-R	927738	55	190	TGGTTTGGGATCAAAAGGAG	
2_10563-F1		55		ACATAATTACATTAATTTTGAGGTAGCATGCAATCAG	G
2_10563-F2		55		CGATATTTTGAGGTAGCATGCAATGAT	T
	(-) sign means downstream of 171-172				

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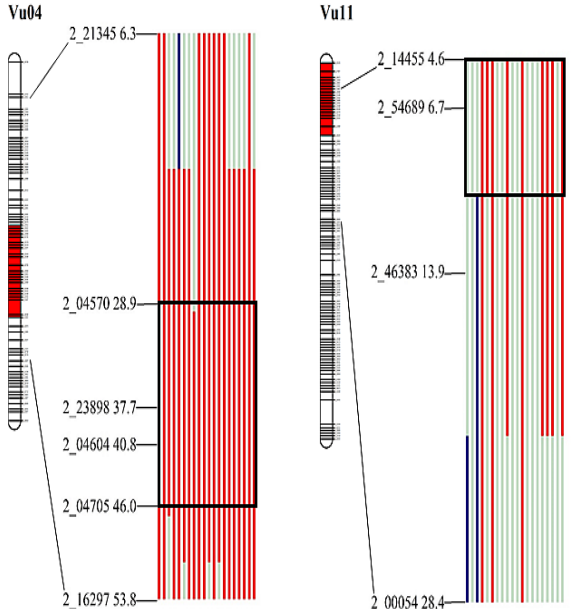
Identification of markers for Alectra resistance

B301 is a well know source of resistance to Alectra vogelii and appears to have two genes involved in the resistance response. We phenotyped and genotyped BC3F2 and BC3S1 families derived from a cross of **524B** **(*Alectra* susceptible)** and **B301** **(*Alectra* resistant)** challenged with Alectra collected in the **Kikomba- Dodoma** regions of Tanzania.



Rav1 Resistance at this loci is conferred by single dominant gene (~ 3:1 segregation and X^2 test $p < 0.05$).
Gene is located on chromosome Vu04 and tagged by SNP markers 2_07872 and 2_04604

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

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The Rav1 (Vu4) and Rav2 (Vu11) resistance intervals are "R-gene hotspots"

9 gene models map to Vu04; 27 gene models map to Vu11

Rav1 interval

One gene, Vigun04g080050, located within the Rav1 interval 11411318 - 11414855 encodes a RPP13 like NBS-LRR (ARC) domain-containing disease resistance protein that is a paralog of the S. gesnerioides resistance gene RSG3-301 (e-value < 4 e 10-7). This gene contains a single exon and has only one mRNA isoform. We have cloned this gene and are characterizing its resistance conferring properties.

Rav2 interval

Contains 27 annotated genes including RSG3-301 gene (conferring resistance to *Striga gesnerioides SG3*). Surprisingly, no clearly identifiable RSG3-301 paralogs were in this interval although there are annotated R-genes.

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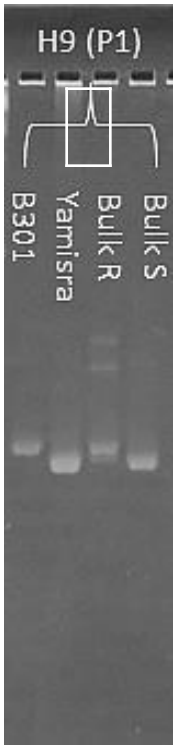
Macsamuel Ugbaa (UAM) phenotyped an F2 population of Yamisra x B301 with Alectra and mapped three different AS-PCR primer combinations

Table 1: χ^2 Analysis of segregation of AS-PCR Markers in the F_2 Generation of Yamisra X B301



Marker	Number of lines	Resistant	Susceptible	Genetic ratio	Calculated χ^2 value	Critical χ^2value (P<0.05)
H9	250	205	45	15:1	58.91	7.815
B10		101	49	15:1	147.19	7.815
D11		195	55	15:1	105.84	7.815

Marker Name	Plate	Well	Annealing Temperature	Mg (mM)	CONSENSUS MAP LINKAGE GROUP	LOCUS	PHYTOZOME CHROMOSOME	LOCUS	Product Size	Sequence	SNP
2_20296R	P1	H9	60	2.5	4	31.4	1	31.4	179	CCTAAGCCTGCCATTTCAAG	
2_20296F1			60	2.5	4	31.4	1	31.4		ACCATACATTACATACTTTTCTTCACCGCCGTT	T
2_20296F2			60	2.5	4	31.4	1	31.4		ATCAACTTTTCTTCACCGCCCTC	C
2_04147F	P1	B10	60	2	4	40.3	1	40.3	218	AGACCCCACTTCTTGTTCCA	
2_04147R1			60	2	4	40.3	1	40.3		ACCATACATTACATACTATCTCTACTAACCGACAGCC	G
2_04147R2			60	2	4	40.3	1	40.3		ATCAACTATCTCTACTAACCGACACCT	A
2_22541R	P1	D11	45	2.5	5	20.9	8	20.9	216	GGTACGTTTTAAAATTGATATGACCA	
2_22541F1			45	2.5	5	20.9	8	20.9		ACCATACATTACATAGATGTTCACAGATGTACTGATG	G
2_22541F2			45	2.5	5	20.9	8	20.9		ATCAAGATGTTCACAGATGTACTGTTA	A

14 % recombination frequency

Recombination frequency R phenotype S Genotype 28/250 – 11%
Recombination frequency S phenotype R Genotype 8/250 – 3%

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Other potential sources of Alectra resistance

In addition to B301, B359 has been implicated as providing Alectra resistance in Botswana.

Dieni et la (2018) have reported that the cultivars/breeding lines KVx414-22-2, KVx165-14-1, Komcalle, IT99K-573-2-1, IT98K-205-8, IT86D10-10, and IT93K-693-2 show some resistance to *Alectra* in Burkina faso.

IT199k-7-21-2-2-1 and IT99K-573-2-1 were used for the selection of VULI AR1 and VULI AR21, respectively and these lines have been released in Tanzania by TARI.

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Fine mapping of Striga SG4z resistance on chromosome Vu10

Suvita-2 (aka Gorom Local) and IT81D-994 are known to carry resistance to the Zakpota race (SG4z). We grew field collected *S. gesnerioides* (SG4z) on B301 and collected the seeds from the emerged plants. This ensured that the *Striga* parasite was specific in overcoming the innate resistance in B301 conferred by SG1/SG4.

F2 mapping populations were developed from crosses of
524B x Suvita-2 and 524B x IT81D-994.

SG4z-B301 interactions at 30 dpi

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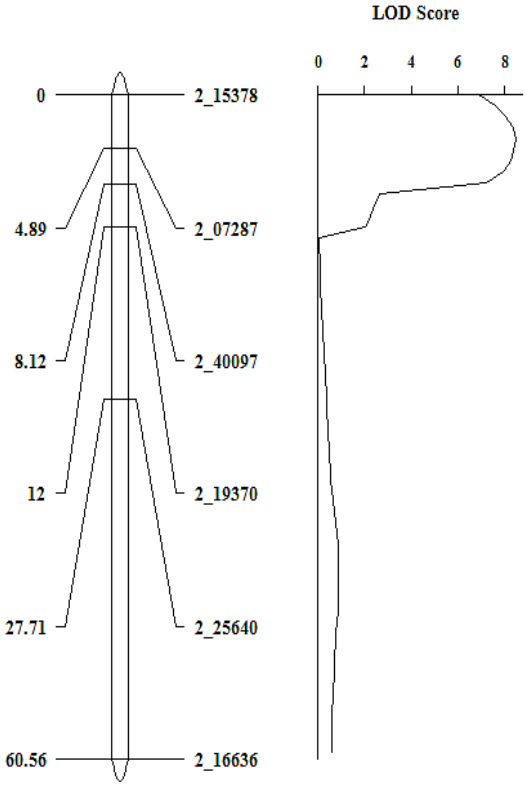

160 individuals from the 524B x Suvita-2 F2 population were phenotyped for resistance to SG4z and genotyped with four SNP markers on LG 10 located in the interval where the resistance gene was expected to be located.

Analysis of 20 BC4F3 families indicated that the **resistance gene falls between the top of Vu10 and the SNP 2_11800, a 783 kb interval.**

Additional markers are being added to the F2 map to improve mapping resolution of the SG4z resistance gene.

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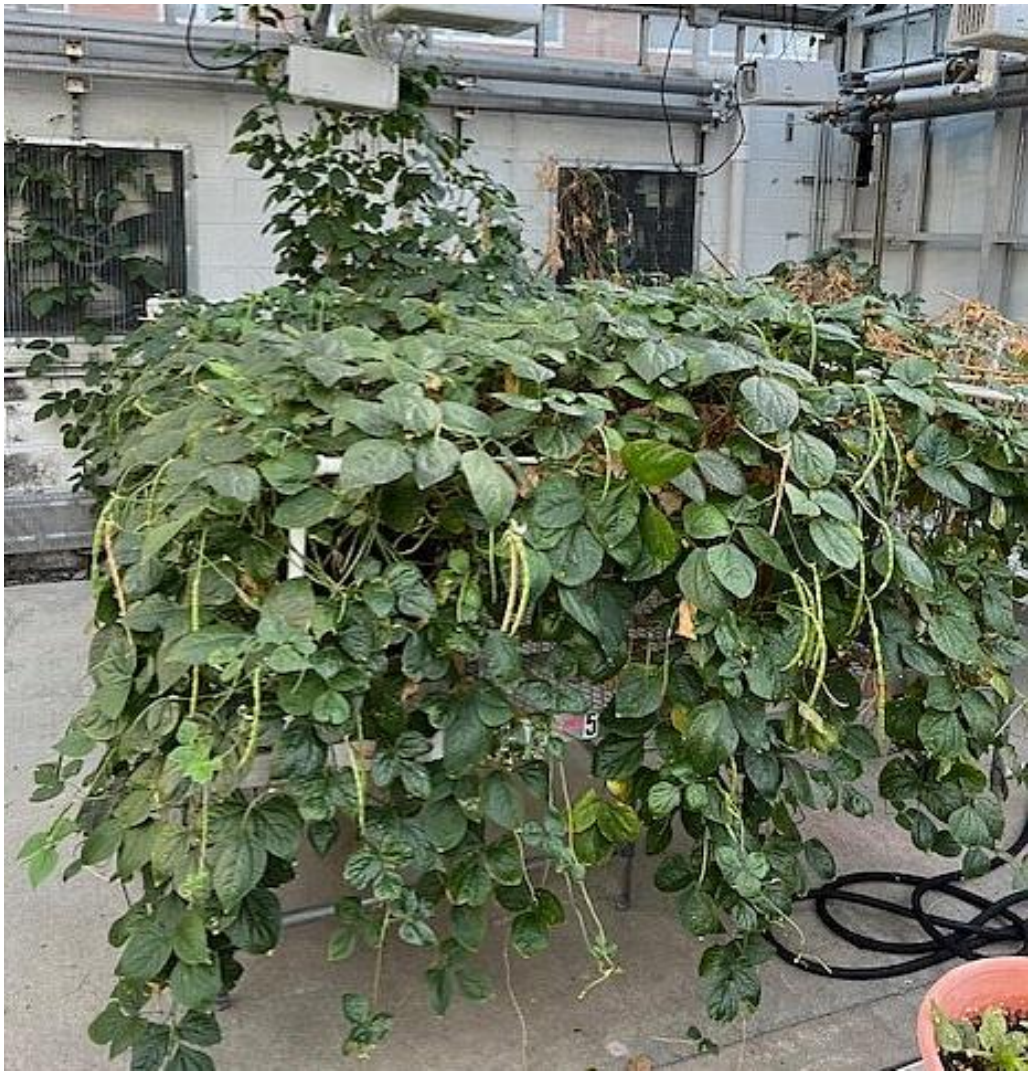
[524B X Suvita 2] X 524B BC1F1 plants show extreme indeterminate growth



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To determine whether **SG4z** resistance conferred by **IT81D-994** maps to the same locus as Suvita-2, a second F2 populations developed from a cross between 524B X IT81D-994 is being phenotyped and markers associated with resistance in Suvita-2 tested to determine if the resistance locus corresponds in the two populations.

In addition to mapping SG4z resistance, the 524B x IT81D-994 F2 population will also be used to map resistance to Alectra vogelii, since IT81D-994 also has resistance to East African Alectra.

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Mapping QTL for Phosphorus Use Efficiency (PUE)

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, IT84S-2246, IT84S-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 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 PO4 and low / no added PO4.

(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.

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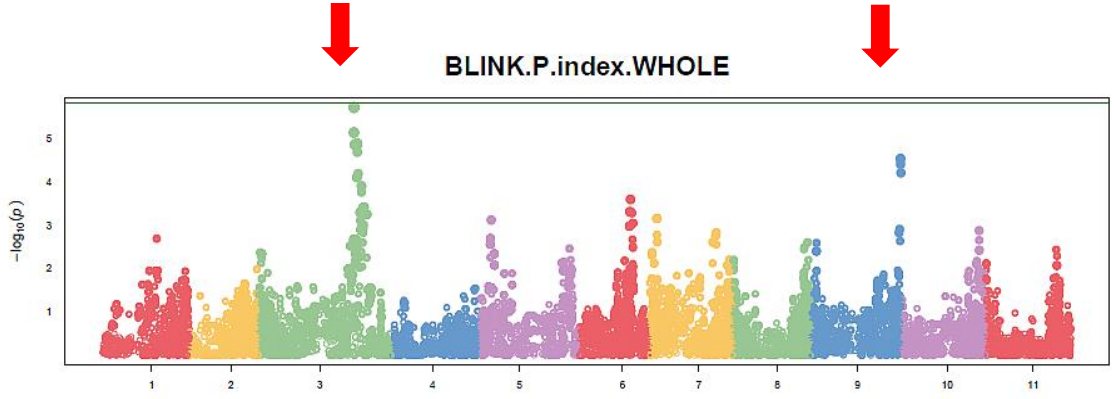
We used three models (BLINK, MLM, and FarmCPU) for analysis and compared the predicted QTL in each model.

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,

Predicted Gene function of SNPs in QTL for PUE

SNP	Chr	Pos	H.B.P.Value	Alleles	In_gene	Function
2_16565	3	46568790	0.019153364	G/A	Vigun03g284700	(1 of 2) PTHR11207//PTHR11207:SF4 - RIBONUCLEASE III // SUBFAMILY NOT NAMED
2_15367	3	46596976	0.019153364	G/A	Vigun03g284900	NA
2_53736	3	46608437	0.019153364	T/C	NA	NA
2_53362	3	46614227	0.019153364	T/G	NA	NA
1_0345	**3**	**46627017**	**0.007986504**	**A/G**	**Vigun03g285000**	**(1 of 2) PTHR31517//PTHR31517:SF3 - FAMILY NOT NAMED // PEROXIDASE 35-RELATED**
2_09131	**3**	**46627117**	**0.007986504**	**C/T**	**Vigun03g285000**	**(1 of 2) PTHR31517//PTHR31517:SF3 - FAMILY NOT NAMED // PEROXIDASE 35-RELATED**
2_09132	**3**	**46627539**	**0.007986504**	**C/T**	**Vigun03g285000**	**(1 of 2) PTHR31517//PTHR31517:SF3 - FAMILY NOT NAMED // PEROXIDASE 35-RELATED**
2_28062	**3**	**46630232**	**0.007986504**	**G/T**	**Vigun03g285100**	**(1 of 4) PF12767 - Transcriptional regulator of RNA polII, SAGA, subunit (SAGA-Tad1)**
2_38772	**3**	**46647927**	**0.007986504**	**G/T**	**NA**	**NA**
2_38773	**3**	**46650779**	**0.007986504**	**C/A**	**NA**	**NA**
2_30865	**3**	**46670477**	**0.007986504**	**C/A**	**NA**	**NA**
2_48922	**3**	**46676934**	**0.007986504**	**C/T**	**NA**	**NA**
2_47283	3	46813741	0.019153364	C/T	NA	NA
2_05364	3	47047999	0.019153364	A/G	Vigun03g288200	NA
2_25490	3	47092220	0.019153364	A/C	NA	NA
2_00381	3	47278916	0.019153364	A/G	Vigun03g290200	(1 of 1) PTHR10641//PTHR10641:SF565 - MYB-LIKE DNA-BINDING PROTEIN MYB // SUBFAMILY NOT NAMED
2_11181	3	47417728	0.019153364	A/G	Vigun03g291000	(1 of 7) K07374 - tubulin alpha (TUBA)
2_54847	3	48152883	0.019153364	C/G	Vigun03g295600	(1 of 1) K01338 - ATP-dependent Lon protease (lon)
2_44975	3	48198677	0.019153364	T/G	NA	NA
2_37593	3	48240049	0.019153364	T/C	NA	NA
2_51883	3	48245745	0.019153364	T/C	NA	NA
2_43139	3	48250078	0.027055146	A/T	NA	NA
2_17678	3	48258564	0.019153364	T/C	Vigun03g296000	(1 of 1) PTHR35696//PTHR35696:SF1 - FAMILY NOT NAMED // F6N18.11
2_48805	3	48262329	0.019153364	T/G	Vigun03g296000	(1 of 1) PTHR35696//PTHR35696:SF1 - FAMILY NOT NAMED // F6N18.11
2_00438	3	48290067	0.019153364	C/T	Vigun03g296300	NA
2_05229	**9**	**43532885**	**0.035706255**	**C/T**	**Vigun09g274700**	**(1 of 3) PTHR10774//PTHR10774:SF50 - EXTENDED SYNAPTOTAGMIN-RELATED // SYNAPTOTAGMIN-1-RELATED**
2_05228	**9**	**43538365**	**0.035706255**	**G/A**	**Vigun09g274800**	**(1 of 2) PTHR11254//PTHR11254:SF73 - HECT DOMAIN UBIQUITIN-PROTEIN LIGASE // E3 UBIQUITIN-PROTEIN LIGASE TRIP12**
2_25076	**9**	**43614081**	**0.046305411**	**C/A**	**Vigun09g275600**	**(1 of 2) PTHR10044//PTHR10044:SF112 - INHIBITOR OF APOPTOSIS // SUBFAMILY NOT NAMED**

Drought tolerance at the seedling stage in a cowpea mutant population

We recently screened a Mutant Diversity Panel consisting of ~650 individuals developed from the cultivar Namuseba by Kelvin Kamfwa (Kenya) as part of a gamma mutagenesis study with the IAEA. Our targets in this analysis were traits associated with seedling drought tolerance:

(i) **Delayed Leaf senescence (DLS) -** 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) -** rated 0 or 1, where 0= not green and 1= green.

(iii) **Plant survival / recovery (SUR) after rewatering** scored at 16 days after resumption of watering.

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

(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.

Seedling drought tolerance

Plants were well-water for about 21 days (through the production of the first trifoliate leaves) and then water was withheld. The response of the parental and mutant lines, as well that of several controls (i.e., cultivars previously reported to have contrasting responses to drought stress such as the tolerant lines Danila, IT96D-602 and TVu 11986 and the highly susceptible line TVu 7778)) was tracked over the next 14 to 21 days.

(Left) Well-watered, day 21 days – Day 0 drought (Right) Drought stress (no water) at Day 14.

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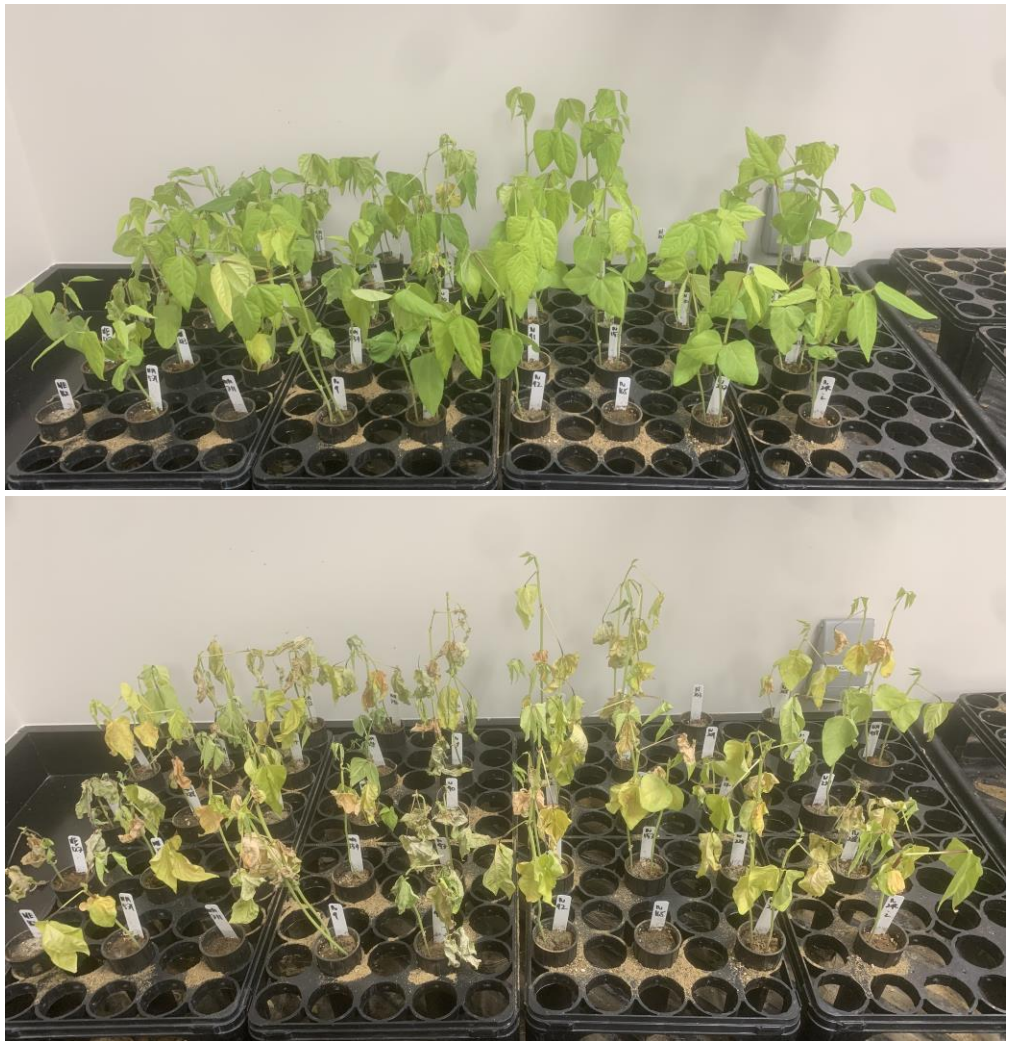
Mutant lines showing greatest tolerance in Trial I were re-screened for DLS, SGM, and SUR

(Top) watered, day 0 (Bottom) no water, day 14.



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General Conclusions:

- 12-15 of the most tolerant lines were selected for further analysis.
- These lines exhibited the strongest SGM response and had reduced DLS (delayed trifoliate leaf senescence) compared to controls.
- We also observed that SGM was also correlated maintenance of stem greening and with DLS.

Interestingly, Muchero et al. (2009) previously mapped several QTL associated with drought-induced trifoliate senescence (DTS), stem greenness (Stg) and survival (Sur) under drought stress at seedling stage in a RIL population generated from a cross of Danila X TVu7778.

QTL for DTS, Stg and Sur overlapped on LG7 and another QTL for Stg and Sur overlapped on LG3, but the QTL for DTS was located elsewhere on LG6.

Whether these QTL have been mutated in the current population is not yet known..

Analysis of stem/root architecture and perenniality potential

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

Mebea Andargie et al. (2014) previously developed an F2 population from a cross of 524B (Blackeye type) x 219-01 (perennial wild cowpea) to study traits associated with domestication; also a good population to study perenniality.

(i) Parents and offspring show heritable differences in root/stem architecture [stem diameter (SD) at soil level, tap root diameter (TRD) at 5, 10, 15 and 20 cm below the soil surface, basal root number (BRN), and adventitious root number (ARN)] as well as differences in WUE, PUE, and photosynthetic partitioning.

(ii) Potential for regrowth (perennialism) after prolonged water [drought] and nutrient stress.

(iii) Potential for regrowth/ratooning/perennialism following grazing or herbivory mimicry.

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Research Team at UVA

Erik Ohlson, PhD – Striga and Alectra mapping, PUE
Pramod Khadka,,PhD – Aphid and Alectra markers

Danhua Zhang, PhD candidate – SG4z resistance
Hai Liu, PhD – General support

Tatyana Katova – Technical support
Chris Claussen – Greenhouse Manager

Rebecca Hicks – PUE analysis
Xiaoxuan Zhang – PUE mapping studies
Reed Burgess – Drought studies

All of our WACC / ACP Partners and collaborators

Thank you
Kirkhouse Trust SCIO

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