

Enhancing Cowpea Resistance against *Alectra vogelii* through Marker assisted breeding





BSC. Agriculture general- Efficacy of TV and Dimethoate on common bean aphids



Tephrosia vogelii

Control

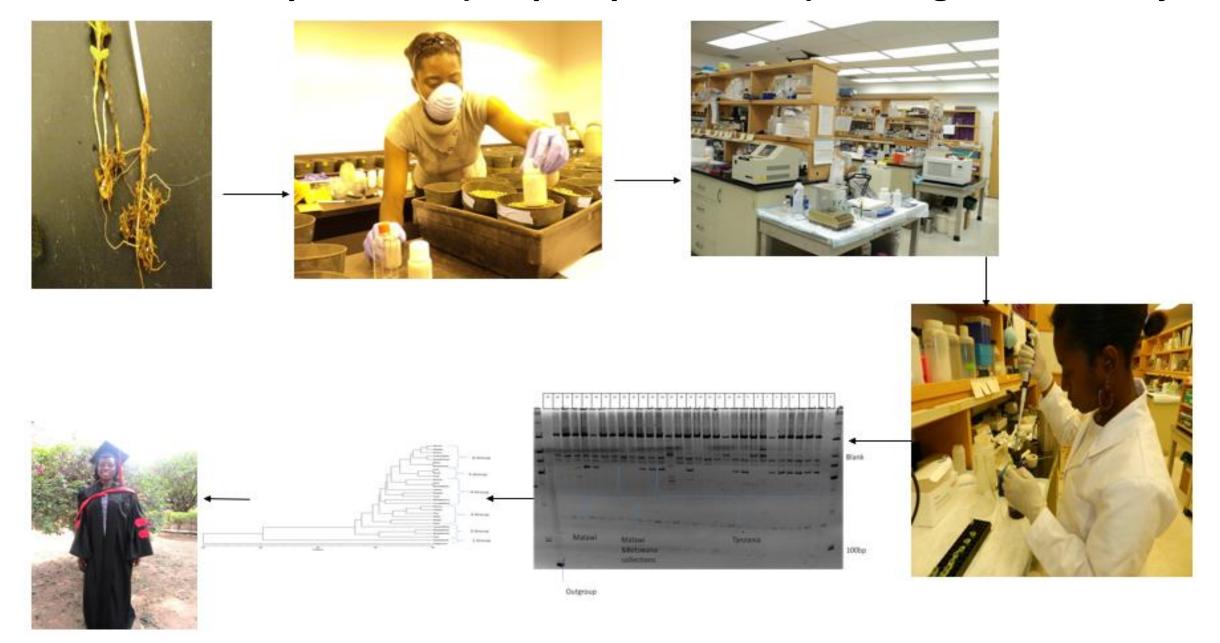


Dimethoate



Common bean aphids

Masters — Crop science (Crop improvement)- A. Vogelii Diversity

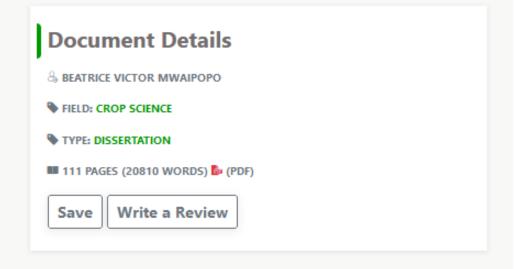


Characterization Of Alectra Vogelii (Witch Weed) Strains Using Molecular Markers In Selected Parts Of Malawi And Tanzania

Overview Reviews Cite Work

BSTRACT

Alectra vogelii has been the major constraint known to attack leguminous species especially to cowpea production. Identification of genetic variation of A.vogelii is a pre-requisite for developing improved cowpea varieties. Hence, the objective of the experiment was the identification of phylogenetically differences and differential responses of A. vogelii found in cowpeas, bambara groundnuts and sunflower from selected parts of Malawi and Tanzania. The first objective, total of 240 SSRs (Rice bean, S. gesnerioides and S. hermothica markers), ISSRs, cpDNA and mtDNA primers used to determine the genetic variability of A.vogelii. The PCR master mix reaction volume of 25μl, containing 2.5μl of 10X PCR buffer, sterile distilled water, 1μM of each primer, 1mM of each dNTPs, 0.5U/μl of TagDNA polymerase and 50 ng DNA PCR and gel electrophoresis rap. High



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PhD. Crop Science- NGS and Sanger sequencing of common bean viruses

 Funded by Common bean PEARL project under Bill and Melinda foundation.

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Vol. 12(18), pp. 1486-1500, 4 May, 2017
DOI: 10.5897/AJAR2017.12236

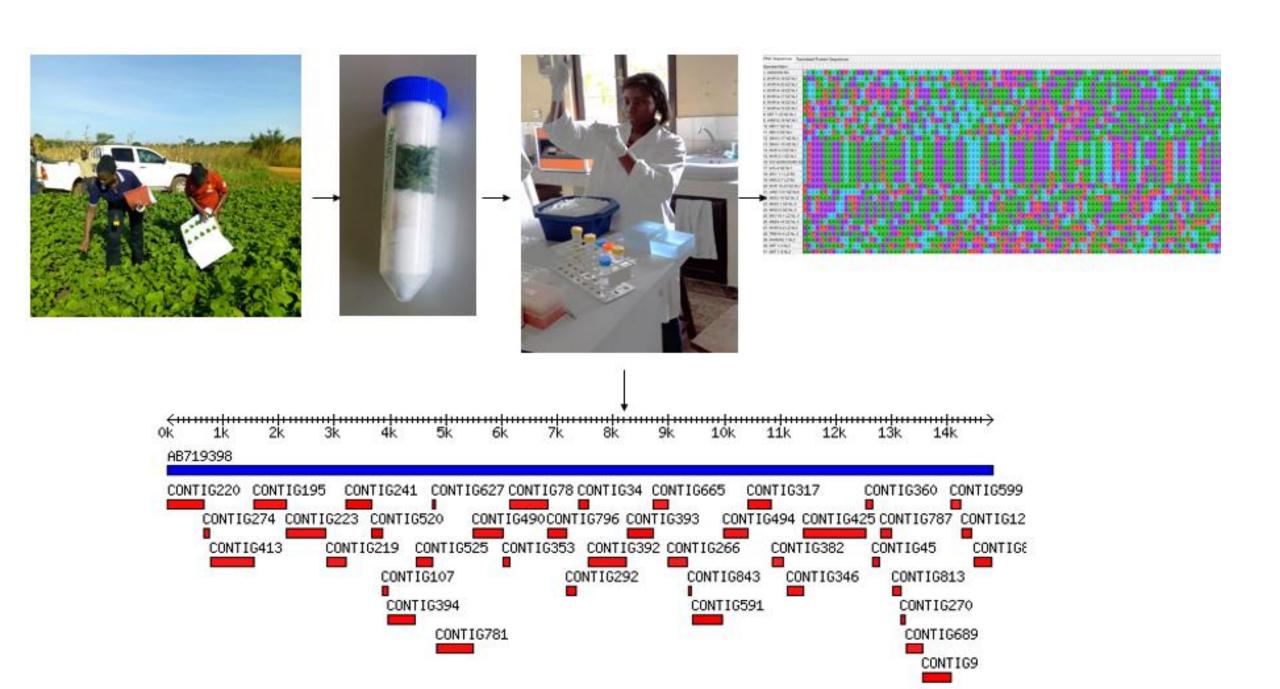
Article Number: 037302264096
ISSN 1991-637X
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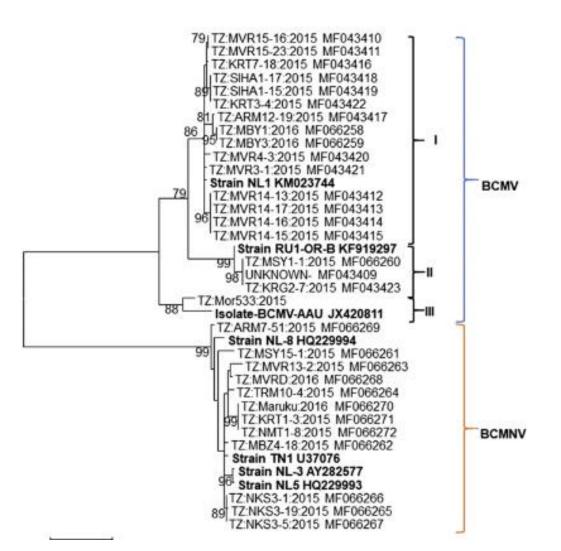
Review

Viruses infecting common bean (*Phaseolus vulgaris* L.) in Tanzania: A review on molecular characterization, detection and disease management options

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 Agricultural Research Institute - Selian, P. O. Box 6024, Arusha, Tanzania.
 Agricultural Research Institute - Uyole, P. O. Box 400, Mbeya, Tanzania.





Zonea	RNA pool sample	Total reads (21–24 nucleotides)	Reads aligned to reference	Viruses detected (reference sequences and percent coverage are shown in parentheses) ^b
SHZ	HXH-1	5,869,348	370,969	BCMNV; +ssRNA; Potyvirus (AY864314; 95.1)
				PeMoV; +ssRNA; Potyvirus (AF023848; 98.6)
				SBMV; +ssRNA; Sobemovirus (DQ875594; 99.9)
				PvEV-1; dsRNA; Alphaendornavirus (KT456287; 95.0)
				PvEV-2; dsRNA; Alphaendornavirus (AB719398; 96.4)
EZ	HXH-2	6,674,109	264,061	BCMNV; +ssRNA; Potyvirus (AY864314; 93.4)
				BCMV; +ssRNA; Potyvirus (KT175569; 100)
				PvEV-1; dsRNA; Alphaendornavirus (KT456287; 86.6)
				PvEV-2; dsRNA; Alphaendornavirus (AB719398; 97.5)
				CPMMV; +ssRNA; Carlavirus (KC774020; 68.4)
				CMV; +ssRNA; Cucumovirus (KJ400004; 86.0)
				CMoV; +ssRNA; Umbravirus (CED51824; 51.5)
				CMoMV; +ssRNA; Umbravirus (ACJ03575; 56.1)
				OPMV; +ssRNA; Umbravirus (AHZ65104; 33.0)
				ETBTV; +ssRNA; Umbravirus (AIL27641; 33.1)
				TBTV; +ssRNA; Umbravirus (TBTV; 77.9)
NZ	HXH-3	5,286,206	203,035	BCMNV; +ssRNA; Potyvirus (AY864314; 86.1)
				BCMV; +ssRNA; Potyvirus (KF114860; 99.9)
				BnYDV; +ssRNA; Crinivirus (EU191905; 77.9)
				CPMMV; +ssRNA; Carlavirus (KJ534277; 73.6)
				NCMV; -ssRNA; Cytorhabdovirus (ADE61669; 24.6)
LZ	HXH-6	11,658,110	378,180	BCMNV; +ssRNA; Potyvirus (AY864314; 95.8)
				CABMV; +ssRNA; Potyvirus (DQ397527; 89.2)
				ToLCArV; +/-ssDNA; Begomovirus (DQ519575; 24)
				ToLCYTV; +/-ssDNA, (AJ865340; 19.3)
				ToLCUV; +/-ssDNA; Begomovirus (DQ127170; 62.8)
				PvEV-1; dsRNA; Alphaendornavirus (KT456287; 61.8)
				PvEV-2; dsRNA; Alphaendornavirus (AB719398; 94.2)
	HXH-7	7.989.740	80.846	TMoV; +ssRNA; Umbravirus (AY007231; 91.1)
				PvEV-1; dsRNA; Alphaendornavirus (KT456287; 91.0)
				PvEV-2; dsRNA; Alphaendornavirus (AB719398; 94.7)
				RuFDV; dsDNA-RT; unassigned (ACL36982; 23.1)
				HRLV; dsDNA-RT; Caulimovirus (AAW56089; 14.3)
				CERV; dsDNA-RT; Caulimovirus (ABX80503; 23.2)
				EVCV; dsDNA-RT; unassigned (ACB69773; 13.1)
				MMV; dsDNA-RT; Caulimovirus (AAM53128; 22.6)
	•••			DMV; dsDNA-RT; Caulimovirus (ABW80581; 24.1)
	•••		•••	SbCMV; dsDNA-RT; Soymovirus (CAA33833; 14.2)
	•••			PEMV; +ssRNA; Umbravirus (AAU20330; 22.3)
	•••			SVBV; dsDNA-RT; Caulimovirus (AKB94072; 36.7)
	•••			GRV; +ssRNA; Umbravirus (CTO57207; 33.7)
			***	SPuV; dsDNA-RT; Caulimovirus (AFP95350; 15.9)
wz	HXH-15	28.223.699	3,793,494	BCMNV; +ssRNA; Potvvirus (AY864314; 77.2)
112			-,,	SBMV; +ssRNA; Sobemovirus (DQ875594; 98.5)
		***	***	
	***	***	***	PvEV-1; dsRNA; Endornavirus (KT456287; 99.3)

Comprehensive Surveys of Bean common mosaic virus and Bean common mosaic necrosis virus and Molecular Evidence for Occurrence of Other Phaseolus vulgaris Viruses in Tanzania

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Abstract

Virus diseases are among the main biotic factors constraining common bean (*Phaseolus vulgaris* L.) production in Tanzania. Disease management requires information on types, distribution, incidence, and genetic variation of the causal viruses, which is currently limited. Thus, a countrywide comprehensive survey was conducted. Use of a next-generation sequencing technique enabled simultaneous detection of 15 viruses belonging to 11 genera. De novo assembly resulted in many contigs, including complete or nearly complete sequences of *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), and *Southern bean mosaic virus* (SBMV). Some viruses (for example, SBMV and *Tomato leaf curl Uganda virus*-related begomovirus) were

detected for the first time in common bean in Tanzania. Visually assessed virus-like disease incidence ranged from 0 to 98% but reverse-transcription polymerase chain reaction-based incidence of BCMV and BCMNV (7,756 samples) was mostly less than 40%. The Sanger-based nucleotide sequences encoding coat proteins of BCMV and BCMNV isolates were 90.2 to 100% and 97.1 to 100% identical to each other, respectively. Phylogenetic analysis showed that BCMV isolates were more diverse than BCMNV isolates. The information generated in this study will contribute to the development of molecular diagnostic tools and strategies for management of virus diseases nationally and internationally.

Tanzania is the largest producer (>1,000,000 tons annually) of common bean (*Phaseolus vulgaris* L.) in sub-Saharan Africa (FAO 2014). Common bean serves as the main source of protein and starch root (Drijfhout 1978; Grogan and Walker 1948). At least five viruses—BCMV, BCMNV, Cowpea mild mottle virus (CPMMV; Carlavirus), Cucumber mosaic virus (CMV; Cucumovirus), and

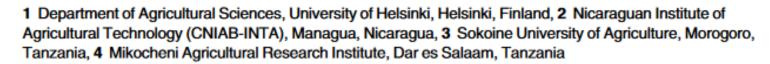




RESEARCH ARTICLE

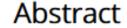
Pathogenic seedborne viruses are rare but Phaseolus vulgaris endornaviruses are common in bean varieties grown in Nicaragua and Tanzania

Noora Nordenstedt¹, Delfia Marcenaro^{1,2}, Daudi Chilagane^{3,4}, Beatrice Mwaipopo^{3,4}, Minna-Liisa Rajamäki¹, Susan Nchimbi-Msolla³, Paul J. R. Njau³, Deusdedith R. Mbanzibwa4*, Jari P. T. Valkonen1*



^{*} mbanzibwad@yahoo.co.uk (DRM); jari.valkonen@helsinki.fi (JPTV)

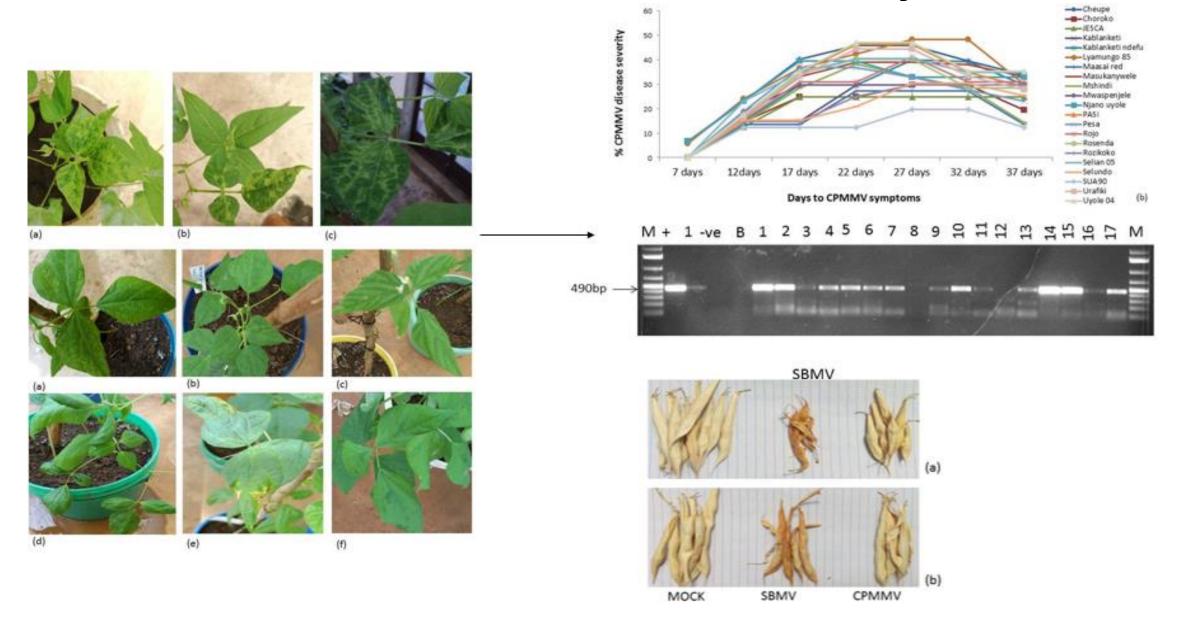




Common bean (*Phaseolus vulgaris*) is an annual grain legume that was domesticated in



Common bean viruses severity



Alternative host experiments







>8P10_11 Ocimum basilicum

>38P5_6 Bolusafra bituminosa

Table 3. Viruses detected in wild plants in Tanzania by siRNA sequencing (based on a BLASTN search of VirusDetect)

Location ^a	Sample pool	Genus	Accession number ^b	Virus	Sequence (length)	Coverage (%)°	Average depth ^d	Average identity (%) ^e
EZ	JDH-1	H-1 Begomovirus AM7		Tomato leaf curl Toliara virus	DNA (2,764 nt)	52.3	85.8	96.2
		Carlavirus	KC774020	Cowpea mild mottle virus	RNA (8,209 nt)	47.1	8.4	97.4
		Potyvirus	JN190431	Yam bean mosaic virus	RNA (9,649 nt)	78	346.1	90.1
		Potyvirus	FJ155666	Sweet potato feathery mottle virus	RNA (11,004 nt)	67.7	75.9	94.7
		Potyvirus	MF997470	African eggplant mosaic virus	RNA (9,694 nt)	50.4	864.3	83.8
		Potyvirus	KP115621	Sweet potato virus C	RNA (10,830 nt)	99.6	301.7	93.8
	JDH-5	Begomovirus	EF194760	Tomato leaf curl Arusha virus	DNA-A (2,762 nt)	34.9	235.5	93.7
			HE616777	African cassava mosaic Burkina Faso virus	DNA-A (2,770 nt)	25.2	300.5	90.0
		Begomovirus	AM701768	Tomato leaf curl Toliara virus	DNA (2,764 nt)	24.6	309.1	94.8
		Begomovirus	AF261885	Tomato curly stunt virus	DNA-A (2,766 nt)	21.1	303.8	91.3
		Begomovirus	KJ016240	Emilia yellow vein virus	DNA-A (2,726 nt)	21.2	197.8	89.6
		Begomovirus	KC953604	Tomato yellow leaf curl Sardinia virus	DNA (2,775 nt)	20.6	212.1	90.5
		Begomovirus	AJ132711	Tomato yellow leaf curl virus	DNA (2,771 nt)	20.6	142.9	91.3
		Begomovirus	DQ127170	Tomato leaf curl Uganda virus	DNA-A (2,747 nt)	71.5	191.4	96.4
		Carlavirus	KC774020	Cowpea mild mottle virus	RNA (8,209 nt)	89.2	24.5	97.3
		Carlavirus	FJ813512	Potato virus S	RNA (8,485 nt)	63.9	8.0	96.5
		Comovirus	X00206	Cowpea mosaic virus	RNA1 (5,889 nt)	52.4	197.4	85.3
		Comovirus	X00729	Cowpea mosaic virus	RNA2 (3,481 nt)	57.7	329.8	85.0
		Potyvirus	JN190431	Yam bean mosaic virus	RNA (9,649 nt)	83.9	618.4	89.8
		Potyvirus	AF348210	Cowpea aphid-borne mosaic virus	RNA (9,465 nt)	66.3	601.2	84.9
NZ	JDH-2	Begomovirus	DQ127170	Tomato leaf curl Uganda virus	DNA-A (2,747 nt)	39.1	58.7	96.9
		Carlavirus	KJ534277	Cowpea mild mottle virus	RNA (1,238 nt) ^f	70.8	9.5	98.4
		Crinivirus	EU191905	Bean yellow disorder virus	RNA-2 (8,530 nt)	54.3	20.4	96.5
		Potyvirus	KU708532	Peanut mottle virus	RNA (9,734 nt)	96.5	274.9	95.8
		Potyvirus	JN190431	Yam bean mosaic virus	RNA (9,649 nt)	83.7	943.7	89.1
	JDH-6	Begomovirus	AJ542539	Hollyhock leaf crumple virus	DNA (2,740 nt)	29.7	70.4	92.3
		Begomovirus	DQ519575	Tomato leaf curl Arusha virus	DNA-A (2,766 nt)	20.0	125	95.7
		Begomovirus	DQ127170	Tomato leaf curl Uganda virus	DNA-A (2,747 nt)	86.8	140.4	97.1
		Begomovirus	AM701757	Cotton leaf curl Gezira virus	DNA (2,754 nt)	57.0	101.9	97.0
		Carlavirus	KJ534277	Cowpea mild mottle virus	RNA (1,238 nt) ^f	58.6	16.0	98.1
		Cucumovirus		Cucumber mosaic virus	RNA1 (3,363 nt)	98.7	103.4	96.6
		Cucumovirus	KX660757	Cucumber mosaic virus	RNA2 (3,046 nt)	93.3	38.0	97.1
		Cucumovirus	KC527774	Cucumber mosaic virus	RNA3 (2,220 nt)	94.2	111.7	97.5
		Polerovirus	KY364847	Cowpea polerovirus 2	RNA (5,945 nt)	26.8	7.4	99.1
SHZ	JDH-3		no detection	FF				
	JDH-4	Cripavirus	MF458892	Aphid lethal paralysis virus	RNA (9,828 nt) insect virus	71.8	50.2	97.0
LZ	HXH-16	Tobamovirus	AB083196	Tomato mosaic virus	RNA (6,385 nt)	90.5	18.2	99.2

Next-Generation Sequencing-Based Detection of Common Bean Viruses in Wild Plants from Tanzania and Their Mechanical Transmission to Common Bean Plants

Beatrice Mwaipopo,^{1,2} Minna-Liisa Rajamäki,^{3,†} Neema Ngowi,¹ Susan Nchimbi-Msolla,² Paul J. R. Njau,² Jari P. T. Valkonen,³ and Deusdedith R. Mbanzibwa^{1,†}

Abstract

Viral diseases are a major threat for common bean production. According to recent surveys, >15 different viruses belonging to 11 genera were shown to infect common bean (*Phaseolus vulgaris* L.) in Tanzania. Virus management requires an understanding of how viruses survive from one season to the next. During this study, we explored the possibility that alternative host plants have a central role in the survival of common bean viruses. We used next-generation sequencing (NGS) techniques to sequence virus-derived small interfering RNAs together with conventional reverse-transcription PCRs (RT-PCRs) to detect viruses in wild plants. Leaf samples for RNA extraction and NGS were collected from 1,430 wild plants around and within common bean fields in four agricultural zones in Tanzania. At least partial genome sequences of viruses potentially belonging to 25 genera were detected. The greatest

virus diversity was detected in the eastern and northern zones, whereas wild plants in the Lake zone and especially in the southern highlands zone showed only a few viruses. The RT-PCR analysis of all collected plant samples confirmed the presence of yam bean mosaic virus and peanut mottle virus in wild legume plants. Of all viruses detected, only two viruses, cucumber mosaic virus and a novel bromovirus related to cowpea chlorotic mottle virus and brome mosaic virus, were mechanically transmitted from wild plants to common bean plants. The data generated during this study are crucial for the development of viral disease management strategies and predicting crop viral disease outbreaks in different agricultural regions in Tanzania and beyond.

Keywords: NGS, Phaseolus vulgaris, plant virus, wild plants

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2012-2021

TARI- Tanzania Agricultural Research Institute

- Germplasm maintenance
- Legume lines evaluation for release (pigeon pea and cowpeas)
- Agronomic activities







UNDER TARI.....

- Sustainable intensification of maize legume system for food security in eastern and southern Africa (SIMLESA).
- TL II Pigeon pea and cowpea project.
- Innovating Strategies to safeguard Food Security using Technology and knowledge Transfer (Trans-SEC).
- Association for strengthening agricultural research in eastern and central Africa (ASARECA) base on sorghum- legume intensification (SLI).

AT SOKOINE UNIVERSITY OF AGRICULTURE

- Supervising students (29 students)
- Research
 - Projects
 - SUARIS 3 30 Million
 - AI4CROPHEALTH 150 Million





On going Project

 Characterization of Southern bean mosaic virus (SBMV) in common bean in Tanzania (SUARIS 3) (just starting)

 Plant pests prediction and Emerging Disease detection using Image processing, webGIS and machine learning Techniques (PREDICT) funded by SUA (Ongoing)

 Development of an Artificial Intelligence-empowered Crop Disease Surveillance, Prediction, and Management (AI4CROPHEALTH) funded by Tanzania Commission for Science and Technology (COSTECH) (Ongoing) Vol. 19(3), pp. 260-271, March, 2023

DOI: 10.5897/AJAR2022.16226 Article Number: 986C91670471

ISSN: 1991-637X
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Full Length Research Paper

Artificial intelligence and deep learning based technologies for emerging disease recognition and pest prediction in beans (phaseolus vulgaris I.): A systematic review

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Received 29 September, 2022; Accepted 15 December, 2022

Artificial Intelligence (AI) and deep learning have the capacity to reduce losses in crop production, such as low crop yields, food insecurity, and the negative impacts on a country's economy caused by crop infections. This study aims to find the knowledge and technological gaps associated with the application of AI-based technologies for plant disease detection and pest prediction at an early stage

Expected to be published as soon as possible

1. Deep learning CNN model for cassava disease detection

2. Digital image based estimation of common beans, cassava and rice diseases Severity.

 Developing weather-based machine learning/artificial intelligence and decision support systems for plant disease prediction crops.

MY DREAMS WORKING WITH KT

Request for submitting the proposal

Yahoo/Sent ☆

Fri, Oct 30, 2020 at 1:27 PM 🏠





Beatrice Mwaipopo

From: beatricemwaipopo@yahoo.com

To: info@kirkhousetrust.org

Dear sir/Madam



RE: Online enquiry submitted via the KT website



Fri, Nov 13, 2020 at 2:04 PM \$\frac{1}{2}\$



Kirkhouse Trust

From: info@kirkhousetrust.org

To: Beatrice Mwaipopo

Dear Beatrice Mwaipopo,

Thank you for your email. Please accept our apologies for the time taken to respond to your application.

Your application has been reviewed and unfortunately at this time the Kirkhouse Trust is also unable to consider unsolicited proposals as its funding capacity has been reached.

The Trust wishes you good luck in securing funding from another source.

TITLE: BREEDING FOR ALECTRA VOGELII RESISTANCE ON TANZANIAN LOCAL COWPEA (VIGNA UNGUICULATA(L.) WALP) LANDRACES USING MOLECULAR MAKER

PI : BEATRICE V. MWAIPOPO

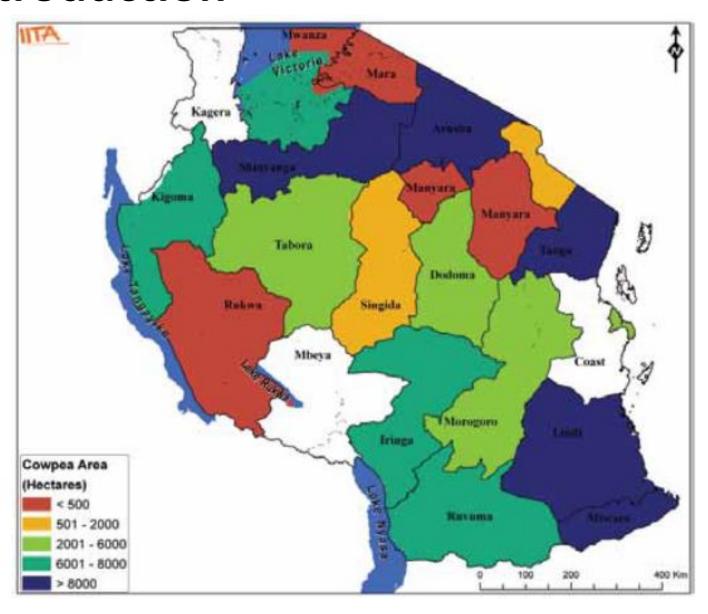
CO-PI: **MESHARK MAKENGE**

Introduction

 Tanzania is among the countries that produce cowpea

 But the yield is still low due to abiotic and biotic factors including A. vogelii.

 Witch weed cause up to 50% yield losses





Advances in Research 7(5): 1-8, 2016, Article no.AIR.11478

ISSN: 2348-0394, NLM ID: 101666096

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Alectra vogelii, a Threat to Bambara Groundnut **Production in Singida and Dodoma Regions, Tanzania**

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¹Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania. ²Agricultural Research Institute Ilonga, Kilosa, Morogoro, Tanzania. ³Horticultural Research Institute, Arusha, Tanzania. ⁴Agricultural Research Institute, Uyole, Mbeya, Tanzania.

Table 1. Bambara groundnut farms covered during the current survey

Farm number	Village	District	Number of A. vogelii per quadrant ^a	Number of plants per quadrant ^b	Number of A. vogelii infestation per plant°	Score
1	Kaselya	Iramba	137	32	4.28	3
2	Kaselya	Iramba	85	33	2.58	3
3	Kaselya	Iramba	126	38	3.32	3
4	Msungua	lkungi	71	28	2.54	3
5	Msungua	lkungi	6	22	0.27	2
6	Msungua	lkungi	37	19	1.95	3
7	Mpunguzi	Dodoma urban	18	19	0.95	3
8	Mpunguzi	Dodoma urban	10	22	0.45	2
9	Mpunguzi	Dodoma urban	4	22	0.18	1
Mean			54.90	26.11	1.84	2.56

• IT99K-573-1 and IT99K-7-21-2-2-1, were evaluated for resistance to *A. Vogelii* under McKnight foundation collaborative crops research project and released



Intro.....

- The adoption of the released varieties is low
- The most preferred varieties are the local varieties but susceptible to the *A. vogelii*.
- Multi-pronged approach incorporating genes of resistance to preferred genotypes is important
- This will help farmers mitigate the economic losses and food security challenges accompanied with *Alectra vogelii* infestations
- Effective breeding for resistance to *A. vogelii* using marker-assisted selection (MAS) in cowpea will be important for rapid improvement of our existing material.

Objectives of project

- I. To generate the F2 mapping population harbouring genes resistant to *A. vogelii*
- II.To investigate the phenotypic response of the cowpea segregating population (F2) to *A. vogelii* isolates under both controlled and field conditions

III. To utilize marker-assisted selection for identifying resistance genes to *A. vogelii* in the F2 cowpea population

Methodology

• Location: Screenhouse and lab work will be conducted at TARI-Ilonga and Sokoine university of Agriculture

Genotypes

No.	Genotypes	Origin	Recipient / Donor parent	Value for cultivation
1	IT99K-573-1(VULI AR 2)	IITA	Donor	Resistance to A. vogelii
2	Local genotype (Chora)	Tanzania	Recipient	Seed size; large, Seed colour: cream, but susceptible to pest and low yield
3	FUAMPEA 3	Nigeria	Recipient	Medium maturing, large seeded, high yielding, Witch weed resistance

Obj 1

Targeting traits

- Seed size
- A. vogelii Resistance
- Development of F2 mapping population

```
(Recurrent genotype) (Donor genotype)

Cowpea genotype A x Cowpea genotype B

F1 population (Selfing)

F2 mapping population
```

Obj 2

□ Screen house experiment (collection of all A. vogelii isolates and set an experiment in screen house)

Testing the F2 progenies to different *A. vogelii* isolates

☐ Field experiment (Morogoro, Iringa and Dodoma)

➤ Check emergence of *A. vogelii* on F2 progenies at the hotspots areas

Obj 3: Marker assisted selection

 Sample collection – The samples will collected starting the development of F1 for lab analysis

• DNA extraction – CTAB Buffer will be used as explained by described by Qi et al. (2021)

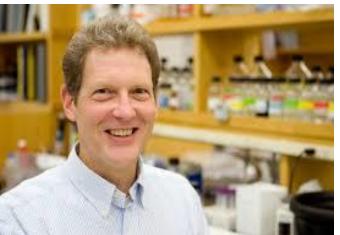
 PCR amplification – Targeting Rav 1 and Rav 2 gene that confer resistance to A. vogelii

Linkage analysis

Markers _ SNP

Marker Name	Annealing Temperature	LOCUS	Product Size	Sequence	SNP
2_20296R	60	31.4	179	CCTAAGCCTGCCATTTCAAG	
2_20296F1	60	31.4		ACCATACATTACATACTTTTCTTCACCGCCGTT	Т
2_20296F2	60	31.4		ATCAACTTTTCTTCACCGCCCTC	С
2_04147F	60	40.3	218	AGACCCCACTTCTTGTTCCA	
2_04147R1	60	40.3		ACCATACATTACATACTATCTCTACTAACCGACAGCC	G
2_04147R2	60	40.3		ATCAACTATCTCTACTAACCGACACCT	А
2_22541R	45	20.9	216	GGTACGTTTTAAAATTGATATGACCA	
2_22541F1	45	20.9		ACCATACATTACATAGATGTTCACAGATGTACTGATG	G
2_22541F2	45	20.9		ATCAAGATGTTCACAGATGTACTGTTA	А











BILL & MELINDA GATES foundation

COLLABORATIVE CROP RESEARCH PROGRAM

MCKNIGHT FOUNDATION

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END