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RESEARCH ARTICLE

Identification of genetic variability among *Alectra vogelii* strains from different geographical locations

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ABSTRACT

Alectra vogelii threatens cowpea production in Sub-Saharan Africa. The weed complicates the development and operation of effective control tactics. For DNA extraction, young leaf samples of *A. vogelii* were taken directly from infested cowpea fields. The study used 23 SSR markers to determine genetic heterogeneity among *A. vogelii* populations, which will help design and implement effective breeding techniques and other parasite control approaches. The effective number of alleles was 1.5648, the observed number of alleles was 8, the anticipated heterozygosity was 0.648, and Shannon's Information index was 0.5169. PIC was 0.8301 on average. The Fst between populations was 0.2986, leaving 70.14 percent genetic variation within populations. A NTSYS-pc (UPGMA) dendrogram developed three clusters. More information on genetic heterogeneity among existing Alectra strains was gathered to help expand the cowpea gene pool for selection and creation of resistant genotypes.

Keywords: *A. vogelii*; Cowpea; SSR markers; Genetic variability; Genetic diversity; Cluster analysis

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INTRODUCTION

Alectra vogelii is a damaging parasitic weed that can cause significant losses to host crops, including food and fodder crops like cowpeas. *A. vogelii* has caused many cowpea crops to be abandoned. This weed is well-known for its outstanding capacity to adapt to varied environmental conditions by producing host-specific variants (Mbega et al. 2016; Njekete et al. 2017).

Controlling *A. vogelii* using hoeing, deep cultivation, herbicides, crop residue destruction, and crop rotation has proven challenging because the parasite causes most damage before it emerges above ground (Geleta, 2010; Shinggu, 2015). Breeding for resistance is one of the most promising economic control techniques available to farmers (Teka, 2014). The approach reduces production costs and *A. vogelii* infestation while increasing cowpea yields.

The genetic resistance gained by breeding at one area may not hold up when transplanted to new regions with different parasite populations (Westwood et al., 2012). The strains make it impossible to generate universally durable resistance, weakening efforts to achieve food security. They must be controlled in any way (Westwood et al., 2012; Atera et al., 2013). The intraspecific Alectra strains swiftly adapt to the host, causing a collapse in host resistance (Welsh and Mohammed 2011; Atera et al. 2013).

The identification of *A. vogelii* genetic diversity helps to understand the weed's distribution and tendencies, and helps design new management solutions (Slotta, 2008). Using SSR markers to study parasitic weed genetic variation allows for strong comparative genetic and genomic research (Appleby et al., 2009; Yoshida et al. 2010; Estep et al. 2011). They determine gene flow and mating system in parasitic Orobanchaceae species (Appleby et al. 2009; Yoshida et al., 2010; Estep et al., 2011; Westood et al. 2012;). The results can be utilised to establish a breeding programme for cowpea genotypes resistant to all Alectra strains. The study's goal was to use SSR analysis to find genetic links among A. vogelii populations from different locations.

MATERIALS AND METHODS

Study area

The present investigation was conducted at Sokoine University of Agriculture, Morogoro, Tanzania. The young leaf samples of *A. vogelii* were collected directly from the infested cowpea fields across the selected locations (Table 1 and Figure 1). All these populations of *A. vogelii* were collected from five different administrative regions between altitudes of 450-1900 m.a.s.l. (Table 1) which are low, mid, to high altitude areas.

Figure 1. Map of Tanzania highlighting sampling locations of *Alectra vogelii*

Collection and preservation of leaf samples

The young leaves were collected with random sampling at each location. The leaf samples were preserved in Eppendorf tubes at -20°C for two weeks to freeze the tissue before DNA extraction was carried out.

DNA Isolation and PCR Analysis

After centrifugation, transfer an aliquot of the supernatant to a fresh tube. 600 µl isopropanol was added, followed by 60 µl 0.75 M ammonium acetate. Gently mix the aqueous. Ammonium acetate was used to remove DNA-bound cellular and histone proteins. Then 30 minutes of freezing and 10 minutes of 14000 rpm centrifugation. It was discovered at the tube's base that the supernatant contained DNA. This was followed by 800 µl of cold 70% alcohol, 10 minutes at -20°C, then 5 minutes at 13000 rpm. After resuspension, the pellets were diluted with 50 µl TE buffer.

The samples' genomic DNA was electrophoresed on agarose gels. Each Alectra sample was tested in a 1% agarose gel to determine DNA concentration. Preparation of the gels: 1 g agarose in 1X 100 mL TBE

buffer, stained with 10 µl Ethidium bromide. The 4 ml genomic DNA mixed with 6 µl loading dye and DNA was run alongside 6 µl of 50 kb genomic DNA ladder This was followed by imaging with a UV transilluminator. The gel was photographed with a digital camera.

With larger sample numbers, clear different bands were identified and employed in this investigation (Yoshinda et al., 2010; Estep et al., 2012). These primers identified DNA from 15 Alectra populations. This was done using SSR primers (Integrated DNA Technologies) and a master mix (New England Bio Labs Inc). The final volume of 25µl PCR reaction mixture contains12.5 µl 2X master mix, 0.5 µl forward and reverse primer, 9.5 µl nuclease-free water, and 2 µl template DNA were used. The PCRs were run in a master cycler. Initial denaturation at 94°C for 1 min, annealing at 45°C for 30 seconds, and final extension at 72°C for 1 min. An extension at 72°C for 10 minutes followed by storage at 4°C.

PCR products were separated on a 3% agarose gel. The electrophoresis used 1X TBE buffer. Pre-staining using Ethidium Bromide (EtBr). An hour of horizontal electrophoresis at 120 V separated the fragments. An UV transilluminator was used to photograph the gels.

Statistical Analysis

The single-population descriptive genetic statistics were were calculated using GenAIEx 6.1 and Popgene 1.32. (Peakall and Smouse, 2006). The PIC values were determined as follows: AMOVA was performed using GenAIEx 6.1 software with 1000 permutations. The UPGMA cluster analysis was performed using NTSYS-pc Version 2.1. (Rohif, 1998). The F-statistics (Wright, 1978) were generated to test for Hardy-Weinberg equilibrium deviation. The outcrossing rate of A. vogelii was estimated using Fst values (Wright, 1978). The Mantel test examined the relationship among 15 populations using a genetic distance matrix.

RESULTS AND DISCUSSION

Variation in efficiency and polymorphism of SSR Markers

Among twenty-three primer pairs tested, only eight generated reproducible amplification products (Table 2). These eight primer combinations were highly informative, distinguished the different populations studied, and can be used to study *A. vogelii* and other parasitic plants at molecular level. The PIC mean value was high, based on Botstein et al. (1980) who divided loci polymorphism in the order of PIC value > 0.5 high, 0.5 > PIC value > 0.25 medium, and PIC values < 0.25 for slightly informative markers. Marker SH 1008 had the highest values for richness and evenness, whereas SH 1061 had the lowest values. The markers SH1008, SH1016, and SH1031 gave the highest Shannon's information index (I) value, whereas SH 1061 gave the lowest Shannon's index value. All loci had almost equal effective alleles, which mean the highest diversity. The data indicated that only 8 pair of primers out of 23 SSR primer combinations exhibited high amplification efficiency; thus, they are reliable in discovering polymorphism (Table 2).

The extra information on A. vogelii genetic variation revealed more genetic variability within populations (Table 3). The genetic diversity analysis ranked the populations from most diverse to least diverse. Ilunda and Iyambi populations had the most alleles, whereas Kondoa had the least. Less than Hardy-Weinberg proportions showed the fixation index (F), also known as inbreeding coefficients. 11 of the 15 populations had excessive homozygosity, while four had excessive heterozygosity due to negative assortative mating or selection for heterozygosity over gene diversity. Ikwega, Nyamahana, Ngamu, and Nkungi had excessive heterozygosity while other 11 populations had excessive homozygosity. These values for random mating were found in Nkungi, Ngamu, and Gawaye populations. *A. vogelii* has a high genetic diversity, according to all genetic statistics.

Genetic Diversity

Nei's genetic distance values demonstrated the genetic link among the 15 *A. vogelii* populations, with smaller values indicating a tighter relationship. Welela and Nyamahana, Welela and Iyambi, Welela and Ilunda, Nyamahana and Mbalawala A. vogelii populations had the most resemblance. *A. vogelii* populations from Lyadebwe and Mangalali, Mbande, and Kondoa had the least genetic similarity (most diverse). The type of colonisation affects this genetic polymorphism because *A. vogelii* is spread by long-distance gene dispersal. The genetic variability of weeds is influenced by founder effects or strong genetic bottlenecks before or after dissemination activities (Barrett and Schluter, 2008; Begg et al., 2012; Gaskin et al., 2012). Genetic drift and selection forces that favour lineages in specific populations are likewise favoured by the founding events (Tremblay et al., 2005).

Table 1. *A. vogelii* collection sites from selected areas of Tanzania

Region	District	Village	Altitude, m	Latitude, S	Longitude, E	Field crop
Njombe	Wanging'ombe	Lyadebwe	1368	08º47'14.8''	034º35'35.9''	cowpea
		Ikwega	1587	08º59'33.1''	034º41'31.5''	cowpea
	Njombe Rural	Welela	1816	09º00'30.5''	034º47'31.0''	cowpea
Iringa	Iringa Rural	Nyamahana	977	07º40'24.6''	035º25'13.1''	Maize/cowpea
		Mangalali	1486	07º45'54.9''	035º34'04.6''	Maize/cowpea
		Kising'a	1390	07º35'13.2''	035º46'06.8''	Maize/cowpea
Dodoma	Dodoma Urban	Gawaye	1092	05º53'29.2''	035º52'45.8''	Maize/cowpea
		Mbalawala	1,121	05º58'57.0''	035º37'38.4''	Cowpea
	Kongwa	Mbande	976	06º06'16.1''	036º20'15.9''	Cowpea
Singida	Singida Rural	Ngamu	1574	04º32'19.1''	035º01'25.4''	Maize/cowpea
		Nkungi	1590	04º20'39.2''	034º51'21.7''	Maize/cowpea
		Iyambi	1579	04º21'49.4''	034º47'39.8''	Cowpea
	Mkalama	Ilunda	1534	04º21'51.3''	034º47'49.4''	Cowpea
Morogoro	Kilosa	Mhenda	580	07º10'19.0''	036º55'42.8''	Maize/cowpea
		Kondoa	485	06º49'21.6''	037º02'15.8''	Cowpea

Table 2. Population genetic structure data of SSR loci linked to *A. vogelii* strains

Locus	Na	Ne	Aa	Ae	I	Ho	He	PIC
SH1007	3	1.2328	8.0328	4.8189	0.3372	0.8194	0.8944	0.9888
SH1008	13	1.8644	8.6644	7.6306	0.6563	0.4183	0.3451	0.5969
SH1016	7	1.65	8.45	6.9391	0.583	0.3132	0.7189	0.9276
SH1029	3	1.2328	8.0328	4.8819	0.3372	0.8127	0.8944	0.9888
SH3031	5	1.4279	8.2279	4.9973	0.4767	0.8152	0.8101	0.9663
SH1032	3	1.2328	8.0328	4.8819	0.3372	0.8127	0.8944	0.9888
SH1042	4	1.3263	8.1263	6.462	0.4115	0.8101	0.8423	0.9794
SH1061	2	1.1475	7.9475	4.2186	0.2512	0.8107	0.9309	0.9952
Mean	5	1.3893	8.1893	5.6038	0.4238	0.7015	0.7913	0.929

Na = Observed number of alleles, Ne = Effective number of alleles, Aa = Allelic diversity (richness), Ae = Effective allelic diversity (evenness), I = Shannon's Information index, Ho = observed heterozygosity, He = expected heterozygosity (gene diversity), PIC=Polymorphic information content.

Table 3. Descriptive population genetic statistics for all *A. vogelii* populations

Populations	Na	Ne	Aa	Ae	I	Ho	He	PIC	F
Lyadebwe	8	1.5003	8.3003	6.1822	0.4929	0.6339	0.7222	0.8918	0.1222
Ikwega	10	1.6462	8.4462	6.314	0.5665	0.6168	0.5776	0.7816	-0.0679
Welela	8	1.6474	8.4474	6.5223	0.572	0.5156	0.6247	0.8303	0.1746
Nyamahana	9	1.6462	8.4462	6.314	0.5665	0.6168	0.5776	0.7816	-0.0679
Mangalali	4	1.3434	8.1434	5.3255	0.3905	0.6478	0.8481	0.9705	0.2362
Kising'a	8	1.5486	8.3486	6.2563	0.4968	0.6155	0.6198	0.7929	0.0069
Gawaye	8	1.5486	8.3486	6.2248	0.4968	0.6189	0.6198	0.7929	0.0015
Mbalawala	8	1.6474	8.4474	6.5223	0.572	0.5156	0.6247	0.8303	0.1746
Mbande	6	1.4255	8.2255	5.6978	0.433	0.6349	0.7567	0.8995	0.161
Ngamu	9	1.5954	8.3954	7.0463	0.5339	0.6142	0.5937	0.7882	-0.0345
Nkungi	9	1.5954	8.3954	7.0463	0.5339	0.6142	0.5937	0.7882	-0.0345
Iyambi	11	1.7572	8.5572	7.2849	0.6197	0.3658	0.532	0.7623	0.3124
Ilunda	11	1.7572	8.5572	7.2849	0.6197	0.3658	0.532	0.7623	0.3124
Mhenda	8	1.5486	8.3486	6.2563	0.4968	0.6155	0.6198	0.7929	0.0069
Kondoa	3	1.264	8.064	5.4086	0.362	0.8118	0.877	0.9857	0.0743
Mean	8	1.5648	8.3648	6.3791	0.5169	0.5869	0.648	0.8301	0.0919

Na = Observed number of alleles, Ne = Effective number of alleles, Aa = Allelic diversity (richness), Ae = Effective allelic diversity (evenness), I = Shannon's Information index, Ho = observed heterozygosity, He = expected heterozygosity (gene diversity), PIC=Polymorphic information content, F = Fixation Index (inbreeding coefficient)

Table 4. Pairwise population matrix of Nei‟s genetic distance for the 15 *A. vogelii* populations

Populations	Lyadebwe	Ikwega	Welela	Nyamahana	Mangalali	Kising'a	Gawaye	Mbalawala	Mbande	Ngamu	Nkungi	Iyambi	Ilunda	Mhenda	Kondoa
Lyadebwe	0.0000														
Ikwega	0.4581	0.0000													
Welela	0.2554	0.2027	0.0000												
Nyamahana	0.4581	0.0000	0.2027	0.0000											
Mangalali	1.3540	0.0000	1.0986	0.0000	0.0000										
Kising'a	1.1513	0.6931	0.8959	0.6931	0.0000	0.0000									
Gawaye	1.1513	0.6931	0.8959	0.6931	0.8959	0.6931	0.0000								
Mbalawala	0.2554	0.2027	0.0000	0.2027	1.0986	0.8959	0.8959	0.0000							
Mbande	0.5108	1.1513	0.6609	1.1513	0.2554	1.1513	0.4581	0.6609	0.0000						
Ngamu	0.4581	0.6931	0.8959	0.6931	0.0000	0.6931	0.6931	0.8959	1.1513	0.0000					
Nkungi	0.4581	0.6931	0.8959	0.6931	0.0000	0.6931	0.6931	0.8959	1.1513	0.0000	0.0000				
Iyambi	0.4581	0.6931	0.2027	0.6931	0.8959	0.6931	0.6931	0.2027	0.4581	0.6931	0.6931	0.0000			
Ilunda	0.4581	0.6931	0.2027	0.6931	0.8959	0.6931	0.6931	0.2027	0.4581	0.6931	0.6931	0.0000	0.0000		
Mhenda	1.1513	0.6931	0.8959	0.6931	0.0000	0.0000	0.6931	0.8959	1.1513	0.6931	0.6931	0.6931	0.6931	0.0000	
Kondoa	0.6609	0.0000	0.0000	0.0000	0.0000	0.8959	0.0000	0.0000	1.3540	0.8959	0.8959	0.0000	0.000	0.8959	0.0000

Table 5. Analysis of molecular variance (AMOVA) for 15 *A. vogelii* populations

Source of variation	Df	SS	MS	Est. Variance	% Variation	*Fst*	P values
Among Populations	14	1063.92	75.99	4.38	29.86	0.2986	0.001
Within Populations	153	1572.59	10.29	10.29	70.14	-	0.001
Total	167	2636.51	-	14.67	100	-	-

df= degree of freedom; SS = Sums of squares; MS = mean squares; Est. variance = estimate of variance; % variation = percentage of total variation; Fst = PhiPT = Phi-statistics probability level after 1000 permutations (Fst = Rst= PhiPT = Gst); P-value = is based on 1000 permutation

Table 6. Geographical distance matrix (km) for the sampling locations

Populations	Lyadebwe	Ikwega	Welela	Nyamahana	Mangalali	Kising'a	Gawaye	Mbalawala	Mbande	Ngamu	Nkungi	Iyambi	Ilunda	Mhenda	Kondoa
Lyadebwe	0														
Ikwega	25.84	0													
Welela	32.92	11.05	0												
Nyamahana	153.72	167.5	163.51	0											
Mangalali	155.11	166.21	161.05	19.04	0										
Kising'a	185.61	196.06	190.5	39.63	30.63	0									
Gawaye	351.38	368.85	366.15	204.3	212.07	189.57	0								
Mbalawala	332.16	350.76	348.73	189.56	199.41	179.89	28.4	0							
Mbande	354.8	369.12	364.82	201.64	204.45	177.04	56.04	78.63	0						
Ngamu	474.38	496.71	497.14	351.23	364.49	349.53	177.38	173.98	226.36	0					
Nkungi	494.75	517.75	518.75	377.2	391.22	377.7	210.02	204.94	259.86	33.87	0				
Iyambi	492.53	515.53	516.52	375.01	389.05	375.57	208.2	202.96	258.19	32.45	2.23	0			
Ilunda	492.58	515.55	516.52	374.8	388.81	375.25	207.56	202.46	257.46	31.59	2.49	1.11	0		
Mhenda	314.11	319.36	311.64	175.73	165.31	136.33	184.52	195.49	136.34	361.17	394.23	392.45	391.78	0	
Kondoa	346.43	353.43	346.24	201.4	193.72	163.44	164.89	180.91	111.44	337.62	371.21	369.55	368.81	40.5	0

The study found communities with remarkably similar genetic diversity from diverse geographical areas. Inbreeding reduces genetic diversity in the separate populations (Yang et al., 2012). Other populations from the same area have distinct genetic diversity. They observed that life history features (period of flowering, fecundity, and dormancy), genetic drift effects, and selection to variable environmental conditions are the causes of genetic difference between individuals and accessions from the same population. The AMOVA revealed considerable diversity among the fifteen *A. vogelii* populations (p<0.001; Table 5). The number of migrants per generation was determined through stepwise mutation in AMOVA. The population gene flow was 0.5872. The AMOVA demonstrated considerable genetic diversity within groups. The study's high significant variations may be attributable to rapid genetic variation in population growth. The observed coefficient of genetic differentiation revealed 29.86% genetic variation between populations and 70.140% within populations. Wright (1978) claims this Fst value was over 0.25, indicating considerable genetic differentiation. AMOVA of *A. vogelii* exhibited high intra-population and minimal inter-population variance (p <0.001). Fst revealed unusually substantial genetic divergence, indicating that populations have deviated from Hardy-Weinberg equilibrium. The significant degree of genetic heterogeneity was caused by seed distribution among groups.

Clustering

The genetic distance between clusters revealed the cluster genetic relationship (Figure 2). Less genetic distance meant a closer link with the most comparable populations. The dendrogram classified the populations into three groupings based on 22% of the variances. The first cluster included seven closely related populations: Lyadebwe, Ikwega, Welela, Mbalawala, Kondoa, Iyambi, and Ilunda. The second cluster included four closely related populations: Nyamahana, Mangalali, Kisng'a, and Ngamu. The third cluster includes Gawaye, Mbande, Nkungi, and Mhenda. This clustering found no link between geographic location and *A. vogelii* genetic divergence. This finding matched Welsh and Mohammed (2011) who found no link between genetic divergences of Striga hermonthica and origin or distance. *A. vogelii's* seed dissemination is a major contributor. Wind, animals, machinery, and humans distribute *A. vogelii* seeds. These seed dispersion parameters altered genetic variability and contributed to gene flow between *A. vogelii* populations. Genetic structure is influenced by gene flow, seed distribution, and reproductive mode

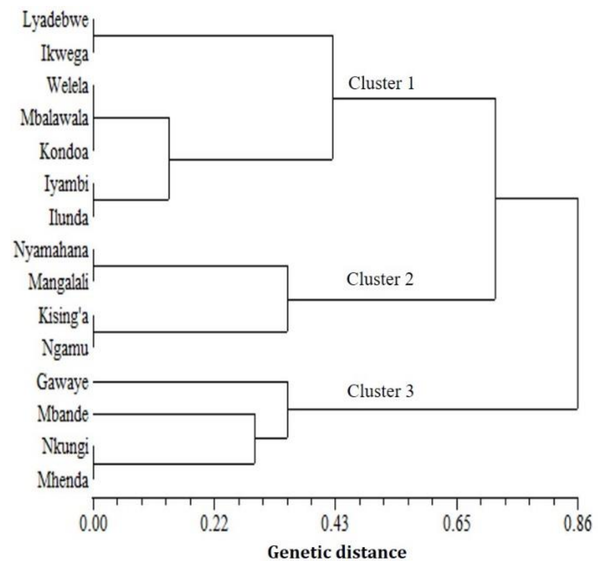

Figure 2. Phylogenetic relationship among the *A. vogelii* populations.

The Mantel's test

The Mantel's test revealed a weak association (r = 0.14917) between genetic and geographic distance (Table 6). However, if there was a meaningful association between the two data sets, the observed correlation should be higher (closer to +1 or -1) than the value generated by random permutation at 1 percent. All of the processes that identify strains in populations were elucidated by the Mantel test. The lack of a significant connection between *A. vogelii* genetic and geographic distances was due to several incidents of introduction and unintentional dispersal mediated by humans. The lack of a significant relationship between genetic and geographic distances between *A. vogelii* populations suggested that their reproductive system and history of colonisation by seed dispersal influenced the spatial distribution of genetic variability. This demonstrates that *A. vogelii* populations were formed as a result of multiple importation episodes.

Allelic variation within populations explains most of the diversity. Based on the number of migrants per generation (Nm=0.5872) and the populations' mating pattern (outcrossing rate of 0.54), this result indicated a degree of gene flow. A few migrants every generation can overcome or hide the process of drift that causes populations to diverge over time (Matt et

al., 2011). *A. vogelii's* genetic differentiation (0.2986) was linked to gene flow. *A. vogelii* had induced the seeds, which were highly influencing the evolution. Thus, high gene flow (Nm = 0.5872) was observed due to the high dispersal of the seeds. The gene flow among the *A. vogelii* populations was caused by human intervention through active trading activities by entrepreneurs on the contaminated cowpeas, sharing of seeds among farmers themselves, dispersal by wind, water, use of machineries and forage animals hence affecting its diversity and variability (Matt et al., 2011). This leads to the gene flow among populations and produces overlapping and intermixing of *Alectra* populations.

CONCLUSION

Only eight pairs of SSR markers were found to be effective and appropriate in distinguishing and identifying genetic variability in *A. vogelii* populations in this investigation. Furthermore, cluster analysis and genetic structure analysis clearly distinguished *A. vogelii* based on genetic similarity and revealed a significant level of genetic variability. According to the findings, there are four categories of physiological A. vogelii strains that are adapted to the cowpea crop. In this situation, to generate resistant/tolerant cowpea genotypes, multisite screening trials during breeding programmes should include representation from each of these three clusters. Further studies on sequencing of *Alectra vogelii* populations should be undertaken in all Alectra infested locations to better understand and define the strains of *Alectra vogelii* for optimal management. Studies involving larger populations in various country locations are recommended to be undertaken during the season and off season to have a better understanding of the genetic variability of each community. Furthermore, understanding how *A. vogelii* reacts to different cowpea genotypes is critical for developing a long-term weed control strategy that has a direct impact on productivity.

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