CHARACTERIZATION OF *ALECTRA VOGELII* (WITCH WEED) STRAINS USING MOLECULAR MARKERS IN SELECTED PARTS OF MALAWI AND TANZANIA

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ABSTRACT

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 *Taq*DNA polymerase and 50 ng DNA. PCR and gel electrophoresis ran. High coefficients of genetic similarity were revelled among *A.vogelii* variants. The 29 *A.vogelii* isolates examined was clustered into six main groups. The identified genetic variability of the *A.vogelii* will help in effective breeding of sunflower, bambara groundnuts and groundnuts. Second objective, the 23 isolates of *A. vogelii* was infested to 11 cowpea varieties/lines (Vuli 1, Vuli 2, Fahari, Tumaini, IT99K-573-1, IT99K-7-21-2-2-1, IT00K-1263, IT99K-1122, IT82K-16, B301, Bunda 1, and TZA 263), made a total of 492 pots. Approximately 500 *A. vogelii* seeds were infested in the prepared pots, 3 seeds of cowpea were sown approximately 5cm deep. The number of emerged *A. vogelii* plants was counted. The results show that, there is high variability in cowpeas depending on number of the parasite emergence and time of emergence. Third objective, a total of 21 *A.vogelii* isolate was infested to Bambara groundnuts, peanuts and soyabeans, which made the total of 126 pots. The plants were watered after every 3 days. The number of emerged *A. vogelii* plants was counted at 6th, 8th, 10th and 12^{th}weeks after pot infestation. Both *A.vogelii* isolates from Tanzania and Malawi showed late emergence and were more reactive on bambara groundnuts than on soyabean and groundnuts.

DECLARATION

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DEDICATION

This dissertation is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

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LIST OF ABBREVIATIONS

SUA	Sokoine University of Agriculture
Spp	Species
MAS	Marker Assisted Selection
USA	United State of America
^{0}C	Degree centigrade
cpDNA	Chlororplast Deoxyribonucleic Acid
mtDNA	Mitochodria Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
µl	Microlitre
dNTPs	Deoxynucleotide Triphosphates.
ng	Nanogram
RB	Rice Bean
NTSYS	Numerical Taxonomy and Multivariate Analysis System
USDA	United State Department of Agriculture
TE	Tris EDTA(buffer)
SSRs	Simple Sequence Reapeats
Conc	Concentration
UV	Utraviolet
V	Voltage
*Mse*I	Restriction endonucleases
*Bcc*I	Restriction endonucleases
*Alu*I	Restriction endonucleases
*Msp*I	Restriction endonucleases
dATP	Deoxyadenosine triphosphate.

dCTP	Deoxycytidine triphosphate
dTTP	Deoxythymidine triphosphate
dGTP	Deoxyguanosine triphosphate
TBE	Tris/ borate/ EDTA(buffer)
ISSR	Inter- Simple Sequence Repeat
A	Absent
E	Early emergence
L	Late emergence
M	Medium Emergence
ARI	Agricultural Resesarch Institute
CA	Canada
Bp	Base pair

CHAPTER ONE

1.0 INTRODUCTION

Alectra vogelii (Benth) is a parasitic plant belonging to the family *Orobanche*, is a hemi parasite that derives its water and nutrients from roots of its host plant (Magani *et al*., 2008). So far *A. vogelii* has been the major constraint known to attack leguminous species (Parker and Riches, 1993) especially in cowpea production.

It infests a number of grain legume crops in an agro-ecological range extending from the northern agricultural regions of South Africa and Swaziland, through Central Africa to Burkina Faso and Mali in the west and east in Kenya (Mbwaga *et al*., 2000). Its climatic requirement is similar to those of *S. gesnerioides* and in many cases the two are sympatric (Mohamed *et al.,* 2006).

Host range tests indicate that populations from Mali, Nigeria and Cameroon can attack groundnut and cowpea (Riches *et al*., 1992). Samples from eastern Botswana, South Africa attack mung bean in addition to cowpea and groundnut. Populations sampled from Kenya, Malawi and eastern areas of Northern Province, South Africa, parasitize bambara as well as crops which are susceptible elsewhere. Geographic variation in host preference has also been observed in *A. vogelii* populations from West Africa and Cameroon attack cowpea and groundnut respectively (Riches, 1992).

The *A.vogelii* isolates from eastern Botswana and northern portions of South Africa parasitize cowpea, groundnut, and mung bean. While those from the eastern portions of South Africa, Kenya, Malawi and Zimbabwe parasitize cowpea, groundnut, mung bean, and Bambara groundnut. *A.vogelii* also has distinct races that differentially parasitize cowpea (Botanga and Timko, 2006; Polniaszek *et al*., 1991; Singh, 1993).

For example, the cowpea landrace B301 is resistant to *A.vogelii* in Kenya, but susceptible to isolates from Malawi, Botswana, and some areas of South Africa (Riches, 1992).

Cowpea (*Vigna unguiculata* L.) is one of the most important food legumes which is prone to *A.vogelii* in the semi-arid tropics covering Asia, Africa, Southern Europe, Southern United States and Central and South America (Singh, 2005 and Timko *et al*., 2007).
It serves a critical role in the lives of millions of people in Africa and other parts of the developing world, where it is a major source of dietary protein that nutritionally complements staple low-protein cereal and tuber crops (Singh, 2002) and Langyintuo *et al*., 2003). In addition to its nutritional value, cowpea is a valuable and dependable commodity that produces income for farmers and helps to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky *et al*., 2002, Tarawali *et al*., 2002 and Sanginga *et al*., 2003).

Yields of cowpea grain are reduced by a variety of biotic and abiotic constraints of which attacked by two root parasitic angiosperms (Parker and Riches, 1993). *A. vogelii* replaces *S. gesnerioides* as an important constraint to cowpea production in east, central, and southern Africa (Parker and Riches, 1993).

In Tanzania *A. vogelii* was observed in cowpea during the 1988/89 season in national trials at Hombolo Research Station. In the same season, it was reported on farmers' fields at Naliendele in southern Tanzania (Mbwaga, 1991). The yield losses of up to 50% have been reported in Tanzania by Mbwaga *et al*. (2000). Bagnall- Oakley *et al.* (1991) reported total crop loss in parts of Kenya.

In Botswana, Riches (1989) reported that losses of 80 – 100% in a susceptible cultivar. Yield losses of up to 15% has been reported in groundnut in Nigeria (Salako, 1984), while in South Africa 30 – 50% reductions in yield of bambara were reported (Beck, 1987).

In addition to cowpea, it infect other crops like soybean, bambara groundnut (*Vigna subterranea*), common bean, mung bean (*Phaseolus radiata*) and many legume fodder crops including *Lablab purpureus*, Siratro (*Macroptilium atropurpureum*) and velvet bean (*Mucuna pruriens*) (Mbwaga *et al*., 2000).

It is increasingly becoming a major pest on sunflower especially in Singida, Dodoma and Iringa (Mbwaga and Massawe, 2013, personal communication). This parasitism is severely detrimental to crop production, and may result in complete yield loss (Morawetz *et al*., 2003).

The parasite is so widespread and severe that susceptible cowpea varieties can be wiped out completely and fields may become so heavily infested with its seeds that farmers cannot grow cowpea. It can also attack members of the family Compositae, Euphorbiaceae, Labiatae, Malvacea and Pedaliacea (Parker and Riches, 1993). There is generally greater availability of resistant genotypes against *Alectra* (Riches, 1989; Mainjeni, 1999; Rubiales *et al*., 2006).

It can produce as many as 600,000 seeds per plant (SP-IPM, 2003) resulting to up to 75% of the crop damage. The damage of crop is done before the *A. vogelii* seeds emerge above the ground (Singh & Emechebe, 1991). These seeds have ability to remain viable in the soil for more than ten years. The intimate physiological interaction with cowpeas also contributes to the main difficulties that limit the development of successful control measures to be used by subsistence farmers.

A. vogelii has a similar life-cycle to that of *Striga species*. Seed germination occurs in response to the root exudates of potential hosts and a hemi-parasitic phase after emergence follows the holo-parasitic development of the plant on the host roots (Botha, 1984).

The most effective method of controlling the weed is through host-plant resistance or tolerance. Several control methods have been tried for the control of parasitic weeds, including cultural, mechanical, physical, chemical, use of resistant varieties, and biological still the weed is a problem (Kroschel, 2001). Host range of this parasitic weed can be variable depending on geographical location. Like in Tanzania, it appears that *Alectra spp* from Singida has a narrower host range and less virulent on cowpea than other *Alectra spp* from different sites. In Malawi, it appears that *Alectra spp* from Bunda and Kasungu are similar but different to that of Zomba in the same country. Mbwaga *et al*., (2010) reported that the *A.vogelii* samples collected from Malawi are different from the parasite collected further south near Blantyre as trials conducted at LongAshtoon.

Resistance against most parasitic weeds, including *A. vogelii* is often difficult to assess due to numerous confounding factors in the field, including parasite variability, unpredictable environmental influences, and imprecise selection criteria. Despite these difficulties, no significant success has been achieved in the identification of heritable sources of resistance to both *S. gesnerioides* and *A. vogelii*, and the inclusion of germplasm having these traits into cowpea selection and breeding programs (Singh, 2005; Timko *et al*., 2007; and Singh, 2002).

The genetic diversity on *A.vogelii* has never done to assess to what extent the weed vary from one geographical location to another, since there is no publication to prove that work, there is needs clear understanding of the geographic distribution and genetic variability (Phylogenetic analyses) of the parasite. A phylogenetic analysis has become essential in

researching the evolutionary tree of life. Phylogenetics is the study of evolutionary relation among groups of organisms (e.g. species, populations), which is discovered through molecular sequencing data and morphological data matrices. The result of phylogenetic studies is a hypothesis about the evolutionary history of taxonomic groups (Arenas, 2008).

The understanding of the ecotypes of the weed will assist in effective breeding for resistance to the particular strain either conventionally or using marker-assisted selection (MAS) in cowpea. Also it is important to determine whether the *A.vogelii* from different places can grow on different legume crop especially groundnuts (*Arachis hypogea* L.), cowpeas (*Vigna unguiculata*) and soybeans (*Glycine max*.); this will help to understand if there is variation of *A.vogelii* isolates from different areas for effective breeding.

This work therefore focuses on identification of different pathotypes or races of *A. vogelii*, which may exist in Malawi and Tanzania for essential cowpea improvement. So without a clear understanding of the geographic distribution and genetic variability of the parasite through the phylogenetic analysis and phenotypic analysis, effective breeding for sustainable resistance either conventionally or using marker assisted selection (MAS) in cowpeas is compromised.

1.1 Objectives

1.1.1 Main objective

Identification of phylogenetically differences and differential responses of *A. vogelii* found in cowpeas, bambara groundnuts and sunflower from selected parts of Malawi and Tanzania

1.1.2 Specific objectives

1. To establish the phylogenetic relationships among populations of *A. vogelii* growing on similar and different hosts.
2. To determine differential response of individual *A.vogelii* growth on different cowpea host varieties to assess host specificity and host range.
3. To determine resistance/susceptibility of pulse crops to *A. vogelii* variants from Tanzania and Malawi.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of introduction and spread *of A. vogelii*

Little is known of the origin of *A. vogelii*, as it is not known in natural vegetation. It has presumably moved with the cowpea crop during human migrations from West and Central Africa (CABI, 2012). Although *A. vogelii* is already widespread in semi-arid areas of Africa, further spread is possible as seed contaminating grain legume shipments to markets or on legume planting seed sold commercially or in samples distributed throughout sub-Saharan Africa for trials by research organizations. The main danger in Africa would be to introduce biotypes with differential host specificity from one area to another. The accidental introduction of the related *S. asiatica*, a noxious parasitic weed of maize and other cereals, into the USA in the 1950s (Parker and Riches, 1993) demonstrates that long-distance spread of the tiny seeds of these root parasites is possible. *A. vogelii* is already prohibited as a noxious weed in the USA (USDA-APHIS, 2003).

2.2 Taxonomy and nomenclature of A. *vogelii*

Engler (1922) split the species into *A. angustifolia, A. merkeri* and *A. scharensis*. Melchior (1941) considered these all to be characteristic of *A. vogelii* on the basis of the specimen collected by Vogel in Guinea in 1843. All previous and subsequent major floras for West Africa (Hutchinson and Dalziel, 1963) and south-eastern Africa (Philcox, 1990) have maintained the name as *A. vogelii*. Although these accounts include the genus in the family Scrophulariaceae, a sequence analysis of three plastid genes suggested that it should be placed in the Orobanchaceae along with other closely related parasitic genera (Olmstead *et al*., 2001). No morphological or anatomical evidence for this reclassification has however been advanced.

2.3 Botanical description

A. vogelii is found in family Scrophulariaceae, it is an Annual herb, 20–50 cm high; stems erect, simple or with several branches arising from the base or above. Base of stem and roots are orange-yellow. Leaves are opposite, ovate having 7–25 mm long, 3–8 mm wide. Inflorescence of terminal racemes is generally compact. Flowers are solitary in the axils of leaf-like bracts; bracts linear to linear-lanceolate, 9–13 mm long, entire or with 1–3 blunt teeth, with 1–3 prominent veins; bracteoles linear, 5–6 mm, acute, hispid; pedicels 1–2 mm. Calyx campanulate, 5–6 mm long, 5-veined, veins not prominent, hispid; lobes ovate, 2–3 mm, acute, ciliate. Corollasare yellow with purple veins, 10–12 mm long with rounded lobes, filaments glabrous with a few hairs present right below the anther.Capsule ovoid, 5–6 mm long, ± 5 mm in diameter, glabrous (Ghazanfar *et al.*, 2008).

2.4 Hosts/species affected with *A. vogelii*

Cowpea is the major crop host of *A. vogelii* throughout its range (Parker and Riches, 1993). Bambara, groundnuts, common bean, soyabean, mung bean, and tepary beans are also common hosts. There have been occasional reports of infestation of chickpea and runner bean. Pigeon pea is the only widely grown grain legume which is not parasitized. Although *A. vogelii* can attack the crops listed there is clear geographic variation in the host range in different regions of Africa. Host range tests by Riches *et al*. (1992), indicate that populations from Mali, Nigeria and Cameroon can attack groundnut and cowpea. Samples from eastern Botswana and northern areas of Northern Province, South Africa attack mung bean in addition to cowpea and groundnut. Populations sampled from Kenya, Malawi and eastern areas of Northern Province, South Africa, parasitize bambara as well as crops that are susceptible elsewhere. No association has been observed between morphological variation, largely in leaf shape, and host preference.

A. vogelii has a wide host range and recorded as parasitic on non-legume weeds including *Acanthospermum hispidum* and *Vernonia poskeana* (Compositae), *Euphorbia* (Euphorbiaceae) and *Hibiscus* (Malvaceae) species in addition to common legume weeds including *Indigofera* and *Tephrosia* species.

2.5 Habitat

A. vogelii is always associated with the cultivation of leguminous crops, on which it is parasitic, in semi-arid savannah areas of sub-Saharan Africa. Reports of the species in association with non-crop legumes and occasionally non-legumes always involve weeds on fallow or cropped arable land. The weed has not been reported as a component of natural vegetation (CABI, 2012).

2.6 Geographical distribution of *A. vogelii*

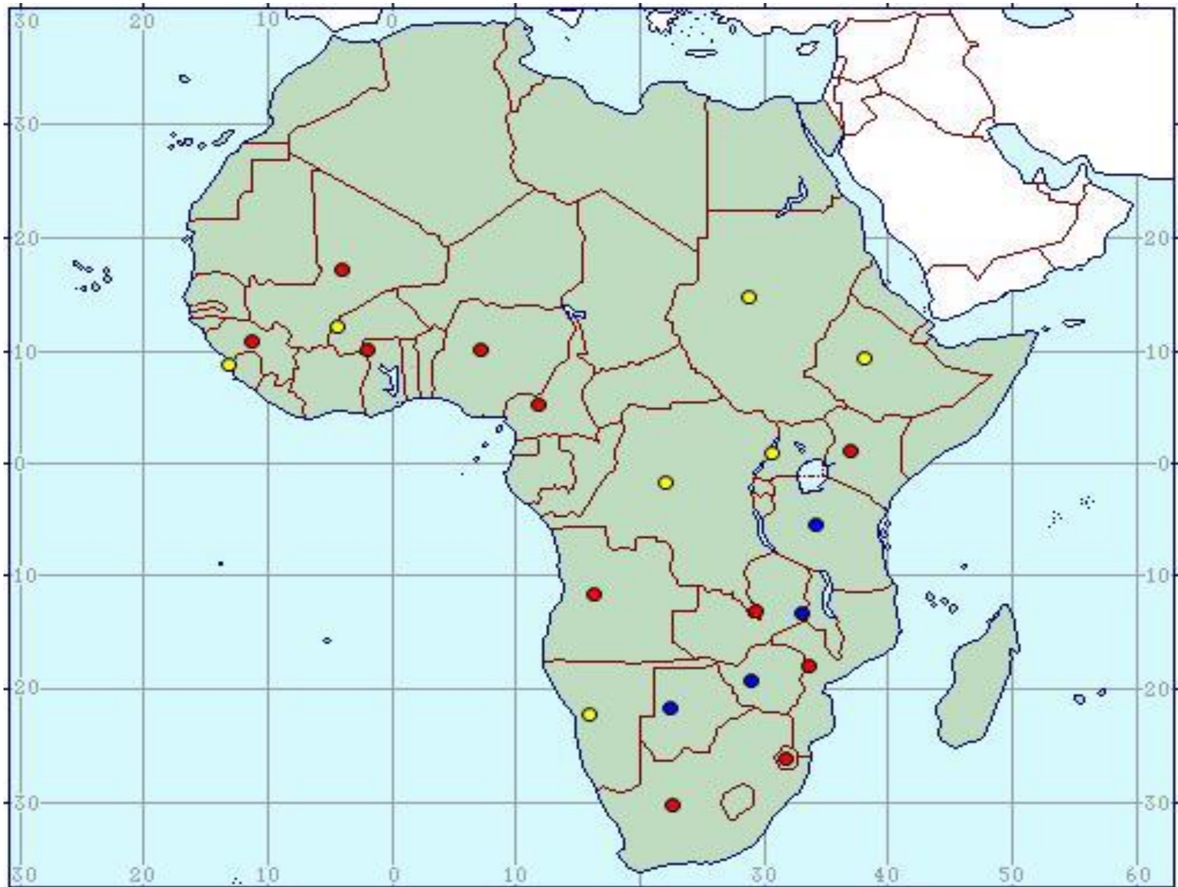
A. vogelii is found at 300–2500m above sea level. The areas in which it is found are as follows: Kenya: Embu, Kapiti Plains, slopes of Mwami Hill, ICIPE Mbita Point Field. Tanzania: Dodoma District, Manyoni, Shinyanga, Tanga District especially Korogwe. Uganda: Serere Farms. Others are Botswana, Congo, Ghana, Guinea, Malawi, Mozambique, Nigeria, Zambia, Zimbabwe (Ghazanfar *et al.*, 2008).The species of the genus *Alectra* are found mainly in tropical Africa and subtropical southern Africa. *A. sessiliflora*, and *A. fluminensis* are also found in subtropical Asia and tropical and subtropical South America, respectively (Parker and Riches 1993). *A.vogelii* is the most important species parasitizing mainly grain legumes in sub-Saharan Africa, which include cowpea, bambara groundnut (*Vigna subterranea* (L.) Verdc.), soybean (*Glycine max* (L.) Merr.), mung bean (*Vigna radiata* (L.) Wilczek), groundnut (*Arachis hypogea* L.) and common bean (*Phaseolus vulgaris* L.). *A. vogelii* is distributed throughout semi-arid areas of tropical and sub-tropical Africa.

In the Nigerian savannah it can be found in cowpea crops that are also attacked by *S. gesnerioides*. It has been reported as the major parasite of the crop in the northern Guinea savannah (Lagoke, 1989). Elsewhere in West Africa, infestations tend to be more localized, as in southern Mali. *A. vogelii* replaces *S. gesnerioides* as an important constraint to cowpea production in East, central and particularly southern Africa.

Table 1: Geographical distribution of *A. vogelii*

Country	Distribution	Original	Invasive	References
Angola	Restricted distribution			Hutchinson & Dalziel, 1963
Botswana	Widespread	Native	Not invasive	Philcox, 1990
Burkina Faso	Present, few occurrences	Native	Not invasive	Ramiah *et al*., 1988
Cameroon	Restricted distribution	Native	Not invasive	Parker & Riches, 1993
DRC Congo	Present, few occurrences	Native	Not invasive	Anota, 1991
Ethiopia	Present, few occurrences	Native	Not invasive	Stroud & Parker, 1989
Ghana	Restricted distribution	Native	Not invasive	Hutchinson & Dalziel, 1963
Guinea	Restricted distribution	Native	Not invasive	Hutchinson & Dalziel, 1963
Kenya	Restricted distribution	Native	Not invasive	Bagnall-Oakeley et al., 1991
Malawi	Widespread			Philcox, 1990
Mali	Restricted distribution			Hoffman & Diara, 1991
Mozambique	Restricted distribution			Philcox, 1990
Namibia	Present, few occurrences			Visser, 1978
Nigeria	Restricted distribution			Hutchinson & Dalziel, 1963
Sierra Leone	Present, few occurrences			Visser, 1978
South Africa	Restricted distribution			Parker & Riches,1993
Sudan	Present, few occurrences			Melchior, 1941
Swaziland	Restricted distribution			Visser, 1978
Tanzania	Widespread			Philcox, 1990
Uganda	Present, few occurrences			Riches, 1989
Zambia	Restricted distribution			Philcox, 1990
Zimbabwe	Widespread			Philcox, 1990

Source: CAB, 2012



Source: (CABI, 2012)

Figure 1: Map of Africa showing areas infested with *Alectra vogelii*

Key: ●= Present, no further details, ●= Widespread ●= Localised, ●= Confined and subject to quarantine ●= Occasional or few reports, ●= Evidence of pathogen, ●= Last reported ●= Presence unconfirmed, ●= See regional map for distribution within the country

2.7 Agricultural significance and yield losses of *A. vogelii* on hosts' plants

A. vogelii is a serious constraint to the production of grain legumes, particularly cowpea, bambara groundnuts, groundnut and soybean in the semi-arid savannahs of sub-Saharan Africa. The parasite infestation does not decrease cowpea dry matter production, but it significantly alters dry matter partitioning by increasing the proportion of root dry matter (Rambakudzibga *et al.,* 2002).

Crop yield losses resulting from *A. vogelii* infestation range from 41% to total crop loss in highly susceptible cultivars, sometimes can be lower than 41% regarding the type of crop or cowpea variety attacked (Lagoke *et al.,* 1993). Before parasite emergence above the ground, affected cowpea plants may appear wilted, delayed flowering, a reduced number of flowers and pods all contribute to yield loss (Mugabe,1983).The extent of yield loss depends on the susceptibility of the cultivar with greatest losses reported for introduced lines rather than landrace types (Parker and Riches, 1993).Yield losses of 20% were reported from Kenya in the 1920s with total crop loss in the 1980s in Embu District (Bagnall-Oakeley *et al*., 1991).

In Botswana, infested fields of the cultivar Black eye, introduced from USA, produced only 20% of the grain harvested from uninfected sites in the late 1970s (Riches, 1989). Losses in groundnut of 15% have been recorded due to the parasite in Nigeria (Salako, 1984), and yield reduction in bambara in South Africa of 30-50% has been observed (Beck, 1987). Late sown crops of soyabean may be completely destroyed by the parasite in northern Nigeria (Lagoke, 1989). *A. vogelii* is also a constraint to common bean production in the Blantyre Shire Highlands of Malawi (Riches, 2001).

In Malawi the parasitic weeds (witch weeds) for cereals and legumes exist simultaneously. The predominant host species for legumes is *A. vogelii*, which causes damage in groundnuts (*Arachis hypogea* L.), cowpeas (*Vigna unguiculata*) and soybeans (*Glycine max*).

A. Vogelii is widely seen in Lilongwe and Kasungu plains many parts of southern region (Riches and Shaxson, 1993; Mainjeni, 1999; Kabambe *et al*., 2005). It is also common in Dowa and districts in central Malawi, the lower lying and Blantyre/Shire Highlands (Mbwaga *et al*., 2008).

In Tanzania, *A. vogelii* is common in Mwanza, Shinyanga, Dodoma, Iringa and Ruvuma regions. The yield a loss of *A. vogelii* is up to 50% have been reported (Mbwaga *et al.,* 2000).

2.8 Environmental conditions for *A.vogelii* growth

A. vogelii is largely dependent on annual cropping and environmental requirements mirror. The parasite is most commonly found in areas of mono-modal rainfall with a long dry season as in Botswana or the Guinea savannah of West Africa, but it is also a pest in bimodal rainfall areas as in northwest and coastal Tanzania (CABI, 2012). Host crops are largely associated with free-draining sands and sandy-loams (CABI, 2012).

Table 2: Factors limiting *A.vogelii* growth

Parameter	Lower limit	Upper limit
Mean annual temperature (ºC)	19	26
Mean annual rainfall(mm)	520	1000

Source: (CABI, 2012)

2.9 Genetics of Resistance to Parasitic Plants in Cowpea

A few laboratories have examined the genetic basis of resistance to *A. vogelii* parasitism in cowpeas. Approximately 650 local cowpea varieties and exotic accessions were screened for resistance to *A. vogelii*. Landraces B301 and B359 from Botswana were among the most resistant genotypes (Riches, 1987 and Riches, 1989).

The superiority of B359 as a source of resistance for southern Africa was demonstrated when it was shown to remain completely resistant to isolates of the parasite from Malawi.

While IT90K-59 and IT90K-76 (two lines derived from B301 as parent), all supported the emergence of parasites of a population from Malawi (Mainjeni, 1999).

B359 was resistant in pot trials to isolates of *A. vogelii* from different locations in east, southern and West Africa, including Botswana, Cameroon, Mali, Malawi, Nigeria and South Africa (Mainjeni, 1999).

2.10 The importance of Population genetic studies of *A. vogelii*

The ability of a parasitic weed to adapt to new ecological niches or to the resistance of host depends on the amount of genetic variation of the entire population.

Studies of molecular variability of populations have applications in the fields of taxonomy and systematic identification of species basing on morphological variability. Most parasitic weeds are highly variable plants, they display extraordinary plasticity in terms of variation of morphological characters as a result taxonomy of parasitic weed of genera *Alectra, Striga* and *Orobanche* is difficult task and almost impossible (Kroschel, 2001). Molecular markers represent a tool that is independent from environmental and developmental factors.

2.11 Possible control methods of *A. vogelii* and their constraints

Compared with non-parasitic weeds, the control of parasitic weeds has proved to be exceptionally difficult due to the ability of the parasite to produce a tremendously high number of seeds, which can remain viable in the soil for more than ten years.

Their intimate physiological interaction with their host plants, are the main difficulties that limit the development of successful control measures that can be accepted and used by subsistence farmers.

However, several control methods have been tried for the control of parasitic weeds; these methods were well reviewed by Parker and Riches (1993), and recently summarized in Kroschel (2001) and Omanya (2001).

2.11.1 Cultural control

Two options catch and trap cropping, are available for reducing the size of the *A. vogelii* seed bank in the soil. Catch crops are susceptible species that are ploughed in or harvested after parasite attachment but before emergence and seed production (Hattingh, 1954). In a season of good rainfall, a quick-maturing crop of sunflower could then be grown with cowpea planted again in the following season. Trap-crops produce the *Alectra* germination stimulant in their root exudates but are not susceptible to be attacked by the parasite seedlings.

In Botswana, grain or fodder cultivars of pearl millet and bambara, which is not attacked by the local biotype of the parasite, are potential stimulators of *A. vogelii* germination. These can be used in a rotation to cause suicidal germination of the parasite and hence reduce the number of seeds in the soil (Parker and Riches, 1993).

Improved cowpea cultivars which combine resistance to *A. vogelii*, the related parasitic weed *S. gesnerioides,* and several insect pests and fungal diseases have been developed in West Africa. These include the cultivars IT90K-76-6 and IT90K-82-2 which have been released for commercial production in Nigeria (Singh, 2000). These are not, however, resistant to biotypes of *A. vogelii* from southern Africa (Riches, 2001). The Botswana landrace accession B359 has been shown to be resistant to samples of the parasite from Botswana, Malawi and Kenya, so could be used as a parent for breeding improved cultivars for East and southern Africa (Riches *et al*., 1992: Riches, 2001).
Potentially useful levels of resistance have also been demonstrated in germplasm of bambara (Riches *et al*., 1992) and cultivars of soyabean (Kureh and Alabi, 2003) but multi-location testing is needed to confirm the value of these lines in the field.

2.11.2 Mechanical control

Hand pulling and destruction of emerged stems before flowering may be useful where infestations are limited in extent to prevent seed production and an expansion of the area infested. However, Beck (1987) found that hand pulling did not directly improve the yield of an infested bambara crop and this is likely to be the same for other susceptible species, as the majority of damage occurs before the parasite emerges above ground. Prompt ploughing of crop residues after harvest will prevent continued seed production as host plants continue to grow on residual moisture.

2.11.3 Chemical control

A. vogelii is predominantly a pest of crops grown by resource-poor small-holder farmers. The potential for controlling the weed by treating cowpea seed with the herbicide imazaquin before planting has been demonstrated (Berner *et al*., 1994).

Magani and Lagoke (2009) report that farmers can reduce cowpea infection by *A.vogelii* when pre-emergence herbicide mixtures containing pre (metazachlor + antidote) are applied followed by post-emergence application of imazaquin at 0.18 kg a.i/ha.

2.11.4 Integrated management

Integrated control should be built around the use of resistant crop cultivars if possible, or choice of the least susceptible cultivar that is currently available. Timely destruction of legume crop residues is important to prevent parasite seed production after harvest and trap-crops should be included in the rotation to reduce the soil seed bank. Hand pulling should be carried out on lightly infested areas, particularly in fields that have not previously had a history of infestation (CABI, 2012).

According to the host/parasite relationship descriptions, habit, geographical descriptions, yield losses caused by *A. vogelii* both in Malawi, Tanzania and other parts of the world, genetic resistance and the of importance genetic studies of *A. vogelii* with connections to few studies which has been published about the weed, there is a need for more studies on *A.vogelii* at physical and molecular level. Thus the objective of the research is the identification of phylogenetically different Pathotypes and differential responses of *A. vogelii* found in cowpeas, bambara groundnuts and sunflower from selected parts of Malawi and Tanzania.

2.12 Geographic variability of *A. vogelii* on hosts

Geographic variability experiment in host response to *A. vogelii* was done under McKnight funded project; Studies undertaken in pot experiment at LongAshton UK indicate that at a species level, there are 3 strains of *A.vogelii* by host range. Where by *Alectra* from Singida attaches and emerges on cowpea and groundnut but not on mung or common bean. *A.vogelii* from Bihawana and Ismani attaches and emerges on cowpea, groundnut, and common bean but not on mung bean**.** *Alectra* from Malawi sites attaches and emerges on cowpea, groundnut, common bean and mung bean. Within cowpea; patterns of virulence were also observed to be evident. *A. vogelii* from all sites in both countries emerges on IT36E-16 and TZA 263 but not on B301.

A.vogelii from Bunda and Kasungu emerges on ITK1207, IT97K 818-35, IT97K819-118 and IT99K7-21-2-2-1. *Alectra* from Zomba emerges on ITK1207, IT97K 818-35, IT97K819-118 but not on IT 99K7-21-2-2-1 (this result for IT99K7-21-2-2-1 same as in 2008 pot trial). *Alectra* from Singida and Ismani did not emerge on ITK1207, IT97K 818-35, IT97K819-118 and IT 99K7-21-2-2-1 (result for Singida same as in 2008 trial). *Alectra* from Bihawana emerged on IT 99K7-21-2-2-1 but not on ITK1207, IT97K 818-35 and IT97K819-118.

The studies done showed the differences on attacking the cowpeas and other legume, but there are no studies done on some varieties in Tanzania and Malawi. At the same time only few isolates of *A. vogelii* was used to test the cowpea varieties, so there was importance of testing more varieties of cowpeas using many *A. vogelii* isolates to get more information, as 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.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Location

The Research was conducted in Tanzania, Malawi and USA. *A.vogelii* isolates were collected in selected parts Tanzania and Malawi. Evaluation of the *A.vogelii* was conducted in the USA, at University of Virginia (Charlottesville, Virginia, USA), at the Department of Biology. Since *Alectra* is considered a noxious weed and it is under quarantine in the USDA APHIS, all experiments involving growth of the parasite was carried out in the quarantine room. The room was maintained under proper temperature of 30°C and humidity for optimal growth of host and parasite.

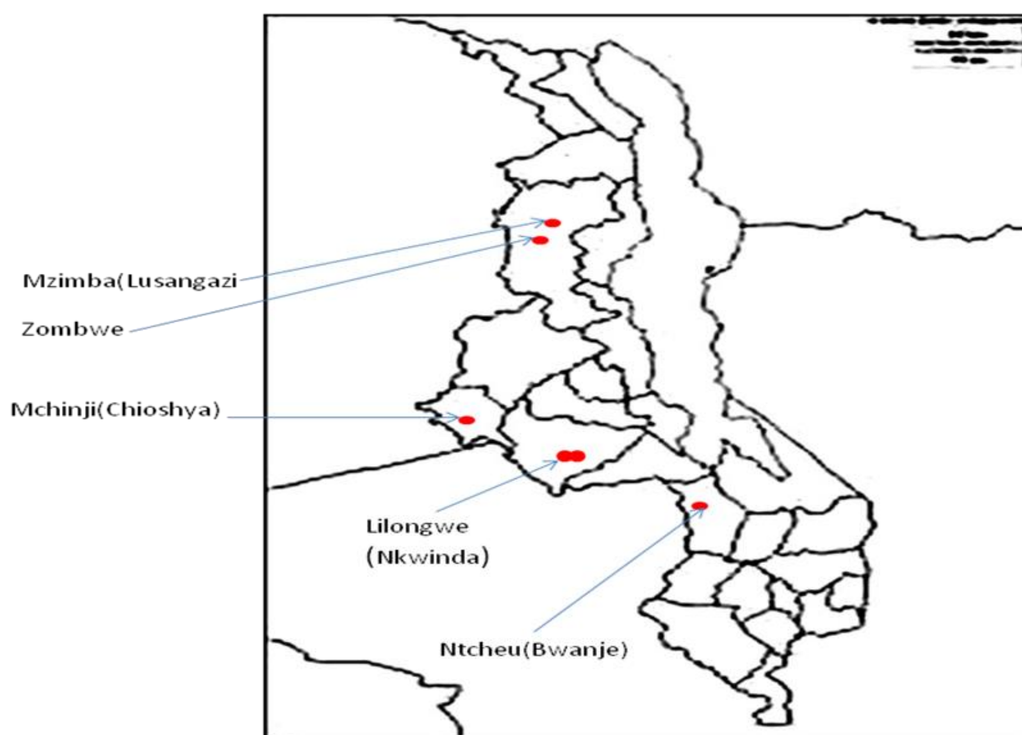
3.2 Materials

3.2.1 *A. vogelii* collected from different parts of Tanzania and Malawi

The *A. vogelii* seeds were collected in Malawi and Tanzania; in Tanzania the isolates came from Singida, Dodoma and Iringa regions. In Malawi, *A.vogelii* was collected in Mzimba, Lilongwe, Ntcheu and Mchinji districts. *Alectra* seeds were collected from sunflower (*Heliuthus annus*), Bambara groundnuts (*Vigna subterranea)* and cowpeas (*Vigna unguiculata*) crops. *A.vogelii* collection sites are shown on table (3) and Figure (2).

Table 3: ***A.vogelii*** **isolates from Malawi, indicating hosts, village, latitude and longitudes of the sites**

No.	PARASITE	HOSTS	VILLAGE	LATITUDES	LONGITUDES
1	*Alectra vogelii*	Groundnuts	Bwanje (Ntcheu)	$14^0$16'59.99″S	$34^0$42' 0″
2	*Alectra vogelii*	Groundnuts	Lusangazi (Mzimba)	13°28'S	31°32'E
3	*Alectra vogelii*	Groundnuts	Zombwe (Mzimba)	11° 19' 0.0012"S	33° 49' 59.9988"E
4	*Alectra vogelii*	Groundnuts	Nkwinda(Lilongwe)	13°59′S	33°47′E
5	*Alectra vogelii*	Cowpeas	Nkwinda(Lilongwe)	13°59′S	33°47′E
6	*Alectra vogelii*	Groundnuts	Mbawa	12^0 07' 00"S	33^0 25' 00"E
7	*Alectra vogelii*	Groundnuts	Chioshya(Mchinji)	$13^0$48'0″S	$33^0$10'0.12″E



Source: World Trade Press (2007)

Figure 2: Maps of Malawi showing the areas where *A. vogelii* was collected.

The map has been partitioned according to districts, where by the arrows and red dots shows the villages in the districts where *A. vogelii* were collected.

Table 4: *A.vogelii* collected from Tanzania, indicating hosts, village, latitude and longitudes of the sites of collection

No.	REGION	DISTRICT	VILLAGE	HOST SEEDS	LATITUDES	LONGITUDES
1	**DODOMA**	Dodoma rural	Kikombo	Cowpea	6°16'0″S	35°38'0″E
2		Dodoma urban	Bihawana	Cowpea	6° 13'0.12″S	35°58'59.88″E
3		Dodoma urban	Mpunguzi	Cowpea	6° 10' 23.00"S	35° 44' 31.00"E
4		Dodoma rural	Homboro	Cowpea	5° 52' 55" S	35° 59' 18" E
5		Dodoma urban	Nkulabi	Cowpea	6° 10' 23.00"S	35° 44' 31.00"E
6	**IRINGA**	Iringa rural	Mkungugu	Cowpea	7°S	34°E
7		Iringa rural	Mkungugu	Bambara groundnuts	7°S	34°E
8		Iringa rural	Mkungugu	Sunflower	7°S	34°E
9		Iringa rural	Mangalali	Cowpea	07°46' S	35°34' E
10		Iringa rural	Ilambilole	Cowpea	7^{0}28'S	35 48'E
11		Njombe	Usuka	Cowpea	9^{0}S	34°46'0.12″E
12		Njombe	Utegi	Cowpea	8°51'0″S	34°37'12″E
13		Njombe	Mayale	Cowpea	8°49'48″S	34°37'12″E
14		Njombe	Utiga	Cowpea	8°51'0″S	34°37'12″E
15		Iringa rural	Ismani	Cowpea	7° 30' 0" S	35° 48' 0"E
16	**MOROGORO**	Kilosa	Ilonga	Cowpea	6°48'S	37°00' E
17	**SINGIDA**	Singida rural	Ikhadoda	Sunflower	04^{0}2'16.19″S	34°33'52.92″ E
18		Singida	Msungua	Cowpea	4^{0}49'S	34^{0}45' E

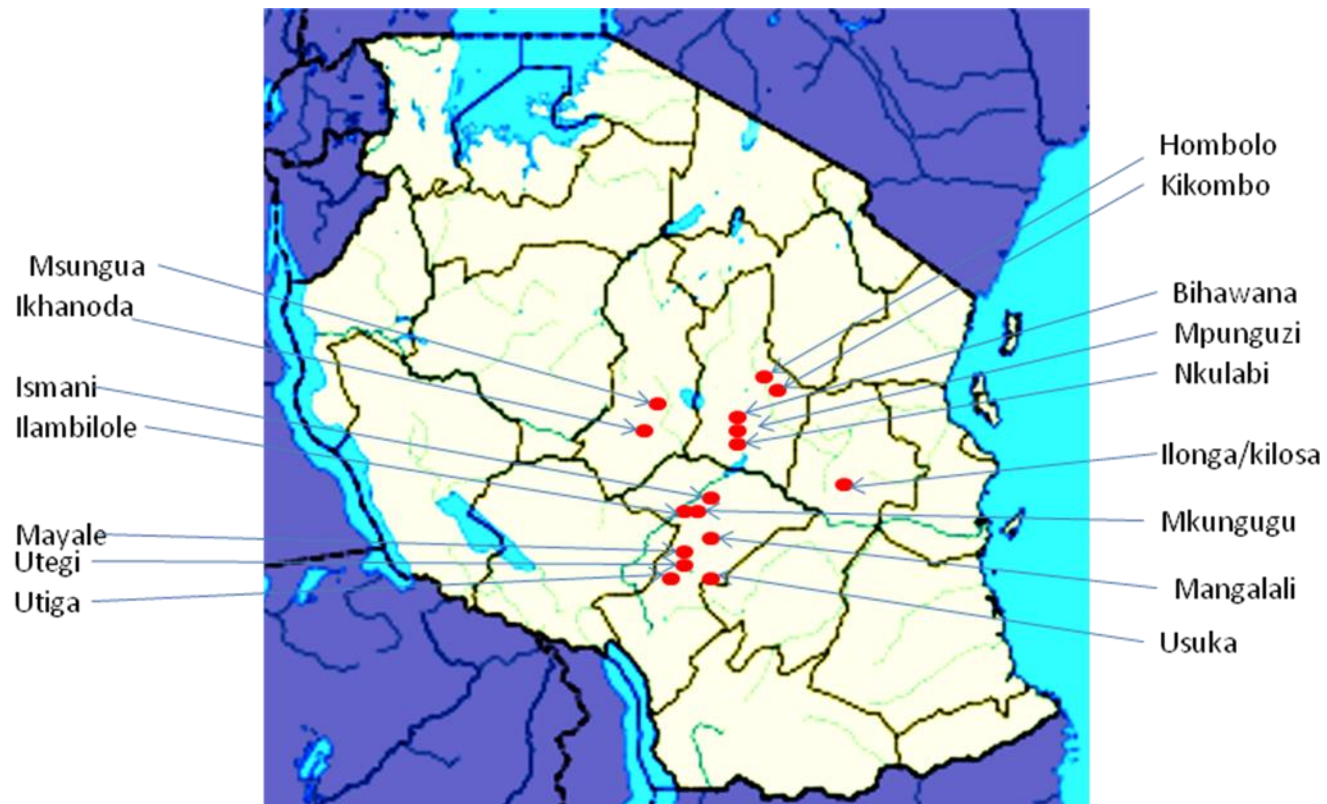


Figure 3: Map of Tanzania showing the areas where *Alectra vogelii* was collected (Source: Bamse (2009).

The red dots and arrows show where the *A. vogelii* was collected in four regions.

Table 5: *A.vogelii* collection sites in Botswana and Malawi showing villages, hosts, year of collection, Latitudes and Longtudes

No.	VILLAGE	HOST	YEAR COLLECTED	LATITUDE	LONGITUDE
1	**Marajone**	*A. vogelii*			
2	**Gathuma**	*A.vogelii*			
3	**Sebele**	*A. vogelii*		24° 34' 25" S	25° 58' 00" E
4	**Malawi**	*A.vogelii*	2002	13º 30´S	34º 00´E

3.2.2 Seed preparation of *A. vogelii* and cowpeas

Alectra seeds were well dried and cleaned. The cleanness was done at Ilonga Agricultural Research Institute (ARI), Kilosa in Morogoro. Where by the *A.vogelii* seeds were sieved in different sieving plates to remove impurities; 4mm sieve was used for removing larger particles, then sample was transferred to 212µm sieve aperture (laboratory test sieve, Endecotts LTD, London, England) followed by the last sieve of 0.0059INS(149MICS) aperture(U.S standard sieve series, Endecotts (test sieves) Limited London England) . Then the seeds were packed in the screw capped bottles and sent to University of Virginia under USAID quarantine permit for analysis. The cowpea seeds were also prepared and parked and sent to University of Virginia.

Table 6: Cowpea varieties/lines used in the screening experiment

NUMBER	VARIETY	SOURCE
1	Vuli 1	Tanzania
2	Vuli 2	Tanzania
3	IT99K-7-21-2-2-1	IITA
4	Tumaini	Tanzania
5	TZA 263	Tanzania gene bank
6	IT99K-1122	IITA
7	IT99K-573-1	IITA
8	IT00K-1263	IITA
9	Bunda 1(IT99K494-6)	Malawi
10	IT82E-16	IITA
11	B301	Botswana

3.3 Methods

3.3.1 Phylogenetic relationships among populations of *A. vogelii* growing on different hosts of cowpeas (*Vigna unguculata*)

3.3.1.1 Infestation of *A. vogelii* to the hosts in pot experiment

The 84 half a liter plastic pots where filled with 70:30 mixture of sand and Metro-Mix®250 or common name is Grace-Sierra (Formulated with Canadian Sphagnum peat moss, vermiculite, coarse perlite, starter nutrient charge (with Gypsum) and dolomitic limestone(Scotts-Sierra Horticultural Product Company, Marysville, OH). Respectively was infested with single spatula of *A. vogelii* seeds, which is equivalent to 500 seeds. The seeds were infested at approximately 5 cm depth. One *A.vogelii* infested pot was planted 3 blackeye pea seeds and the second infested pot was planted with the cowpea host seeds collected in the farm during *A.vogelii* collection. The soils were kept moist by watering 3 times per week. At six weeks, *A.vogelii* plants emerged and they were left to 8 weeks before collection. Ten *A.vogelii* plants for each isolate were individually collected, placed into heavy aluminum foil and then was quickly frozen in liquid nitrogen.
Immediately the DNA was extracted, and the frozen remains of the samples were stored at –80˚C until used for DNA extraction.

3.3.1.2 Isolates and quantification of *A.vogelii* genomic DNA

Total DNA was extracted using the DNAzol ES® (Molecular Research Center Inc. Cincinnati, OH) as per the protocol described by the manufacturer with minor modifications. Leaf samples were collected and pulverized in liquid nitrogen before grinding, then the samples were ground in liquid nitrogen by using mortar and pestle cooled with liquid nitrogen and the powder was transferred immediately to a 2.0 ml eppendorf tube containing 750µl DNAzol.

Samples were well mixed and incubated at room temperature for 10 min. 750µl chloroform was added and then the mixture was vortexed for 20s and incubated at room temperature for 10 minutes. Then the mixture was centrifuged in a micro-centrifuge at 13,200 rpm for 10 min. supernatant was transferred to another 2.0ml tube, 750µl isopropyl alcohol (2-propanol) was added and mixed well by gently inverting for 5 minutes. The tubes were incubated at room temperature for 5 min and centrifuged at 5000 rpm for 4 minutes. Pelleted DNA was washed with 2000 µl 70% ethanol and centrifuged at 5000 rpm for 5 min. DNA was dried at room temperature for 2 hours, then 100µl TE buffer was added and stored overnight in refrigerator at 4^0C , then DNA was dissolved in the water bath at 65^0C for 15 minutes. 13 µl of H_2O, 5 µl DNA and 2 µl of loading dye were mixed and the agarose gel electrophoresis was run for checking the quality of DNA. The DNA was quantified by using Qubit Fluorometer (Life Technologies, Carlsbad, CA) as described by the manufacture.

3.3.1.3 PCR amplification and gel electrophoresis of the *A.vogelii* genomic DNA

a) *Striga hermonthica* SSRs primers for amplification of *A.vogelii* genomic DNA

The informative SSR markers which have been developed specifically for *Striga species* by Estep *et al.*, (2011) were used to determine the genetic diversity of *Alectra* (Table 7). Initially microsatellite markers were tested to assess the genetic diversity of the DNA collected from *A.vogelii*. The PCR master mix was prepared with a reaction volume of 25 µl, containing 2.5 µl of 10X PCR buffer, sterile distilled water , 1 µM of each primer, 1mM of each dNTPs, 0.5U/ µl of *Taq*DNA polymerase and 50 ng DNA. The tubes were placed in an BioRad-DNA engine® peltier Thermal cycler programmed for initial denaturation at 94°C for 1 min followed by 35 cycles of 30 s at 94°C, 30 s at 45^0C, 1 min at 72°C, and a final extension of 10 min at 72°C.

PCR products were resolved on a 6% polyacrylamide gel (mixture of 10mL of 10X TBE, 30ml of acrylamide 40% solution acrylamide: Bis-acrylamide, 29:1 (Fisher Bioreagent® USA), 160ml of distilled water, 600µl of TEMED solution (NNNN-Tetranetlylenediamine, C6H16N2) and 600µl 10% ammonium persulfate.

The fragments were separated by vertical electrophoresis apparatus at 450V containing 40µl of 1 mg/ mL ethidium bromide. The gels were photographed using Canon EOS Rebel Tzi and Bio-Rad Molecular Imager® Gel DOCTMXR+ with image LabTM software under UV transilluminator.

Table 7: List of SSRs primers targeting nuclei/DNA of *A.vogelii* isolates

Primer name	Left Primer	Right Primer	Product size
1000	CTGATGCTGTGGATGTTTGG	GACCAACGAGAAGGTCGATG	286
1001	AATCAAGCAATTCACCCAAGA	TGGGAGATGAACCCTAGCAC	248
1002	CTTGGCGCGTAGTCTAGGTC	CCCACGCCTCTCTCTCAAT	296
1003	TCCCTTTGCGTTCTAGTGCT	GATTCGATTGCGACGAAAAT	293
1004	GCCCCGTTCGTTTATGAAT	CGGTTCGGCACAAATTAAGA	296
1005	CGATCGCCTCCTGGATACTA	TCGGAAAAATTGCGAAAAAC	209
1006	GGCAACAATTATTCCCATGC	GGCCTTCACAAAGATCCAAA	274
1007	CTAGGCTCGCCATTGTTGTT	CAAACCCGTCTCACACACAC	290
1008	CCGTGACCTCGATGAAGATT	CCGCAACGTAAATTCCAAGT	286
1009	GCATCCAGATAAGGCTGCTT	TGGGTTGTGTTGAGTGAGTGA	241
1010	TCTGCGTGATTTTCGCATTA	GCCAATTTAAGGGCCTTTTT	282
1011	GCTCAAAAACGATCACACGA	TCCGAGGACGAAATTCTTTG	283
1012	TGGATAAGGCCTTTTGTGAGA	GCAACAGCCCATTTGAGTCT	295
1013	GGCTGACATGACGAGTCTGA	CGGCTTGACTTGAACAACAA	286
1014	AGGGACATTATGCAGCCAAC	CGCATGACGAACAAGAAGAA	167
1015	GGCCTTGTTACGGAACCATT	CCAGCATGTCGACTTGTCACT	181
1016	GATTTGGATATCGCGGTTGT	TTCTGGCGATGAAAATGACA	273
1017	CAATAGATGGGGGAGCTCTTT	CCATGAGTTCGACACCACAC	281
1018	TCAAGGGACCTGATGGAGTC	TGCAGGTGAATGTGTGTGTG	283
1019	TTTCATAAGGGTGGGAGAATG	CCATTCTCATGTGAACCCAAC	242
1020	TTATTCAAAACGCGATGGTG	AACTTGTCCAACAAGACGAGA	257
1021	CTTCCCCGAGGATGACATTA	GGTAACCCCCGAATCAGTTT	264
1022	GAAGCTCAAGAGCGAGAGGA	ACAAACGTTGGCTTTTGCTT	169
1023	GGGTGGGCTGTATTCAACAA	AACGAGACGGAGGCTATCAG	297
1024	GATCTAATGCCTTGGCTTGC	TAGACGGGCGTAGGAAACAC	176
1025	CAAAGCTCGAATGAGAACTGA	CCATGTCTAGCTGACCCACA	225
1026	CGGTTCCAGGTCATTGTTTT	ACGGTCTGCTGGAACTCTGT	286
1027	CAAGCCACCCTTGATAATCC	CGTCCGTTTTCATCCCTAAC	185
1028	CGTGCATGGTCATGTTTGTA	GCTCGGTTCGACGAAAATTA	296
1029	CTTATAGCCCGCATGCAATC	CCCCTCCGTTCAGTTCAGTA	269
1030	TGTCTCGTTCCGTCCTCTCT	GCAATGCAGGTAGCCTCCTA	219
1031	CGGTTCCATATGTGTGTGTG	GCTCGGGAGGGTGTGTTAT	231
1032	TATCGAGTCGGGAAAGATGC	CATTCCACACCCACTACACG	280

1033	AAGAAACCGCAAGCGAAATA	TGGCCATTGATATGTTGGAG	299
1034	ACCAGAAAGCTGCATTATTGA	CAGCTTGCGAGTGTTGTCAT	277
1035	AAATGTTAGGGCGAAATCGTT	CAATCATCAACTCCCCCAAT	259
1036	TCAGTGGTGCAGGTTAACGA	CTGCAGCATGGAAGTTCGTA	182
1037	GTTTATGTGTGTGGCGATGC	GGGCCAAATGTAGTGGTGTT	284
1038	TCAAAATCAACTCCGCAAAA	TTCTCCAGTGTGAGCGTGTC	241
1039	GGCAACAATTATTCCCATGC	CCAAGTCAACCGGGATTCTA	159
1040	ATGCGATTGACTGCGAATTA	CCCGCTTTATCGTGGATTAT	260
1041	GGAGTGGCCAGGATCATTTA	TCCCCGGGCTCTTAGTTAAT	177
1042	CTCATTCCCTCGCTTTCTTG	TTTCTGCGTTTTGTTTTGGA	261
1043	AAGCCGAGTGGAAGTTGAGA	TCGGTTAGGTGGTCTTCTCG	274
1044	CACACGATCCTCATCATTGG	TTTGTGGACTCGTGGTTGAG	219
1045	TTCTGCCAAACTGTGAGTGC	TCTTATTTTGGGGGAGCTGA	297
1046	CACAAAACCCCACTTTACCC	AAGGGAACCAAAGGATGAGG	252
1047	GCATAACGATATGGGCCAAG	TCCAATGCCTATGTCAGTGC	173
1048	GTTTGCCTTTTGCTGAGAGG	ACTCCCTCTCCAAACCTTCG	252
1049	TCATCCCGGACATTGTACCT	TGTGGCAGACTTCACAGAGG	265
1050	ATAGGTTCGACCGGAAGCTC	TAGACTGTAACGCCCCGTTC	294
1051	ACAACCATGGAATTGCGTCT	GTTTTGGTCTTCGGTTTGGA	183
1052	ATGGCCACCGTAAGTTTGAG	GATGCTCGTCAAGCAAACAA	256
1053	TACGAAACGCGACAATACGA	CGGTACCCGAACTTTGAGTTT	234
1054	TTCAAGGAAAGGGCGTAATG	CCGGTTCGACGAAAATTAAA	289
1055	CCGGGAGAAGTGTCTGGTAA	CAAACACGTCTTCCATGGTG	179
1056	GCAGGCTTTCTCTTTCATGG	CCTTTTTCAGAAGGGGCTCT	242
1057	ACGGCTCCAGTGCTGACTAT	CCCGGTTTGACTTGAACAAC	202
1058	TTGTTTTGGGGCTTAGGAAA	AATGGCTTGAATGAGCAGAA	289
1059	TCGAAAACGAACGAAGTCAA	CCCACTCGTATGCTTCCCTA	288
1060	CAATCTCAAAATGTGCCAAA	AGGTGCAAAAGCGACAAACT	163
1061	GATTCTGCCTGGTCCACAAT	TGCTTCCATTCCAAAACACA	219
1062	GGCTCGGATCACACGATATT	CGGTTTAATCGCGTAGTTCG	262
1063	GATGGCTTGAACGAGGAAAA	ATAAGGCTTGTCCGGATGTG	292
1064	CGAGGTCGGAATCCTTTGTA	TCACCTGCCAAGTAACACGA	251
1065	GGCTAAATGGGGAGAGCAAT	CCCAAGTCTCCTAATCCCAAG	154
1066	CCATCTCGAATCTTCCCTTC	ATGGTGTACGGCAAATGGAT	280
1067	TTTCCAATCATCCGTTCACA	GCGTGCAAAAGATGCAATAG	256
1068	GTGCGATTGAGTATCGCAAA	GGAAGTTCAGTTCGACGAAAA	287
1069	TTGGCAGCTTTTCTCCATCT	GGAAACGAGAGGGCCTAAAC	166
1070	AAGTTCAGCCCAAAACATCC	TCGGGCTATACCCAAATGAC	287
1071	GGTAACCCCCGAATCAGTTT	TCAGCACTTACCTGGTGTCG	237
1072	CAAACACCCTTCTTCAACTCG	CTCCCAAGGGTCCCTACTGT	211
1073	GGCAACAATTATTCCCATGC	GGCCTTCACAAAGATCCAAA	274
1074	GTTTGACTTTGGCTCGGTTT	GTACATGTAACGCCCCGTTT	287
1075	TCCACATTCAAAGTCCACCA	ACTCGATTTTTAGGCGGTTG	295
1076	GGTCACGAAGGGAGAAGAGA	TCCTCCAGCCCTAATCCTCT	163
1077	CGGGCTCGACTAAAATCGTA	ATACCCCATTTCATCGTGGA	285
1078	CCACGGTGTACAGCTCAAGA	CCACGGTGTACAGCTCAAGA	170
1079	CTAATTCAGTGGCCCCACAC	AGGACGGAGCTGAGTTCTCC	233
1080	CCGAATCAATTGTTGAACTGG	GACCTTGACTCGGTTGACCA	299
1081	AATCAAATTTAATGGGCCTGA	GCTCGCGTTAGTGGTTCATT	246
2000	TCCAGAGTCCCTAACTTGTCA	ATGGCTCCGTAGCAAAACTG	300
2001	ACCGGTAGCTTGGTTCGAC	GCCCCGTTCGTTTATGAAT	299
2002	CGGAACGAGGATAAGATCCA	CTAAACTGCCGCATGAGACC	299
2003	TGAGGAAAGCCAATAGCAAGA	TCGATGATTGTGCGTTGAAT	298
2004	CAGTGGGAGTTCCAGTTGGT	GGGAAGCCCACACTATCAAT	297
2005	GGGAAGCCCACACTATCAAT	CAGTGGGAGTTCCAGTTGGT	296
2006	GGCTTGAACGAAAGATGATGA	AGCGACTACCAGCCCTCTTA	296

2007	TAGGATTCCGAAGCACCAGA	CAGGGAAGTTCACAGGGTGT	295
2008	GAACTCTGGACGGGCAATTA	TTGAGAACTCGGTACGACGA	292
2009	CGGACATGGTACCTGCATTA	AAATGCGGTAAGTCCCACAG	292
2010	CGAGCTCTGCCTCAGAAAAT	TGTTCCGGATGAGTTTTGTTT	292
2011	CGATTCACTCAACAGCTGGA	GCCGCTCCTCATATTTTCAC	291
2012	GGCCTTCACAAAGATCCAAA	TCTATGCACACAAGGCAACA	283
2013	ACAGCATTGGTCCATCACAA	CGGTGCCTGTGAGGTTACTT	283
2014	TAAATCGAACGGGGCATTAG	CCGAGGACGAAATTCCTTGT	282
2015	TGTCCCGTTTGTCCCTTTTA	ATTTGACAGTTTGGGCGACT	282
2016	CTGCAAGATCCCGAAACAAT	CATAGCCTTGGAGGCAGGTA	281
2017	TTGTGGATTATTTGGGAATGA	TCGACAAACGACGAATAACAA	280
2018	GATTGTCGAGCAACAATACCA	TGAGAAACCCGGTGTAATGC	280
2019	TGCGATACACGGGAAAATTA	TTTGGTGGGTCTCACTCGAT	279
2020	TTTTCGGTTTGAACGGTTTC	TGGGCTTGATCCATACCATA	278
2021	AGCCCAAACTTCTCGACAGA	CCGGTCTTAACCCCACTCTT	278
2022	ACAAGGTCCTCCTCCAGGTT	CGCTCCTCTCTGTTGTACCC	271
2023	TCTAAGCATCCGGTTTGACC	ATTCCGAGGACGGAATTCTT	262
2024	ACCCCGTTTTAATGTGGATT	CGATTGACGACGAGCAATAA	253
2025	TTGTGGAGCAGTTGAAGACG	CCATGACACAAAAGGAGAGGA	250
2026	CGACCACTGCCTCTGAAAAT	TATTGGACGGCTCTTGGTTT	245
2027	TCAGCAAAATTCCCAAATCA	CTGTTAGGGTTTGGCATTGG	237
2028	CAATAGATGGGGAGCCCTTT	AATCCTGCTCAACCGAGAGA	236
2029	TGTTGCACTACCGTGCATTT	GAGCCGACACATACCGAGAT	231
2030	CCACTGGAACGGGAATTTTT	TTTACCTCTCTCCGGTGCTC	223
2031	CGTGGTATGGTCACTTGCTG	GGAGAGACAATCCCACAACC	223
2032	CATAGGGGCCGAATTTTTCT	CTCCAAGCTTTCTTCCAACG	205
2033	CGATCTTGCCAACTTTGGTT	AATGGCAGGCTCAGTTGTCT	205
2034	AGTACGCCAGCGAAGAAGAA	GCCCATGCTTTTTAAGTCCA	199
2035	AAGACCATTGGGCACGTTAC	CGTACTCCACACTTGCCAAA	198
2036	TCATCTTGGCCCATTTTCTC	GGTGGCGACTTGAAATGAGT	194
2037	TGTCATCCCAAAGCAATGAA	TAGAAATCGCGGAGGTTGTT	187
2038	TCCGTGTAATATCGATTGGTT	ACGAAATTAACTGCCCAACG	186
2039	ACCGCGTACTGGTAACTCGT	CACCGTCGCTCCCAAACTA	181
2040	AATTGACGTTCATGTGGGTTC	CACGGGAACCATGTATGTTG	153

b) PCR Amplifications of Chloroplast/ Mitochondria microsattelite of *A.vogelii* genomic DNA

The total number of 16 primers (Table 8) targeting chroloplast and mitochondria DNA were used to determine polymorphism among isolates of *A.vogelii*. Initially they were tested on 23 isolates to access the genetic diversity of *A.vogelii* collected from different places. The PCR master mix was prepared with a reaction volume of 26 µl, it contained 10X PCR buffer, water , 1µM of each primer, 1mM of each dNTPs, 0.5U/ µl TaqDNA

polymerase and 50 ng DNA. PCR amplification and electrophoresis was done as described on SSRs markers for *S.hermothica*

c) Digestion of mitochondrial microsattelites of *A.vogelii* DNA with four base cutter enzymes

The mtDNA PCR products were digested with *Mse*I, *Bcc*I, *Alu*I and *Msp*I enzymes. The aim of 4 base cutter enzymes is to cut DNA sample at or near specific recognition nucleotide sequences known as restriction sites. It recognizes a specific sequence of nucleotides of DNA. The differences and similarities of different samples can be observed. This method was used after the failure of tested markers to show the differences and similalities of *A.vogelii* isolates.

The master mix was composed of 12 µl of water, 2 µl of 10X buffer (NEB 2 buffer for *Mse*I, *Msp*I and *Alu*I enzymes and NEB 1 buffer for *Bcc*I),enzymes and 0.25 µl BSA(100X) for *Bcc*I and *Mse*I enzymes, 5µl of the PCR product. Then the mixture was incubated for 12 hours at 37^0C, the digested sample was removed from incubator and added 15 µl loading dye, then the digested products were resolved on 0.8% agarose gel and ran at 176V. The gel was photographed using Canon EOS Rebel Tzi and Bio-Rad Molecular Imager® Gel DOCTMXR+ with image LabTM software under UV transilluminator.

Table 8: Description of 16 pairs of cpDNA and mtDNA primers used to amplify chloplast and mitochondria DNAfrom *A. vogelii*

Forward primers	Sequence for forward primer	Reverse primer	Sequence for reverse primer	Optimal annealing temperature(^{0}C)	Expected fragment size (Bp)	Reference
CP primer						
trnK2	TAA AAG CCG AGT ACT CTA CCG TTG	trnQ	CTA TTC GGA GGT TCG AAT CCT TCC	47.5	3075	Dumolin,1997
trnQ	GGG ACG GAA GGA TTC GAA CC	trnRr	ATT GCG TCC AAT AGG ATT TGA A	56.5	3086	#
rpoC1	CGA CAA ATT CCR CTT TTT ATR GG	trnCr	CGA CAC CCR GAT TTG AAC TGG	47.5	4795	#
trnT	GCC CTT TTA ACT CAG TGG TA	psbCr	GAG CTT GAG AAG CTT CTG GT	52.5	3236	#
trnfM	GAA CCC GTG ACC TCA AGG TTA TG	psaAr	ATT CGT TCG CCG GAA CCA GAA GT	47.5	5108	#
trnF	CTC GTG TCA CCA GTT CAA AT	trnVr	CCG AGA AGG TCT ACG GTT CG	57.5	3492	#
trnV	CGA ACC GTA GAC CTT CTC GG	rbcLr	GCT TTA GTC TCT GTT TGT GG	57.5	3850	#
Mt primers						
cox2/1	TTT TCT TCC TCA TTC TKA TTT	cox2/2r	CCA CTC TAT TGT CCA CTT CTA	50	378	Dumolin, 1997
nad 1/4	GCC AAT ATG ATC TTA ATG AG	nad1/5r	TCA CCT TGA TAC TAA ACC AG	47	3264	#
nad4/2[c]	CTC CTC AGT AGC CCA TAT GA	nad4/3r	AAC CAG TCC ATG ACT TAA CA	55	2722	#
nad4/3[c]	GGA GCT TTC CAA AGA AAT AG	nad4/4r	GCC ATG TTG CAC TAA GTT AC	57	1011	#
nad5/1	TTT TTT CGG ACG TTT TCT AG	nad5/2r	TTT GGC CAA GTA TCC TAC AA	57	2258	#
nad5/4	CCA ATT TTT GGG CCA ATT CC	nad5/5r	CAT TGC AAA GGC ATA ATG AT	47	1349	#
nad7/1	ACC TCA ACA TCC TGC TGC TC	nad7/2r	CGA TCA GAA TAA GGT AAA GC	47	1064	#
nad7/2	GCT TTA CCT TAT TCT GAT CG	nad7/3r	TGT TCT TGG GCC ATC ATA GA	57	1508	#
nad 7/3	TCT ATG ATG GCC CAA GAA CA	nad7/4r	ACA CCA AAT TCT CCT TTA GG	47	2546	#

d) RB (Rice bean) SSR primers used for *A.vogelii* DNA amplification

The total numbers of 48 RB (Rice Bean) primers were used to amplify the genomic DNA of *A.vogelii,* the purpose was to find out wether these primers will work on *A.vogelii* by giving good polymorphic band due to failure of other primers to work on *A.vogelii*. Initially the RB primers were tested to access the genetic diversity of the collected *A.vogelii* individuals and then screened for its polymorphism. PCR master mix was prepared with a reaction volume of 25.5 µl, it contained 2.5 µl 10X PCR buffer, sterile water, 1 µM of each primer, 1mM of each dNTPs, 0.5U/ µl *Taq*DNA polymerase and 50 ng DNA. PCR amplification and gel electrophoresis was done as described on *S. hermothic*a primers.

e) ISSR (Inter simple sequence repeats) primers used for *A.vogelii* DNA amplification

ISSR primers were purchased from the University of British Columbia, Nucleic Acid Protein Service Unit, Vancouver , BC (Table 9). Since most of primers seem not to work well on *A.vogelii*, ISSR primers were also tested to *A.vogelii* for good polymorphism. Eleven set of ISSRs primers (808, 809, 811, 835, 836, 840, 857, 873, 876, 879 and 880) were used to test for possible amplification of DNA fragements from extracted genomic DNA of 29 populations of *A. vogelii*. PCR master mix was prepared with a reaction volume of 25µl; it contained 2.5 µl 10X PCR buffer, distilled water, 1 µM of each primer, 1mM of each dNTPs, 0.5U/µl*Taq*DNA polymerase and 50 ng DNA. PCR amplification and gel electrophoresis was done as described on *S.hermothic*a primers.

Table 9: The sequence and annealing temperature descriptions of ISSR primers used during PCR reactions of *A.vogelii* DNA amplification

Primer name	Sequence	Annealing temperature(^{0}c)
808	AGA GAG AGA GAG AGA GC	45
809	AGA GAG AGA GAG AGA GG	45
811	GAG AGA GAG AGA GAG AC	45
835	AGA GAG AGA GAG AGA GYC	45
836	AGA GAG AGA GAG AGA GYC	45
840	GAG AGA GAG AGA GAG AYT	45
847	CAC ACA CAC ACA CAC ARC	45
873	GAC AGA CAG ACA GAC A	45
876	GAT AGA TAG ACA GAC A	45
879	CTT CAC TTC ACT TCA	45
880	GGA GAG GAG AGG AGA	45

f) *Striga gesneroides* SSR's primers used during *A.vogelii* DNA amplification

The SSR markers developed for *S.gesnerioides* were used to characterize *A. vogelii.* The purpose of using these primers was to target the SSRs regions on *A.vogelii* because there are no specific primers developed for *A.vogelii*. For this reason, more different primers were to be tested to obtain the genetic information of *A.vogelii* without succeses. The microsatellite markers were tested to access the genetic diversity of the collected *Alectra* individuals. PCR master mix was prepared with a reaction volume of 25 µl, it contained 2.5 µl 10X PCR buffer, distilled water, 1 µM of each primer, 1mM of each dNTPs, 0.5U/µl TaqDNA polymerase and 50 ng DNA. PCR amplification and gel electrophoresis was done as described on *S. hermothic*a primers.

3.3.2 Determining differential response of individual *A.vogelii* growth on different cowpea host varieties to assess host specificity and host range

The experiment consisted of two pots infestation per sample. The total of 23 isolates of *A.vogelii* was grown with cowpea varieties. Each isolate was grown with 11 cowpea varieties; only 16 isolates of *A.vogelii* collected from Tanzania were infested to IT99K-7-21-2-2-1line due to presence smaller amount of seeds, selection of isolate based on the aggressiveness of the *A.vogelii*, the one which was more aggressive was infested on IT99K-7-21-2-2-1line. The experiment was large that the conditioned room was not able to accommodate more pots, so two pots per sample were used. The total of 492 pots were filled with sand mixed with with Canadian Sphagnum peat moss, vermiculite, coarse perlite, starter nutrient charge and dolomitic limestone, then approximately 500 *A.vogelii* seeds were infested in the prepared pots, 3 seeds of cowpea were sown in the same inoculated pots at approximately 5cm deep. The plants were watered three times per week for 10 weeks. Clear follow-up on emergence of *A.vogelii,* number of *Alectra* plants emerged were counted from each pot in the 2nd, 4th 6th, 8th and 10th week from planting. Then the number of unemerged was counted in the 12th week.

3.3.3 Determining resistance/susceptibility of pulse crops to *A. vogelii* variants from Tanzania and Malawi.

The experiment was done in the conditioned room under quarantine and required condition for host and parasite growth. The experiment was too large that the conditioned room was not able to accommodate more pots, this lad to set experiment without using appropriate design as explained below. Each 21 *A.vogelii* isolates was infested to bambara groundnuts, peanuts and soybeans which was collected from farmers fields, these was local varieties and was not known by their names.

Each *A.vogelii* isolate was infested on half a liter two pots for each legume used, that made total of 126 pots. These pots were field with sand mixed with Metro-Mix®250, common name is Grace-Sierra (Formulated with Canadian Sphagnum peat moss, vermiculite, coarse perlite, starter nutrient charge (with Gypsum) and dolomite limestone)(Scotts-Sierra Horticultural Product Company, Marysville, OH).

The 21 different isolates were used; one spatula containing 500 seeds were infested in the two pots containing 3 seeds of bambara groundnuts. The same thing was done to soybeans as well as groundnuts; the pots were watered three times per week for 10weeks. The numbers of *Alectra* were counted in the 6th week, 8th week and 10th week. At 12th week the number of attachment was counted.

3.4 Data analysis

Objective 1, the polyacrylamide gel pictures were scored, according to the presence or absence of the band, presence of band was scored as 1 and absence of band was scored as 0. The data was then scored, and data was analyzed by using NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) version 2.0 to form the genetic diversity tree/ dendogram which lay to clustering of *A.vogelii* isolates into groups according to their similarities and differences. Objective 1 and 3, there was no replications, no statistical analysis was done, the data was scored as absent (A), early (E), medium (M) and late (L) emergence of *A. vogelii* plants. Mean was calculated on excel and graph was drawn to show *A.vogelii* isolates growth on cowpea varieties/ lines and different bambara groundnuts, groundnuts and soybean.

CHAPTER FOUR

4.0 RESULTS

4.1 PCR Amplification and polymorphific bands

A total of 240 primers from *S. hermothica*, *S. gesnerioides*, RB, cpDNA and mtDNA, and ISSRs screened for polymorphism (Table 9). Only 26(22%) *S. hermothica* primers out of 120 primers were able to amplify the genomic regions of *A.vogelii* DNA and only 7 *S. hermothica* primers (27%) out of 26 primers amplified the genomic regions showed polymorphic bands. Also 8 *S.gesnerioides* (17%) out of 45 primers amplified the genomic region, no primer showed the polymorphic bands. 18 RB primers (40)% amplified the SSRs on genomic DNA of *A.vogelii* and only 8 primers(44%) out of 18 primers amplified the genomic DNA were able to show the polymorphism. 16 chloroplast and mitochondria primers tested, only 4 mitochondria primers out of screened primers did amplify the genomic DNA where by nad5/4 produced 1600bp, nad7/1 produced 1000bp, nad7/2 produced 1500bp and nad7/3 produced 1700bp and none of them showed polymorphic bands. Since mitochondria primers didn't show any polymorphism, the monomorphic PCR products digested with 4 base cutter enzymes (*Mse*I, *Bcc*I, *Alu*I and *Msp*I) (Table 14) also didnot produce polymorphic bands. Lastly 9 ISSR (82%) primers out of 11 primers tested were able to amplify the genomic DNA and all 9 primers showed polymorphic bands, but they were inconsistency, this is due to lower annealing temperature which means lower stringent primer annealing conditions is the one lead to inconsistency, no scoring of bands were done.

Table 10: The number of PCR Amplification and polymorphism of the bands produced when genomic DNA of *A.vogelii* was ran into PCR machine

primers	Primers Screened	No. of Primers Amplified the genomic regions	Primers showed polymorphism
Striga hermorthica	120	26(22%)	7(27%)
Striga gesnerioides	48	8(17%)	0(0%)
Rice Bean primers	45	18(40%)	8(44%)
CP&Mt primers	16	4(25%)	0(0%)
ISSR primers	11	9(82%)	9(100%)

4.2 Polymorphic bands scored when *S. hermothica* primers were used to amplify the *A.vogelii* DNA

Among the 120 primers developed for *S.hermothica,* only 7 primers (1007, 1025, 1031, 1032, 1045, 1055 and 1061) showed polymorphic bands. In general the scored number of bands obtained, that showed polymorphism ranges from 4-12. The list of *S.hermothica* primers that showed polymorphic SSRs regions on *A.vogelii* DNA samples are shown on (Table 11).

Table 11: Number of Polymorphic bands scored when *S.hermothica* primers used

Primer code	Forward primer (5-3')	reverse primer (5-3')	No. of bands
1007	CTAGGCTCGCCATTGTTGTT,	CAAACCCGTCTCACACACAC,	8
1025	CAAAGCTCGAATGAGAACTGA,	CCATGTCTAGCTGACCCACA,	4
1031	CGGTTCCATATGTGTGTGTG,	GCTCGGGAGGGTGTGTTAT,	12
1032	TATCGAGTCGGGAAAGATGC,	CATTCCACACCCACTACACG,	4
1045	TTCTGCCAAACTGTGAGTGC,	TCTTATTTTGGGGGAGCTGA,	5
1055	CCGGGAGAAGTGTCTGGTAA,	CAAACACGTCTTCCATGGTG,	10
1061	GATTCTGCCTGGTCCACAAT,	TGCTTCCATTCCAAAACACA,	5

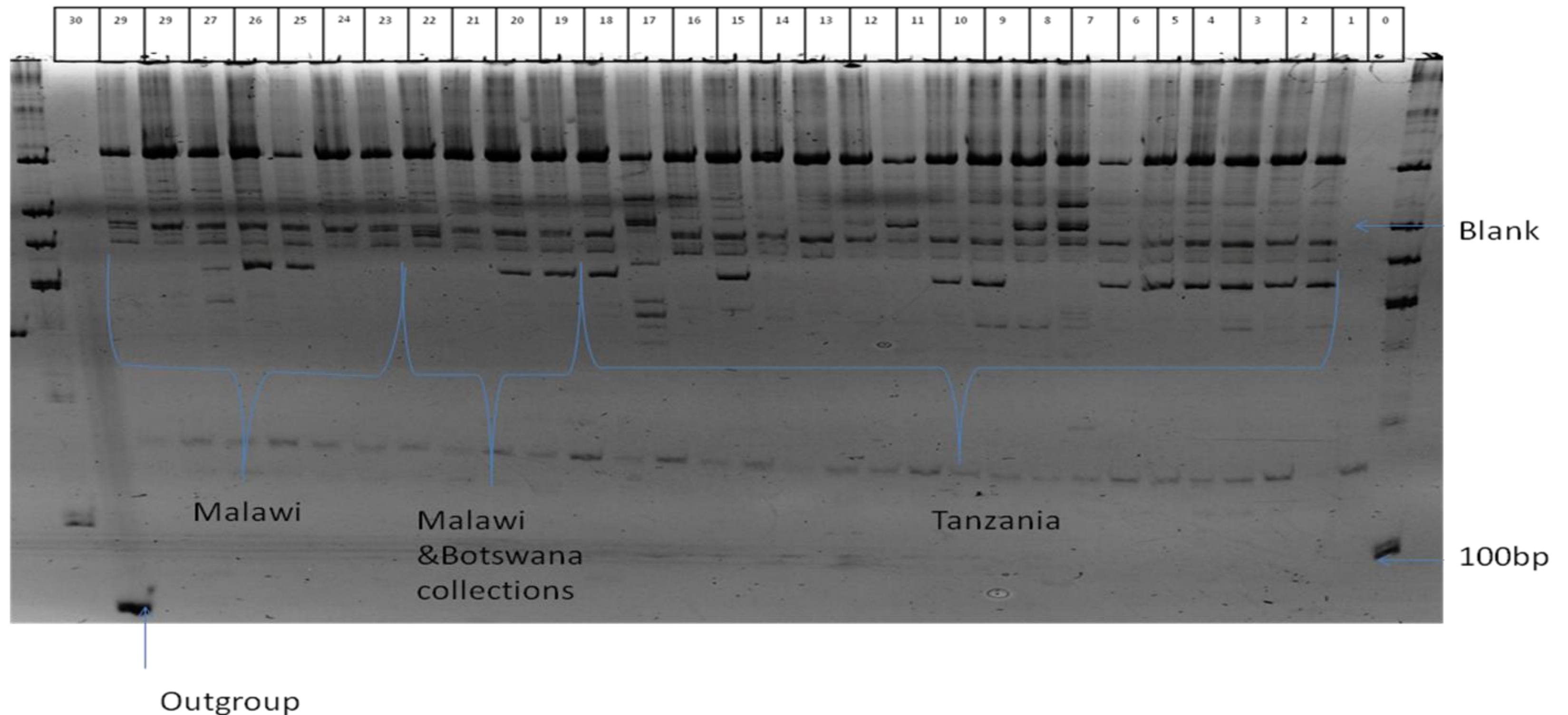


Figure 4: **Polyacrylamide gel showing polymorphic bands produced from PCR amplification of SSR primers from *Striga hermothica*.** From right, the first lane was blank, lanes1-29 was *A.vogelii* amplified bands with primer 1055 and lane # 30 was *S. gesnerioides* amplified bands with primer 1055.Gel is presenting PCR amplification of *A. vogelii* and *S. Gesneriodes*(Outgroup) DNA using the SSR 1055 Primer from *S.hermothica.*

4.3 Polymorphic bands obtained from SSR Rice Bean (RB) primers amplifications on *A.vogelii* DNA

The total numbers of 45 RB primers screened for its polymorphism on *A.vogelii* genomic DNA. Out of 45 markers, only 8 primers were able to amplify and show polymorphic bands. The scored number of band amplified showed polymorphism ranges from 2-6 as shown on (Table 11).

Table 12: Number of Polymorphic bands obtained from Rice Bean (RB) SSRs primers amplificationson *A.vogelii* DNA

Primer code	Bands scored
RB 5	4
RB 6	5
RB 7	2
RB 8	4
RB 11	4
RB 14	6
RB21	2
RB 40	5

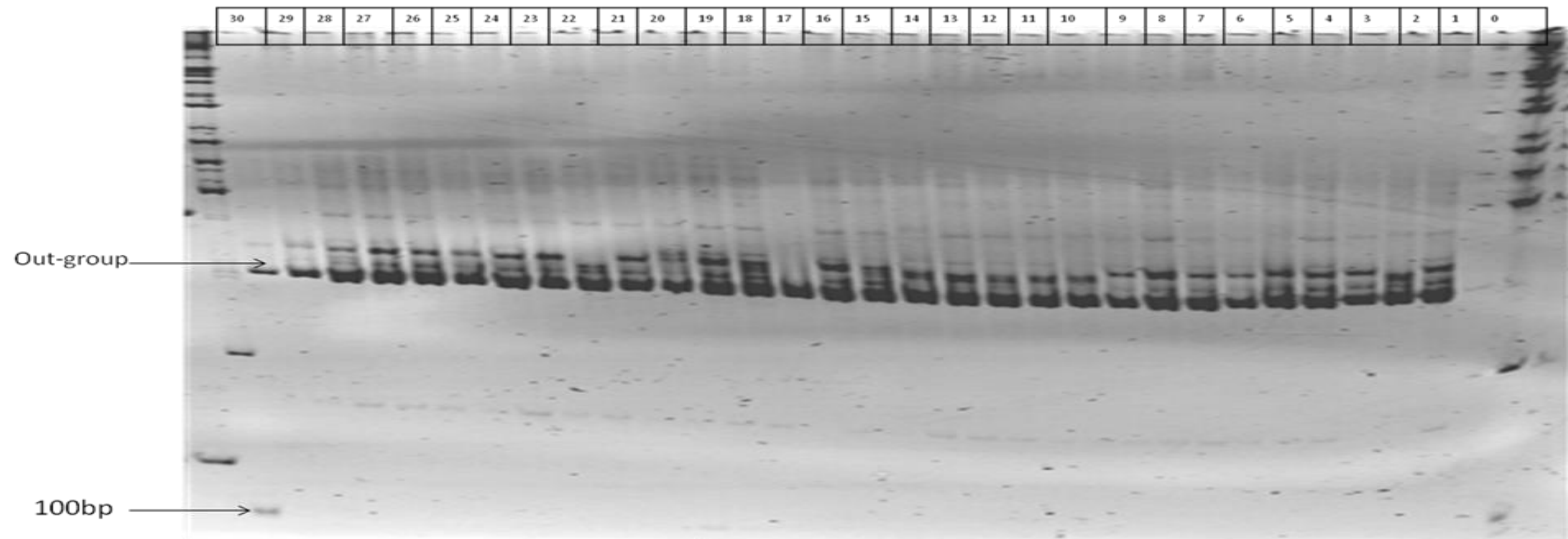


Figure 5: **Polyacrylamide gel showing polymorphic band produced by PCR.**

The gel plate is the polymorphic bands amplified amplified by RB 14 primer. From right, the first lane was blank, lanes1-29 was *A.vogelii* accessions from different areas amplified bands with RB 14 and lane 30 was *S. gesnerioides* which is the outgroup amplified bands with RB 14.

4.4 Mitochondria microsatellites DNA scored during amplification of *A.vogelii* genomic DNA

A total of 16 Mitochondria and chloroplast primers were screened, out of 16 primers only 4 mitochondria primers amplified the *A.vogelii* genomic region. Each primer amplified single band with different size (Table 13). nad5/4, nad7/1, nad7/2 and nad7/3 produce non-polymorphic amplifiable DNA fragments with 1600, 1000, 1500 and 1700bp respectively from each of the accessions when used. No polymorphism was produced on *A.vogelii* DNA samples when these primers were used during PCR reaction (Fig 6).

Table 13: Number and size of mitochondria microsatellites DNA bands scored during amplification of *A.vogelii* genomic DNA

Primer code	Number of bands	Size of band(bp)
nad5/4	1	1600
nad7/1	1	1000,1325
nad7/2	1	1500
nad7/3	1	1700

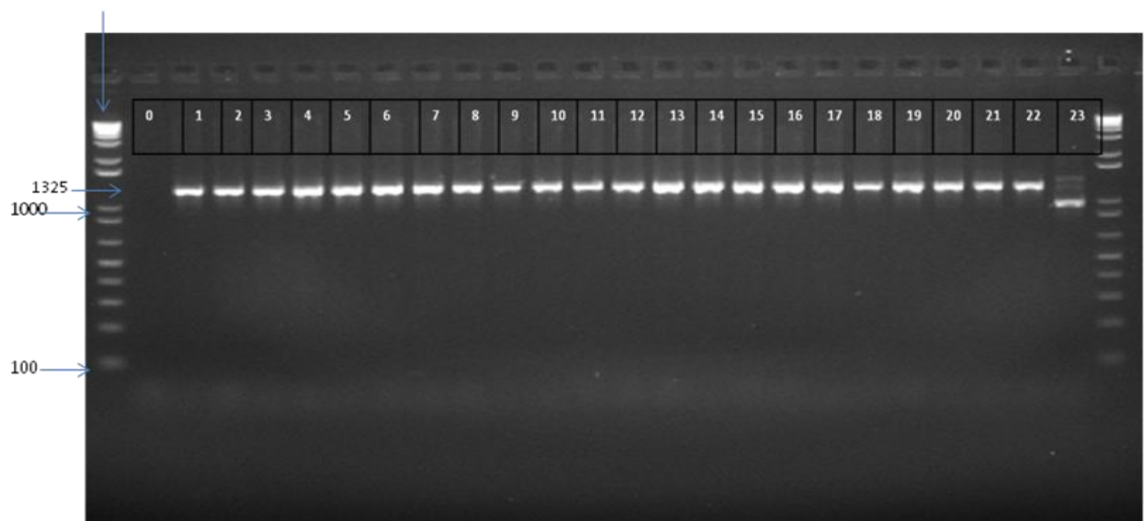


Figure 6: 0.8% Agarose gel showing monomorphic band produced by PCR amplification of mitochondria primers. Lane 0: blank, lanes1-22:*A.vogelii* amplified bands with primers nad7/1 and lane 23: *S. gesnerioides* as the outgroupamplified with primers nad7/1

4.5 Digestion of mitochondria microsatellite PCR products of *A.vogelii* genomic DNA digested by 4 base cutter enzymes

The PCR products of nad5/4, nad7/1 and nad7/2(mitochondria microsatellites) digested with four base cutter enzymes and thus restriction digestion of PCR products using enzymes on mtSSR produces variable information with number of restriction sites giving different sizes of DNA fragements (Table 14). Digestion with the 4 base cutter enzymes used did no tproduce polymorphic bands.

Table 14: Number of bands of mitochondria microsatellite PCR products of *A.vogelii* DNA after digestion with 4 base cutter enzymes

Primer code	Number of bands			
	Mse*I*	**Bcc*I***	**Alu*I***	**Msp*I***
nad5/4	**5**	**4**	**7**	**2**
nad7/1	**1**	**1**	**1**	**2**
nad7/2	**1**	**1**	**5**	**5**

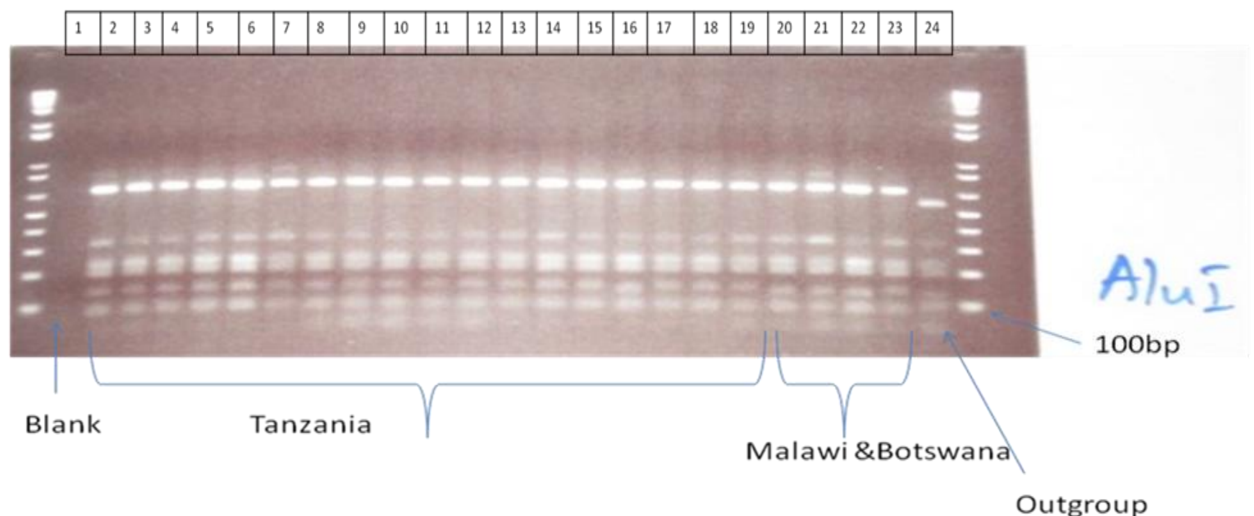


Figure 7: 0.8% Agarose gel showing monomorphic band produced by digestion of PCR amplification of mitochondria primers with Alu*I* enzyme.

4.6 Genetic diversity of *A.vogelii* isolates collected from Tanzania, Malawi and Botswana generated by NTSYS program

The tree diagram (pg 39) for genetic diversity of *A. vogelii* shows the collection of isolates from Tanzania, Malawi and other isolates from Botswana. *S.gesnerioides* was used as outgroup in this genetic evaluation. The tree was generated by using 15 primers out of 240 screened for genetic polymorphism and ability for amplification.

The primers used to discriminate the *A.vogelii* isolates were 8 RB primers (RB5, RB6, RB7, RB8, RB11, RB14, RB21 and RB40) and 7 *S.hermothica* SSR markers (1007, 1025, 1031, 1032, 1045, 1055 and 1061) were targeted to *A.vogelii*. The coeficients to show the distant relationship between the individuals ranges from 0.49 to 0.96.

The genetic diversity was grouped into six groups focusing on their similarities and differences.

The first group comprising of the *A.vogelii* collected from sunflower at Ikhanoda village in Singida region was quite different from the rest of the *A.vogelii* collected from other parts of Malawi and Tanzania.

The second group (Figure 7) demonstrated 84% similarity of *A.vogelii* collected from cowpea at Usuka village in njombe region, sunflower and bambara groundnuts collection from Mkungugu village in Iringa region and *A.vogelii* collected from groundnuts farms at Chioshya village from Malawi, in addition the *A.vogelii* collected from Bambara groundnuts and sunflower at Mkugungu village was similar by 92%.

The third group contains *A.vogelii* isolates collected from cowpeas in all villages. The *A.vogelii* from Malawi-2002 collection, Msungua village in Singida region, Nkulabi and

Bihawana from Dodoma region, Utiga from Njombe region and Ilambilole from Iringa region were grouped together.

The fourth group was the one collected from Lusangazi village in Malawi, Mkungugu in cowpea from Tanzania in Iringa region, Ismani, Mangalali, Ghathuma from Botswana, Nkwinda from cowpea in Malawi, Sebele (Botswana) and Marajone (Botswana).

The fifth group comprising the *A.vogelii* collected from Utegi, Mayale and Ilonga, both villages are from Tanzania.

The last is group sixth contain the *A.vogelii* from Bwanje(Malawi), Malawi collection 2008, Nkwinda from groundnuts (Malawi), zombwe (Malawi), Hombolo (Tanzania), Mpunguzi (Tanzania) and Kikombo (Tanzania).

4.7 Geographical distribution of *A.vogelii* generated by markers

According to marker results, there were six groups of *A.vogelii* distribution in Tanzania and Malawi as indicated on Fig.8. Few selected markers were able to indicate the geographical distribution of *A.vogelii* in Tanzania and Malawi.

According to this study *A.vogelii* do not differ according to distance of collected areas, but two distant locations can have similar genetic make up. Some nearer locations were observed to have the same genetical characteristics. Fig.8 shows how the *Alectra* are distributed in selected parts of Tanzania and Malawi. The same colours show that the *A.vogelii* isolates are related.

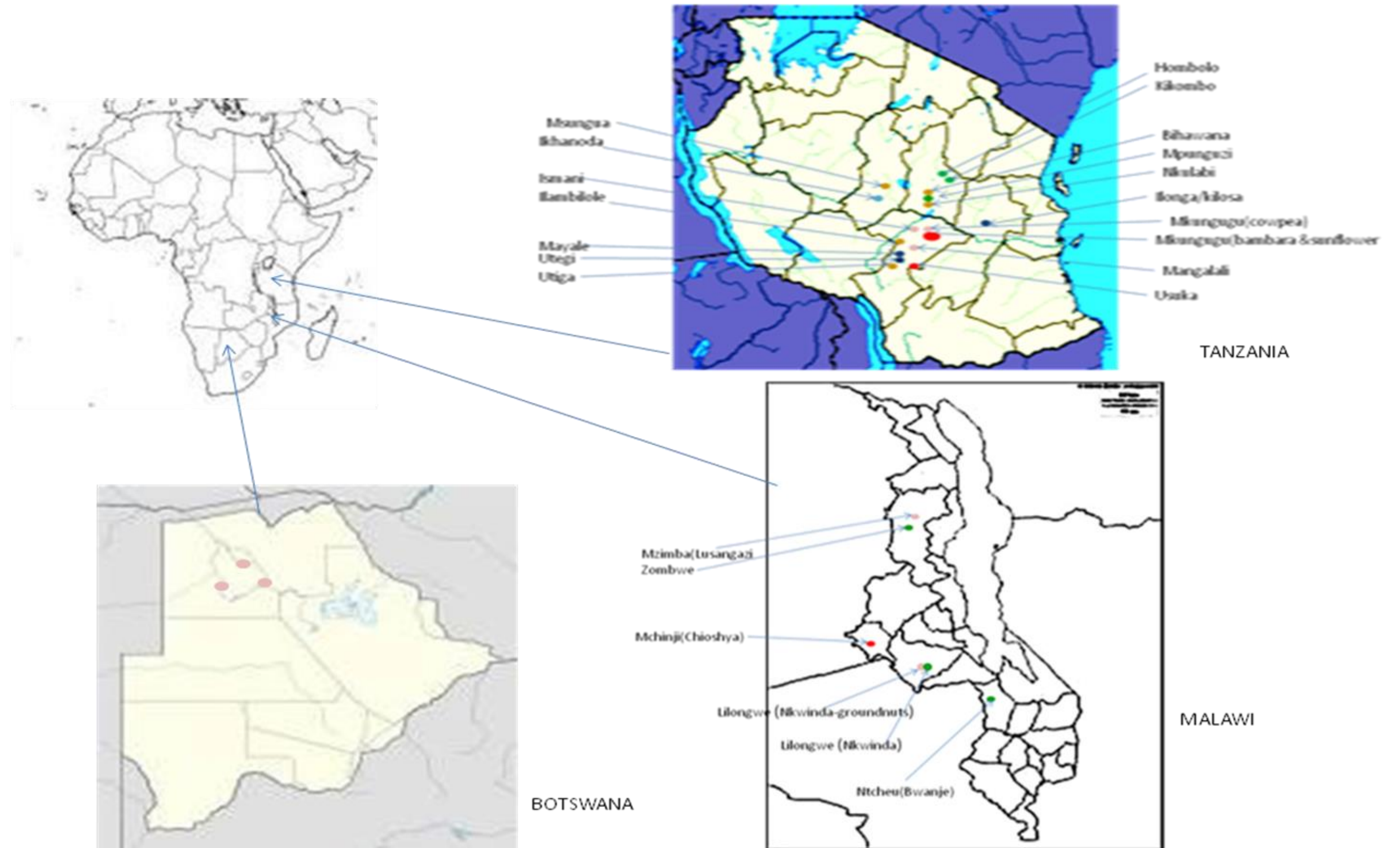


Figure 8: Map of diversity groups generated in by markers work of *A.vogelii* collection sites

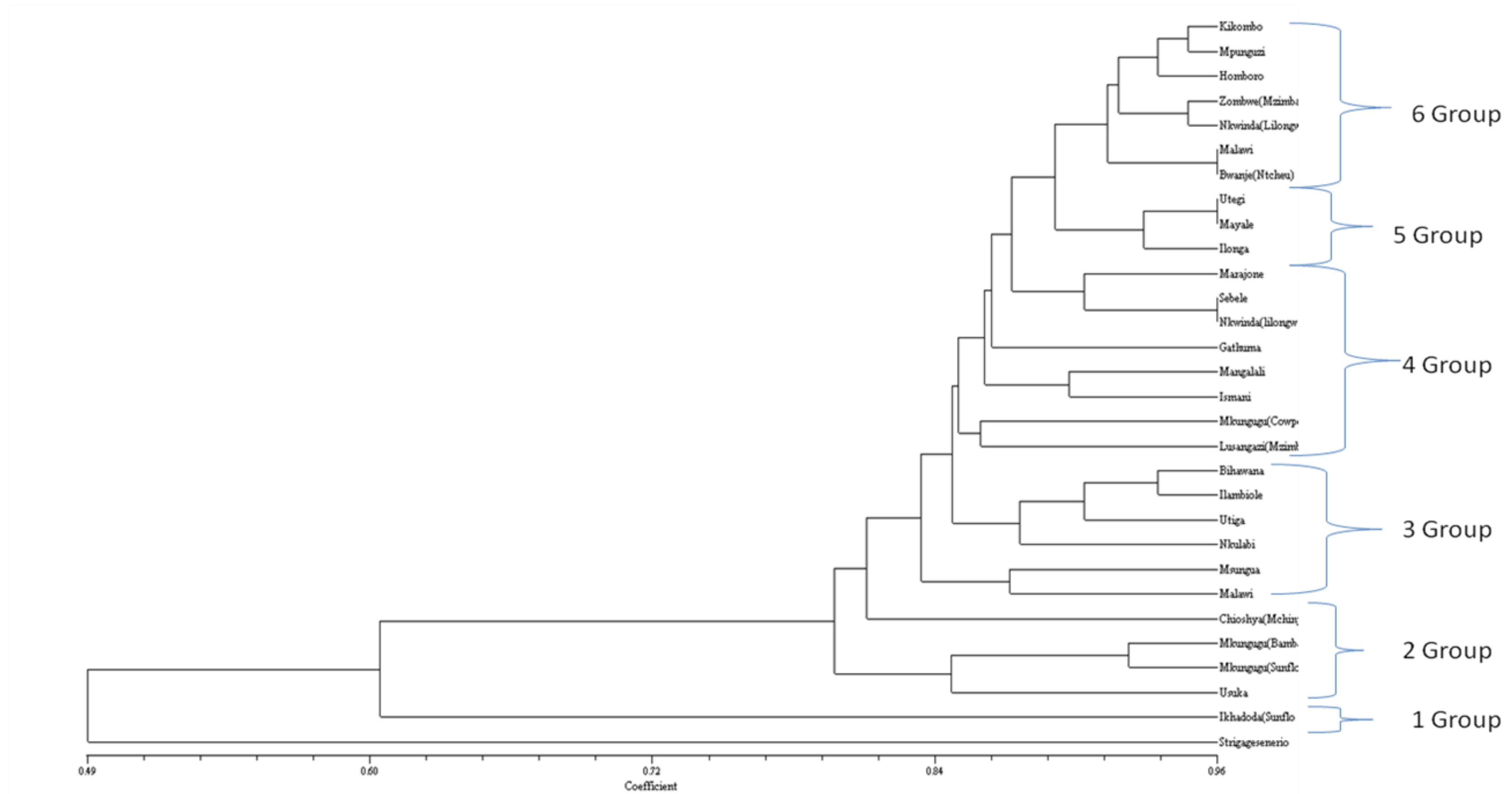


Figure 9: An UPGMA cluster dendrogram showing the genetic relationships among 29 *A.vogelii* isolates from Tanzania, Malawi and Botswana, developed from 15 SSRs molecular data

4.8 Response of individual *A.vogelii* growth on different cowpea host varieties

A total of 5 cowpea varieties and 6 lines were used for determination of differential response of individuals. Of 5 cowpea varieties and 6 lines, only IT99K-7-21-2-2-1 and B301 supported few isolates from *A.vogelii*. IT99K-7-21-2-2-1 supports only 4 *A.vogelii* isolates and B301 supports only 2 *A.vogelii o*ut of 16 and 23 isolates of *A.vogelii* used in the experiment respectively. The data are shown in Figure 12 and Figure 18 respectively. Vuli 1, Vuli 2, Tumaini, IT00K-1263 and IT82E-16 were highly susceptible to *A.vogelii* as indicated by high emergence from 6th week (Figs. 11,13,14,20 and 21) respectively. TZA 263, IT99K-1122, Bunda 1 and IT99K-573-1-1 cowpeas succumbed to approximately 12 isolates out of the 23 *A.vogelii* used in the screening experiment (Figures: 15,16,17and 19).

Plate 1: *A.vogelii* attachment on cowpea roots as observed on the 6th week from inoculation. The arrow shows where the emerging shoots of *A. vogelii* have been attached.

4.8.1 Emerged and un-emerged *A. Vogelii* on the cowpea hosts

The total numbers of 11 cowpea variety/lines were screened for *A.vogelii* collected from Tanzania and Malawi (Table 16). The number of *A.vogelii* isolations emerged and those didn't emerge on all cowpea varieties/lines are indicated on Table (16).

The time of emergence ranges from no emergenge to late emergence and the remark shows the overall time of *A. vogelii* isolates emergence on a particular variety/line, the results are shown on table (16).

Table 15: Number of emergence and un-emerged *A. Vogelii* on the cowpea hosts

Variety/Line	No. of *A. vogelii* isolation emerged/ Un-emerged		*A.vogelii* emergence on host(Absent(A) early(E), medium(M) and late(L) emergence)																							Remaks
	Emerged	Not emerged	Mkungugu(Cowpea)	Mkungugu(sunflower)	Utegi	Kikombo	Mpunguzi	Msungua	Nkulabi	Mkungungu(bambara)	Usuka	mangalali	bihawana	Utiga	kilosa	Ikhanoda	Mayale	Hombolo	Bwanje(Ntcheu)	Lusangazi(Mzimba)	Zombwe(mzimba)	Nkwinda 1(Lilongwe)	Nkwinda 2(Lilongwe)	mbawa	Chioshya(mchinji)	
Vuli 1	18	5	E	E	E	E	E	A	E	E	E	E	M	E	A	L	E	E	E	A	E	A	E	A	E	E
IT99K-7-21-2-2-1	4	12	A	A	A	A	E	A	L	L	A	A	A	A	A	A	L	A	-	-	-	-	-	-	-	L
VULI 2	21	2	E	M	L	M	M	E	E	E	E	E	E	M	A	M	E	E	E	M	L	M	L	M	M	E/M
TUMAINI	21	2	M	E	E	E	M	E	E	E	E	E	E	E	A	M	A	E	E	E	E	E	E	E	E	E
TZA 263	12	11	A	A	A	L	A	M	M	E	E	E	A	E	A	A	E	A	A	E	A	A	L	E	A	A/E
IT99K-1122	10	13	A	A	L	L	A	A	A	E	A	A	L	A	A	E	E	A	E	E	A	M	M	A	A	E
BUNDA 1	12	11	A	A	L	L	L	M	A	A	A	A	A	E	A	A	E	L	L	E	A	A	E	A	M	A/E
B301	2	21	A	A	L	A	A	A	A	A	A	A	A	A	A	A	E	A	A	A	A	A	A	A	A	L
IT99K-573-1-1	14	9	A	A	L	L	A	A	A	M	E	M	L	L	A	A	M	E	M	E	M	E	A	L	A	A/L
IT00K-1263	1	22	E	E	E	E	E	E	E	E	E	E	E	E	A	M	L	L	E	E	E	E	E	E	M	E
IT82E-16	1	22	E	M	E	L	E	E	A	E	M	E	M	L	E	E	E	E	E	M	L	E	E	E	E	E

4.8.2 Number of emerged and un-emerged *A.vogelii*on Vuli 1 cowpea variety

The number of *A.vogelii* emerged on Vuli 1 cowpea variety was high ranging from 0 to 27 *A.vogelii*/pot. Specifically, the *A.vogelii* isolates collected from Mkungugu in cowpea, mbambara groundnuts and sunflower, from Kikombo, Nkulabi, Usuka, Mangalali, Utiga, Mayale, Hombolo, Bwanje, Zombwe, Nkwinda in cowpea and Chioshya was observed early at 6th weeks from planting, but the most aggressive *A.vogelii* was the one collected from Mkungugu in cowpea, Utiga and Mayale in which their number of *A.vogelii* ranges from 9-14/pot at 6th week while others was below 5 *A.vogelii*/pot. The *A.vogelii* collected from Bihawana emerged was observed at 8thweeks from planting. The one from Ikhanoda emerged on 10th week from planting while that collected from Msungua, Kilosa, Lusangazi and Mbawa villages didn't emerged on vuli 1 variety. The 16 *A. vogeli* isolates showed early emergence at 6th week from planting, few of them showed late or no emergence.Variety Vuli 1 cowpea variety was susceptible many to *A.vogelii* isolates (Fig. 10)

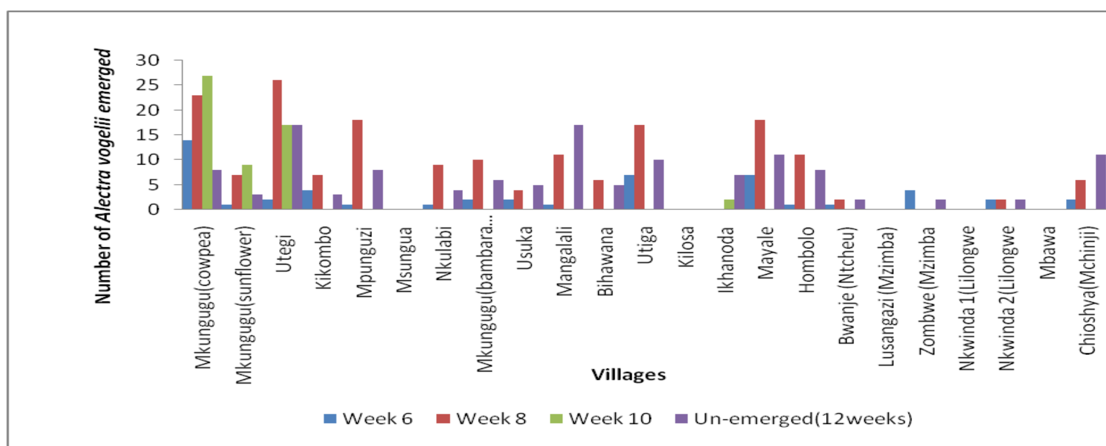


Figure 10: Number of emerged and un-emerged *A.vogelii* as observed on Vuli 1 cowpea variety over a period of 12 weeks.

Scale used : 1cm =5 number of *A.vogelii.* **Note**: Nkwinda 1(lilongwe) on graph is area of *A.vogelii* isolates from Groundnuts,Nkwinda 2(lilongwe) on graph is area of *A.vogelii* collection from cowpeas.

4.8.3 Number of emerged and un-emerged *A.vogelii* on IT99K-21-2-2-1 cowpea line

The number of emerged *A.vogelii* observed on IT99K-21-2-2-1 cowpea line ranges from 0-2 A.*vogelii*/pot**.** The *A.vogelii* isolates collected from Mpunguzi was observed at 6th week from planting, the one collected from Nkulabi, Mkungugu in bambara groundnuts and that of Mayale villages showed the nodules at 12th week from planting. The most aggressive isolate on IT99K-21-2-2-1 cowpea line was that from Mpunguzi, where by it showed early emergence, though the number of *A.vogelii* emerged was 2/pot. The rest showed late emergence and most of all infestation *A.vogelii* did not emerge. For this reason this is resistant line to *A.vogelii* isolations. see fig 11 below.

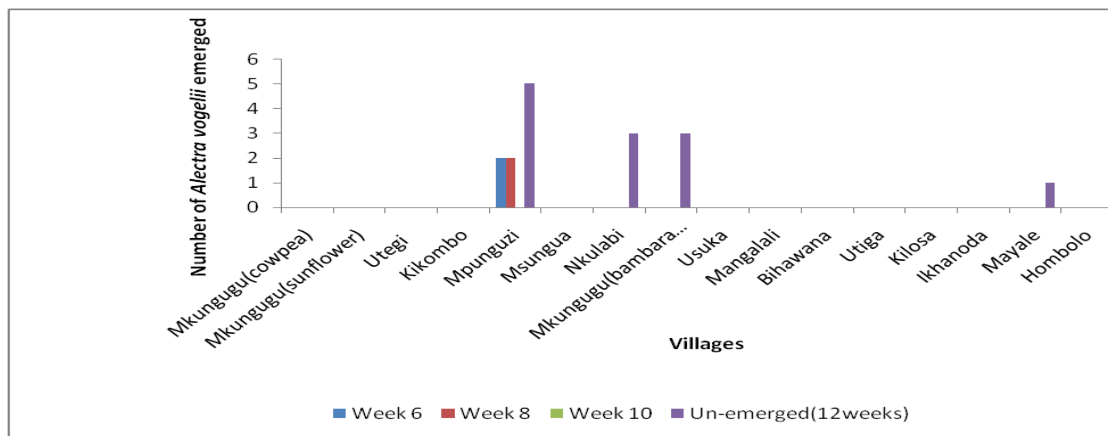


Figure 11: Number of emerged and unemerged *A.vogelii* as observed on IT99K-21-2-2-1 cowpea line over a period of 12 weeks.

Scale used : 1cm =1 number of *A.vogelii*. **Note**: Nkwinda 1(lilongwe) and Nkwinda 2(lilongwe) villages refer fig 10.

4.8.4 Number of emergence and un-emerged *A.vogelii* on Vuli 2 cowpea variety

The *A.vogelii* isolates collected from Mkungugu in cowpea and bambara, Msungua, Nkulabi, Usuka, Mangalali, Bihawana, Mayale, Hombolo and Bwanje villages showed early emergence which is 6th week from planting.

The isolates collected from Mkungugu in sunflower, Kikombo, Mpunguzi,Utiga, Ikhanoda, Lusangazi, Nkwinda from groundnuts, Mbawa and Chioshya emerged at 8th week from planting while that of Zombwe and Nkwinda from cowpea showed late emergence which was 10th week from planting. The *A.vogelii* collected from Utegi and Kilosa did not emerge in this variety. Most of *A. vogelii* collected from all villages emerged at 6th and 8th week from planting, few of them emerged at 10th week and 12th week. The most aggressive *A.vogelii* are the one collected from Tanzania especially that of usuka, Mayale and Hombolo which had more than 20 *A.vogelii*/pot, the one from Malawi was not aggressive compared to Tanzania isolates. According to results this variety is susceptible. see fig 12

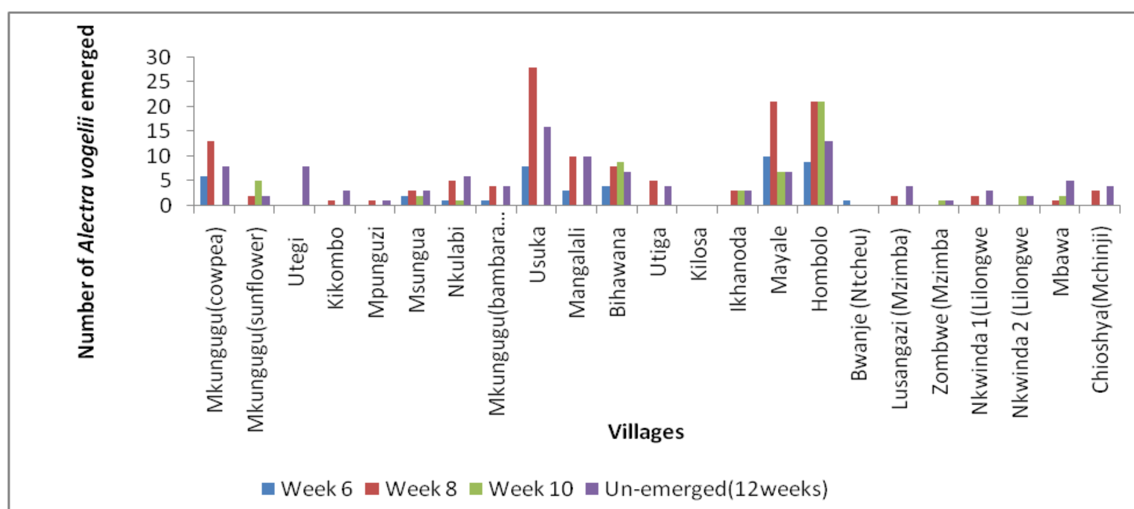


Figure 12:Number of emerged and un-emerged *A.vogelii* as observed on Vuli 2 cowpea variety over a period of 12 weeks.

Scale used : 1cm =5 number of *A.vogelii*, Nkwinda 1(lilongwe) and Nkwinda 2 (lilongwe) villages refer fig 10.

4.8.5 Number of emerged and un-emerged *A.vogelii* on Tumaini cowpea variety

The *A.vogelii* collected from Mkungugu in sunflower and bambara groundnuts, Utegi, Kikombo, Msungua, Nkulabi, Usuka, Mangalali, Bihawana, Utiga, Hombolo, Bwanje, Lusangazi, zombwe, Nkwinda in groundnuts and cowpea, Mbawa and Chioshya was observed early at 6th week from planting.While that of Mkungugu from cowpea, Mpunguzi and Ikhanoda emerged at 8th week from planting. That of Kilosa and Mayale did not emerge at all on Tumaini cowpea variety**.** In this variety many isolates emerged at 6thweek. At 6thweek the number of *A.vogelii* went up to 25/pot while at 8thweek went up 45 *A.vogelii*/pot. The most agressive *A.vogelii* was that collected from Tanzania than that of Malawi. The results suggest that Tumaini variety is susceptible to *A.vogelii* isolates.

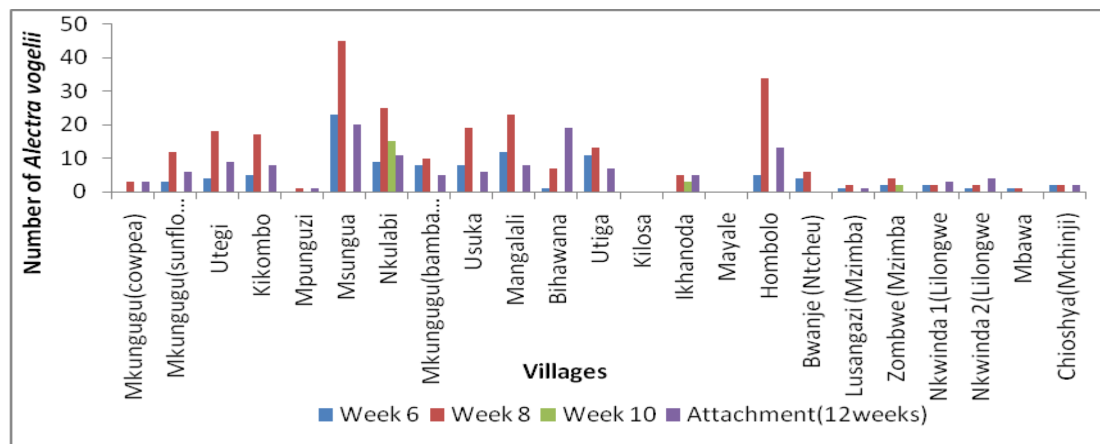


Figure 13: Number of emerged and un-emerged *A.vogelii* as observed on Tumaini cowpea variety over a period of 12 weeks.

Scale used : 1cm =10 number of *A.vogelii*, Nkwinda 1(lilongwe) and Nkwinda 2 (lilongwe) villages refer fig 10.

4.8.6 Number of emerged and un-emerged*A.vogelii* on TZA 263 cowpea line

The *A.vogelii* isolates of Mkungugu from Bambara groundnuts, Usuka, Mangalali, Utiga, Mayale, Bwanje and Mbawa emerged early at 6th week on TZA 263 cowpea line from planting. That of Msungua and Nkulabi emerged at 8th week, while that of Nkwinda from cowpea emerged at 10th week. Isolate collected from kikombo was not able showed only un-emerged *A.vogelii*. The one collected from Mkungugu in cowpea and sunflower, Utegi, Mpunguzi, Bihawana, Kilosa, Ikhanoda, Hombolo, Bwanje, Zombwe, Nkwinda from groundnuts and Chioshya did not show any emergence of *A.vogelii*. On this cowpea variety the the number of *A.vogelii* went up to 14/pot. According to data the *A.vogelii* collected from Iringa and Njombe regions in Tanzania where more aggressive to TZA 263 than other regions in Tanzania and Malawi. This variety was resitance to some of *A.vogelii* and susceptible to other isolates (Fig.14).

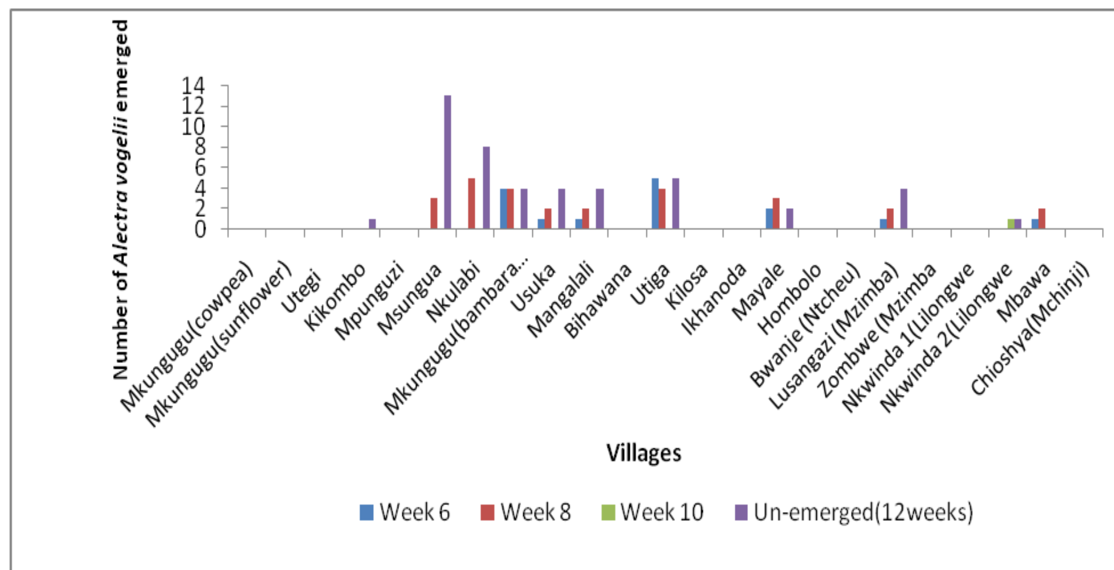


Figure 14: Number of emerged and un-emerged *A.vogelii* as observed on TZA 263 cowpea line over a period of 12 weeks. Scale used : 1cm =2 Number of *A.vogelii.* Note: Nkwinda 1(lilongwe) and Nkwinda 2(lilongwe) villages refer fig 10.

4.8.7 Number of emerged and un-emerged *A.vogelii* on IT99K-1122 cowpea line

The number of *A.vogelii* emerged on IT99K-1122 cowpea variety ranges from 0-6/pot. The *A.vogelii* isolates collected from Mkungungu on Bambara groundnuts, Mangalali, Ikhanoda, Mayale , Bwanje and Lusangazi villages was obseved early at 6th week from planting.While that of Nkwinda from groundnuts and cowpea emerged at 8th week, the isolates from Utegi , Kikombo, and Bihawana showed only un-emerged at 12th week from planting. The *A.vogelii* isolates which did not emerge on IT99K-1122 cowpea line was that from Mkungungu both from cowpea and sunflower, Mpunguzi, Msungua, Nkulabi, Usuka,Utiga, Kilosa, Hombolo, Zombwe, Mbawa and Chioshya. This variety did not support high number of *A.vogelii* since the number of *A.vogelii* ranges from 0 -6/pot. This shows that IT99K-1122 was resistance to most of isolates from parts of Tanzania and Malawi, most of isolates emerged late, only 6 *A.vogelii* isolates out of 23 emerged early. But the *A.vogelii* isolates from Tanzania was more aggressive on this line than that of Malawi.

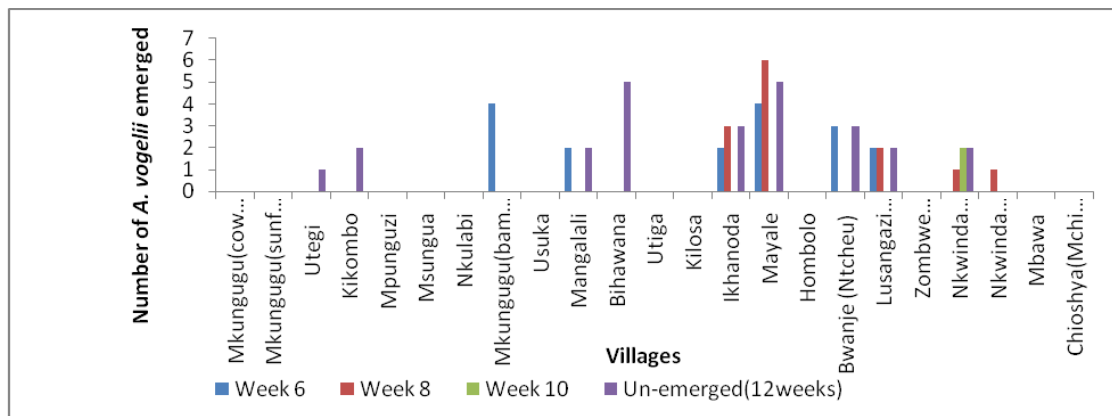


Figure 15: Number of emergence and un-emerged *A.vogelii* as observed on IT99K-1122 line over a period of 12 weeks.

Scale used : 1cm =1 number of *A.vogelii*, Nkwinda 1(lilongwe) and Nkwinda 2 (lilongwe) refer fig 11.

4.8.8 Number of emergence and un-emerged *A.vogelii* on Bunda 1 cowpea variety.

The *A.vogelii* isolates from Utiga, Mayale, Lusangazi and Nkwinda from cowpea emerged early at 6th week from planting.While that of Msungua and Chioshya *A. vogelii* isolates was observed at 8th weeks, that of utegi, Kikombo, Mpunguzi, Hombolo and Bwanje showed only attachments. All isolates from Mkungungu, Nkulabi, Usuka, Mangalali, Bihawana, Kilosa, Ikhanoda, Zombwe, Mbawa, and Nkwinda from groundnuts did not emerge on bunda 1. The number of *A.vogelii* observed on Bunda 1 ranges 0-4.5/pot and most of *A.vogelii* showed late emergence. The data suggest that, the variety showed some resistance to most of *A.vogelii* isolates, since it showed less number of *A.vogelii*

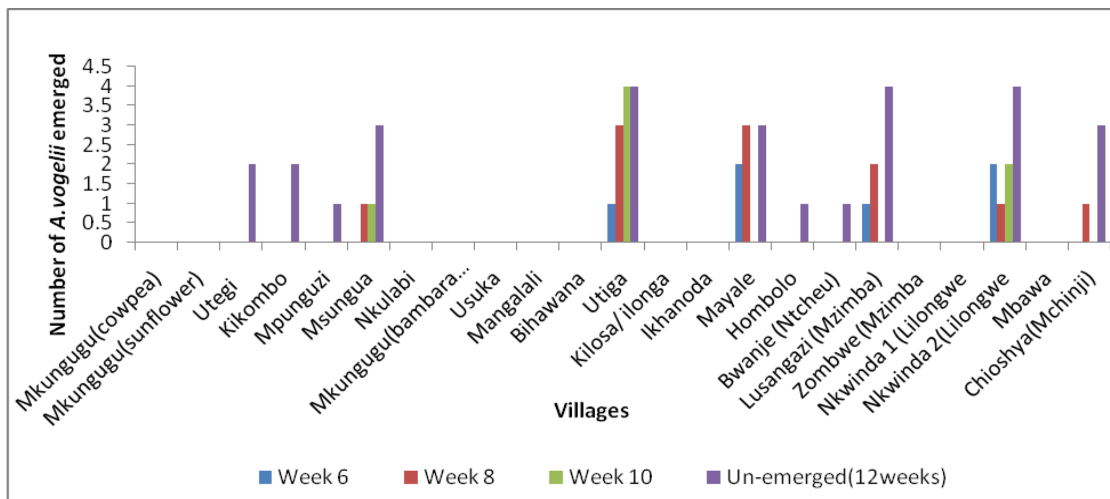


Figure 16: Number of emergence and un-emerged *A.vogelii* as observed on Bunda 1 cowpea variety over a period of 12 weeks.

Scale used : 1cm =0.5 Number of *A.vogelii.* **Note**: Nkwinda 1(lilongwe) and Nkwinda 2 (lilongwe) villages refer fig.10

4.8.9 Number of emergence and un-emeged of *A.vogelii* on B301 cowpea line

The *A.vogelii* Isolates from Utegi and Mayale was observed on B301, that of Mayale emerged at 6th week from planting while that of Utegi emerged at 10th week from planting. The

A.vogelii isolates fro Utegi emerged on B301 was 1/pot and 3 un-emerged *A.vogelii* at 12th week. While that of Mayale had 1 *A.vogelii*/ pot at 6th, 8th,10th week and 4 un-emerged at 12th week from planting. Generally on this line the number of *A.vogelii* emerged on B301 cowpea variety ranges from 0-1/pot. According to data, the cowpea variety B301 was resistant to all *Alectra* isolates except that of Njombe region at Mayale and Utegi villages.

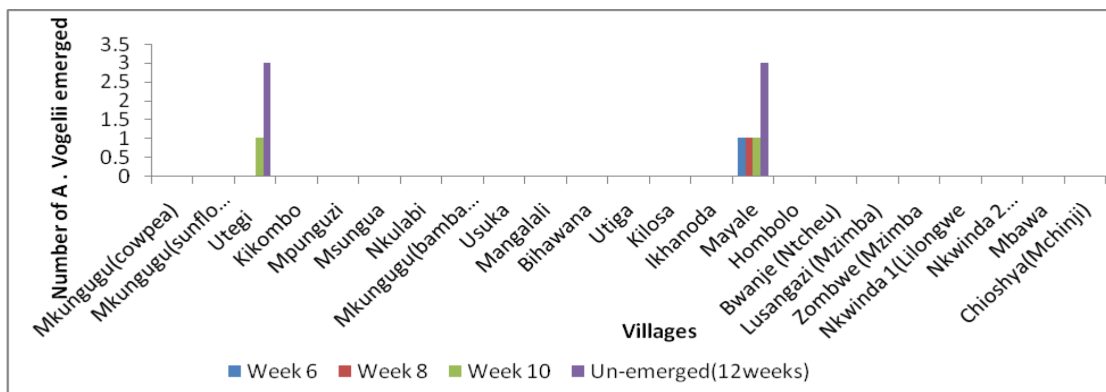


Figure 17: Number of emergence and un-emerged *A.vogelii* as observed on B301 cowpea line over a period of 12 weeks.

Scale used : 1cm = 0.5 number of *A.vogelii*, Nkwinda 1(lilongwe) and Nkwinda 2 (lilongwe) villages refer fig 16.

4.8.10 Number of emerged and un-emerged *A.vogelii* on IT99K-573-1 cowpea line

In general the number of *A.vogelii* emerged on IT99K-573-1-1 cowpea line ranges from 0-7/pot. The *A.vogelii* from Usuka, Hombolo, Lusangazi and Nkwinda from groundnuts emerged at 6th weeks from planting(early emergence).

That of Mkungugu from bambara, Mangalali, Mayale, Bwanje and Zombwe emerged at 8th week from planting, while that of Utegi and Mbawa was observed at 10th week from planting which is termed as late emergence. The one from Kikombo, Bihawana and Utiga didn't emerge, while that of Mkungugu from cowpea and sunflower, Mpunguzi, Msungua, Nkulabi,

Kilosa, Ikhanoda, Nkwinda from groundnuts and Chioshya was not observed. During data collection the *A.vogelii* was observed more at 8th and 10th weeks than at 6th week, the number of attachment was lower at 12th week. 9 isolates was not able to emerge on IT99K-573-1cowpea variety. The most aggressive *A.vogelii* Isolates was that from Hombolo, where by 7 no.of *A.vogelii*/ pot was observed compared to other isolates which had less number of *A.vogelii* per pot.This variety is susceptible to some *Alectra* and resistance to other *A.vogelii* isolates.

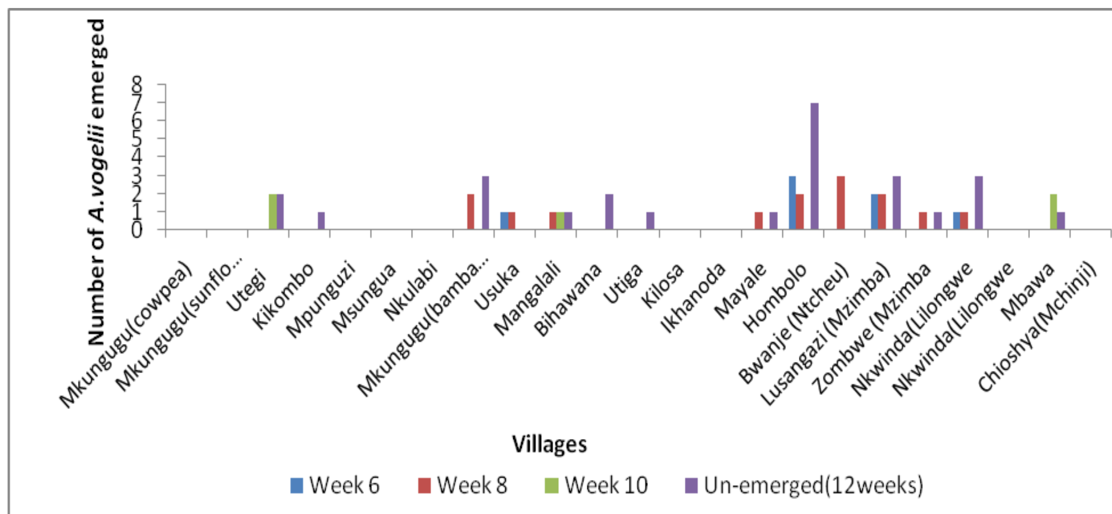


Figure 18: Number of emerged and un-emerged *A.vogelii* as observed on IT99K-573-1 cowpea line over a period of 12 weeks

Scale used : 1cm =1 number of *A.vogelii.*, Nkwinda 1(lilongwe) and Nkwinda 2 (lilongwe) villages explanations, refer fig 11.

4.8.11 Number of emerged and un-emerge *A.vogelii* on IT00K-1263 cowpea line

The *A.vogelii* from Mkungugu from sunflower, bambara groundnuts and cowpea, Utegi, Kikombo, Mpunguzi, Msungua, Nkulabi, Usuka, Mangalali, Bihawana, Utiga, Mayale, Lusangazi, Mbawa and Nkwinda from cowpea and groundnuts was observed early at 6th week

from planting. That of Ikhanoda emerged at 8th week from planting, while that of Bwanje from Malawi and Hombolo from Tanzania showed only attachment but didn't emerge over the ground. The isolate from kilosa didn't emerge at all on IT00K-1263 cowpea variety. The high number of *A. vogelii* was high at 8th weeks and decreasing as time goes. The number of *A.vogelii* emerged on IT00K-1263 cowpea line ranges from 0-9/pot. The most aggressive *A.vogelii* isolates was that from Tanzania compared to that from Malawi where by the number of *A.vogelii* was high at 6th week from planting. The isolate from Hombolo was most aggressive compared to other isolates since it had 9 No.of *A.vogelii*/pot. Due to number of *A.vogelii* isolates observed on IT00K-1263 cowpea line, this line was susceptible to *A.vogelii.* see fig 19.

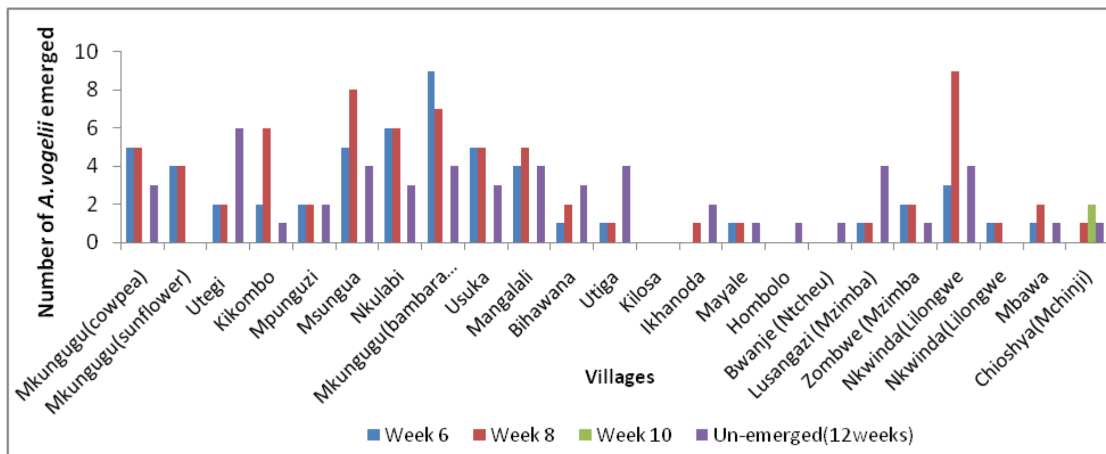


Figure 19: Number of emerged and un-emerged *A.vogelii* as observed on IT00K-1263 cowpea line over a period of 12 weeks.

Scale used : 1cm =2 number of *A.vogelii.*, Nkwinda 1(lilongwe) and Nkwinda 2 (lilongwe) as explained on fig 14.

4.8.12 Number of emerged and un-emerged *A.vogelii* on IT82E-16 cowpea variety

The isolates collected from Mkungugu in cowpea and bambara groundnuts, Utegi, Mpunguzi, Msungua, Mangalali, Ikhanoda, Kilosa, Mayale, Hombolo, Bwanje,Chioshya, Mbawa and Nkwinda from cowpea and groundnuts villages was observed early at 6th week after planting. That of Mkungugu from sunflower, Usuka, Bihawana and Lusangazi emerged at 8th weeks, while that of Utiga, Kikombo and Lusangazi was observed late at 12th week from planting. Only *A.vogelii* from Nkulabi village was not observed on IT82E-16 cowpea line. The number of *A.vogelii* emerged on IT82E-16 cowpea line ranges from 0-9/pot, but all *A.vogelii* isolates collected from Tanzania and Malawi seems to be aggressive to this cowpea variety.The data suggest that, the IT82E-16 cowpea variety is susceptible to *A.vogelii*.

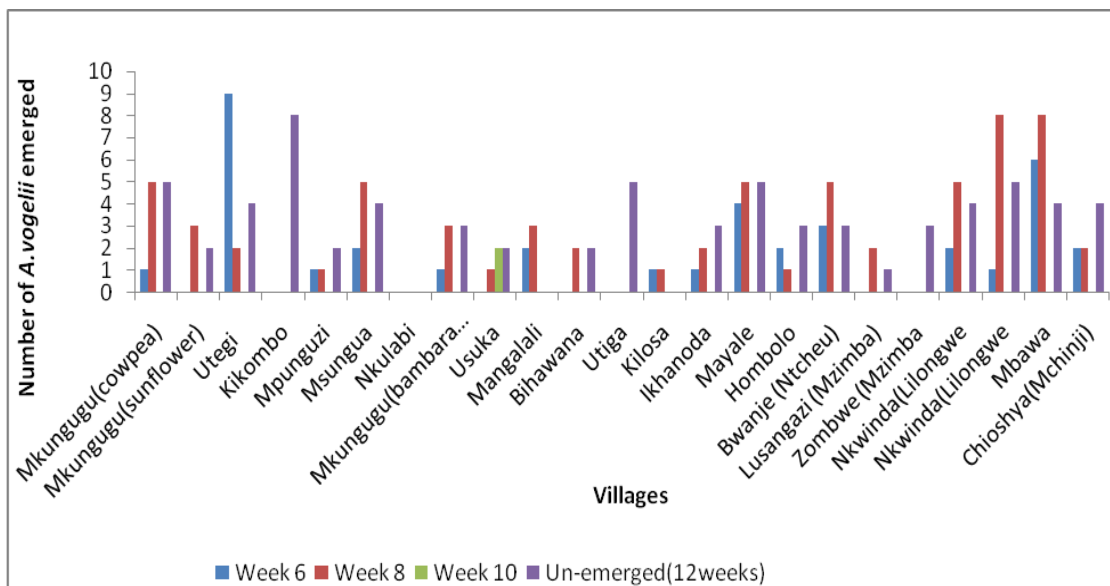


Figure 20: Number of emergence and nodules of *A.vogelii* as observed on IT82E-16 cowpea variety over a period of 12 weeks.

Scale used : 1cm =1 number of *A.vogelii.*, Nkwinda 1(lilongwe) and Nkwinda 2 (lilongwe) as explained on fig 11.

4.9 Resistance/susceptibility of pulse crops to *A. vogelii* variants from Tanzania and Malawi.

4.10 Number of *A.vogelii* emerged and on Bambara groundnuts, soybean and groundnuts

The total number of three hosts was screened for *A.vogelii* collected from Tanzania and Malawi as indicated on table 17. The number of *A.vogelii* isolates un-emerged on soybean was 9 and not grow was 12. On bambara groundnuts, the number of *A.vogelii* isolates un-emerged was 6 and not grow was 15. While the number of *A.vogelii* isolates un-emerged on groundnut was 13 and the one did not grow were 8. Both on soybeans, bambara groundnuts and groundnuts late emergence of *A.vogelii* was observed. The results are shown on the table (17).

Table 16: Number of emergences of *A.vogelii* on Bambara groundnuts, soybean and groundnuts

HOST GROWN	No. of isolates emerged		*Alectra vogelii* emergence on host(late-L emergence)																					Remarks
	Un-emerged	Zero emergence	Bwanje (Ntcheu)	Lusangazi (Mzimba)	Zombwe (Mzimba	Nkwinda(Lilongwe	Nkwinda(Lilongwe	Mbawa	Chioshya(Mchinji)	Usuka	Mangalali	Utiga	Mkungungu(cowpea)	Utegi	Kikombo	Nkulabi	Bihawana	Hombolo	Ilonga/Kilosa	Msungua	Mkungugu(Bambara)	Mpunguzi	Mayale	
Soybean	9	12	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Bambara groundnuts	6	15	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Groundnuts	13	8	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

4.12 Number of *A.vogelii* emerged observed on soyabean

In this study, *A. vogelii* collected from Malawi emerged on soyabean by 57% of all isolates collected from Malawi. While that of Tanzania emerged by 37% of all *A.vogelii* collected. The average number of *A.vogelii* observed ranges from 0-3/pot. No *A.vogelii* from bambara groundnuts was able to grow on soyabeans but didn't emerge over the ground.Only that collected from cowpea and groundnuts grew on soyabeans. According to results the *A.vogelii* from Mayale, Msungua, Nkulabi, Kikombo,Mangalali, Mbawa, Nkwinda from Cowpea, Zombwe and Bwanje from Groundnuts was just started to attach to soyabean at 12th week after planting which was the late emergence, and that is the time the number of attachments were counted. No isolate was aggressive than the other but the one observed to be in high number was that from Nkwinda collected from cowpea and the one collected at Mbawa in groundnuts.

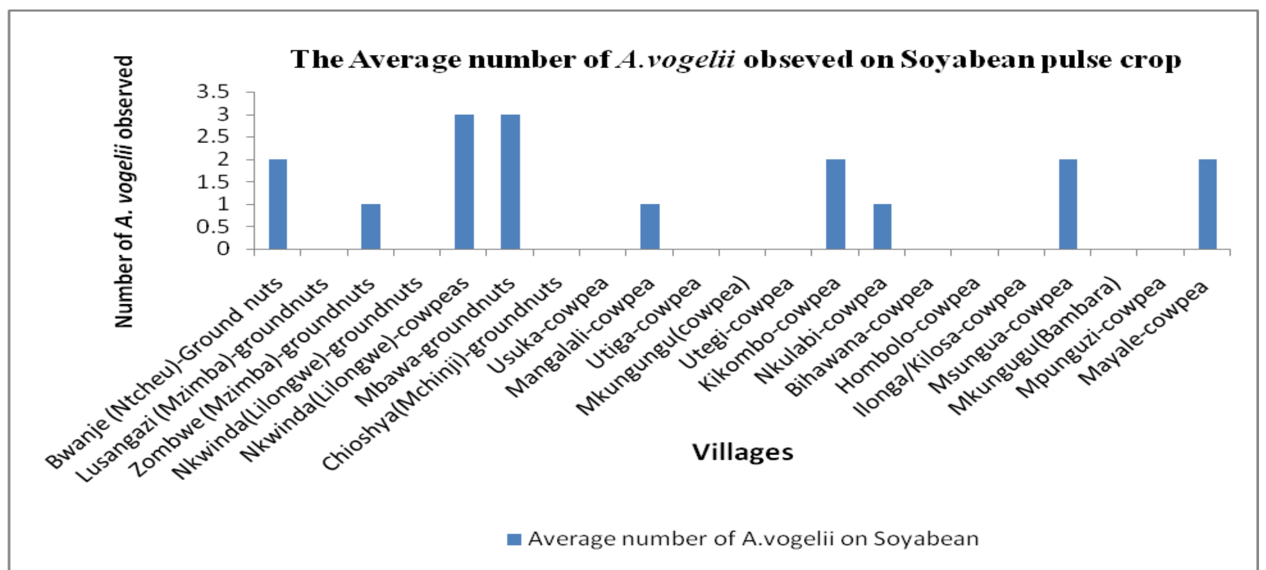


Figure 21: Number of un-emerged and zero emergence of *A.vogelii* as observed on soyabean over a period of 12 weeks.

Scale:1cm= 0.5 number of attachments.

4.12.1 Number of *A.vogelii* emergence observed on bambara groundnuts

The experiment done on bambara groundnuts showed that, 71% of *A.vogelii* isolates collected from Malawi showed attachments on bambara groundnuts. Only 57% of *A.vogelii* collected from Tanzania was able attach on bambara groundnuts. The *A. vogelii* collected from cowpea and groundnuts was able to germinate on bambara groundnuts except that collected from bambara groundnuts it self. The number of *A.vogelii* attachments observed ranges from 0-7/ pot. The *A.vogelii* isolates collected at Bwanje from groundnuts, Lusangazi from groundnuts, Nkwinda from both groundnuts and cowpea, Mbawa from groundnuts, Usuka, Utiga, Mkungugu from cowpea, Bihawana, Mpunguzi and Mayale was observed at late during 12th week after planting. Other isolates were not able to grow on bambara groundnuts. The *A.vogelii* collected from Malawi showed to be more aggressive than that from Tanzania, the isolate which showed highest number of un-emerged *A.vogelii* is that collected at Nkwinda from cowpea.

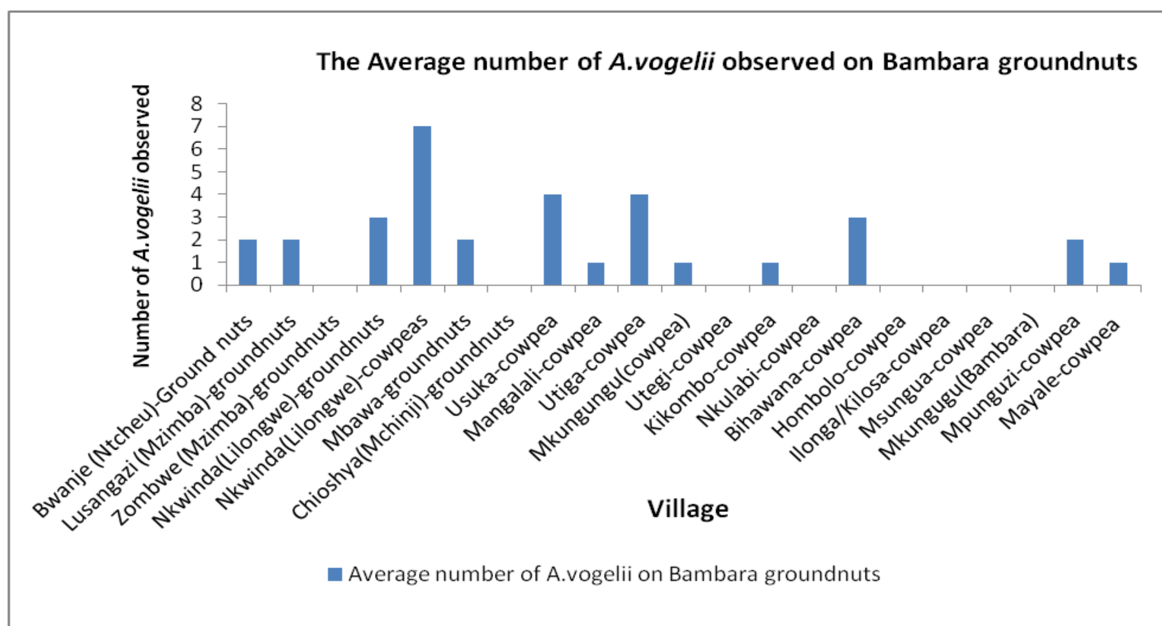


Figure 22: Number of un-emerged of *A.vogelii* as observed on bambara groundnuts pulse cropover a period of 12 weeks.

Scale:1cm= 1 number of attachments

4.12.2 Number of emergence of *A.vogelii* observed on groundnuts/peanuts

The study done on peanuts showed that, 57% of *A.vogelii* isolates collected from Malawi and 14% of *A.vogelii* collected from Tanzania was able to germinate on groundnuts. *A.vogelii* collected from cowpea germinates on peanuts. The 4 isolates of *A.vogelii* collected from peanuts germinate on groungnuts. The number of un-emerged *A.vogelii* observe on groundnuts ranges from 0-6/pot. The *A.vogelii* isolates collected from Bwanje, Lusangazi, Zombwe, Chioshya, Usuka and Ilonga was observed on groundnuts at 12th week after planting. The isolate which was observed to be in high number, was the one collected from Ilonga (6 *A.vogelii*/pot). The *A.vogelii* from Malawi were observed to be more aggressive than that collected from Tanzania.

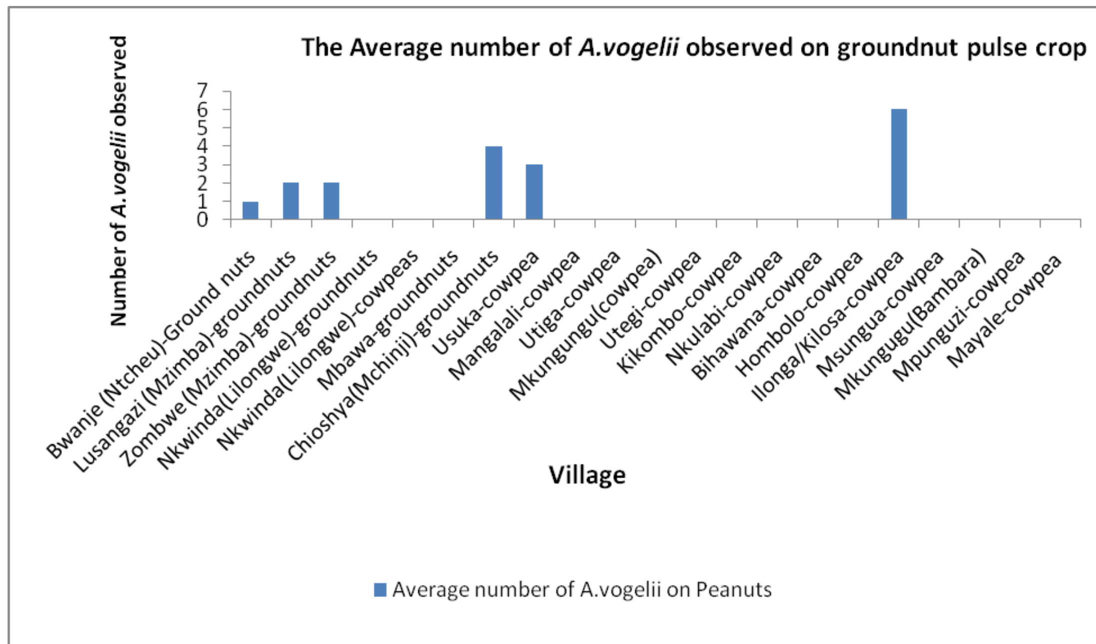


Figure 23: Number of zero emergence and un-emerged *A.vogelii* as observed on peanuts/ groundnuts over a period of 12 weeks.

Scale:1cm= 1 number of attachments.

CHAPTER FIVE

5.0 DISCUSSION

In objective one, the partitioning of *A. vogelii* isolates were established by using 15 SSR markers developed from Rice bean and *S. hermothica.* According to genetic diversity tree, the *Alectra* collected from bambara groundnuts, cowpea and sunflower field showed some level of similarity. *A. vogelii* collected from Mkungugu village in sunflower and bambara groundnuts were the same, but differed to that collected from cowpea of the same place. The *A.vogelii* collected from sunflower in Singida was significant different from the rest of the group, even during its growth stage, there was different phenotypic characteristics observed compared to *A.vogelii* from bambara, cowpea and sunflower from Mkungugu. More investigation is needed in order to understand more the relationship of this kind of *Alectra* observed in sunflower to other *A. vogelii*.

The study also confirmed that *A. vogelii* from Tanzania, Malawi and that of Botswana had some similar sharing characteristics. Some of Tanzania *A. vogelii* isolates *are* similar to that of Malawi; there some of Tanzania is similar to that of Botswana, and some of Malawi is similar to that of Botswana also. This shows that *A. vogelii* collected from the same area can fall into the same group depending on similarities, but also can be clustered to other group depending on differences of races in the same area.

A. vogelii collected from Mangalali and Ismani are the same but they are different from that of Ilambilole and Mkungugu, althought these are the villages from the same region and the same district.

The one collected from Utegi and Mayale fall into the same group on genetic diversity tree, they were both collected in Wanging'ombe ward, also the phenotypic data proves that Utegi and Mayale isolates are the same, since they were able to grow on B301 cowpea (resistant landrance) during the pot infestation, but these two differs to that of Utiga and Usuka villages which are from the same area.

The *A.vogelii* collected from Marajone and Sebele villages from Botswana have similar *A.vogelii*, are also have some similar characters from that of Nkwinda in Malawi collected from groundnuts and Msungua village from Tanzania.

A.vogelii from Zombwe and Nkwinda (from cowpea) falls into the same group they were both collected from Malawi and are all similar. *A. vogelii* collected from Nkwinda (Lilongwe) in cowpea and that collected from Nkwinda (Lilongwe) from Groundnuts were not similar even though they were collected from the same place. That of Malawi 2002 collection are similar from that of Bwanje in Malawi, but they have similar characteristics with some villages from Tanzania. The one collected from Lusangazi (Mzimba) in Malawi is more similar to that of Mkungugu in Tanzania. *A.vogelii* collected from Kikombo and Mpunguzi are the same, but differs slightly from that of Hombolo.

The one collected from Bihawana is the same of isolate from Ilambilole in Iringa region. *A.vogelii* collected from Nkulabi is quite different from all places were *A.vogelii* collected in Dodoma. This is the full picture of the results obtained from the markers generated from the selected primers.

No supportive document has been done on this; this will be the first documentation on genetic diversity of *A.vogelii* collection from Tanzania and Malawi. Most has been done on *Striga Spp.*

Apart from the partitioning and polymorphism obtained, lack of enough polymorphic bands was experienced due to the use of markers which are not related to *A.vogelii* genomic DNA. No genomic sequence of *A.vogelii* for producing specific primers for *A.vogelii* has been developed, which will enhance studies on *A. vogelii*. The use of *S. hermorthica* markers was based on the understanding that there might be a sufficient genetic synteny between these two parasitic weed species though one is specific to legume and the other to cereals. The *S. hermothica* primers generated by Estep *et al*., (2001) were used in this study to generate the amplifications on genomic DNA of *A.vogelii*. Estep *et al*., (2001) work with eleven populations of witch weed, *S. hermonthica*, which were collected in four regions of Mali, Extensive genetic diversity was observed, with most plants heterozygous for most markers.

Till now no molecular markers studies for *A.vogeli* has been published. Other studies have been done to different *Alectra* Spp. The monophyly of *Alectra* was assessed using DNA sequences from the nuclear (internal transcribed spacer) and chloroplast *(rpl16, trnT-L*) genomes, including 11 of 12 species by Morawetz (2009); the monophyly of the remaining species of *Alectra* was highly supported, which is contrary to this study results. In this genomic study using mitochondria and chloroplast primers, a total of 16 cpDNA and mtDNA SSR primers were used to amplify the chloroplast, mitochondria and genomic regions, only 4 mtDNA primers were able to amplify the SSR genomic region of *A.vogelii*, the rest of primers didn't amplify including all cpDNA primers.

In addition, it shows that mtDNA primers demonstrated higher degree of conservation than for the cpDNA primer study by Dumolin *et al*., (1997). The difficulty of detecting polymorphism in chloroplast microsatellites using cpDNA and mtDNA primers has been recognized and discussed by other researchers Kaundun and Matsumoto (2002). Fineschi *et al.,*(2005) did not find any variation in the chloroplast genome of *Crataegus* spp. Kaundun and Matsumoto(2002) used 7 cpSSR primers, but only four produced amplicons in tea (*Camilla sinensis*) and only one (ccmp6) revealed polymorphism.

ISSR primers have been also extensively used in Orobanchaceae. An average of 23.4 and 22.2 polymorphic bands per primer was detected for *Hyobanche* spp and *Orobanche crenata* populations respectively. Wolfe and Randle (2001), Román *et al.*,(2002) and Benharrat *et al.,* (2005*)* obtained 4 polymorphic bands with only one primer. But in our study, ISSR primers seem to be a less functional in *A. vogelii* due to its inconsistency of results.

In objective two, the 5 cowpea varieties and 6 lines were tested to different *A.vogelii* isolates collected from Malawi and Tanzania. These varieties/ lines were Vuli 1, Vuli 2, IT99K-7-21-2-2-1, Tumaini, TZA 263, Bunda 1, IT99K-1122, B301 and IT99K-573-1. Each of these were tested to see differential response of individual *A.vogelii* isolates collected from Tanzania at Mkungugu from cowpea, Mkungugu from sunflower, Utegi, Kikombo, Mpunguzi, Msungua, Nkulabi, Mkungugu from Bambara, Usuka, Mangalali, Bihawana, Utiga, Kilosa, Ikhanoda, Mayale, Hombolo villages. The one from Malawi were collected at Bwanje, Lusangazi, Zombwe, Nkwinda from groundnuts, Nkwinda from cowpea, Mbawa and Chioshya villages. Few authors have been working with these cowpea varieties and lines.

In this study, Varieties and lines of cowpea differed in stimulating *Alectra* seeds germination and allowing emergence of shoots demonstrating presence of genetic divergence of *A.vogelii* collected from Tanzania and Malawi. Proportionally the number of resistant accessions observed on eleven cowpea varieties tested was low than expectation. These observed differential responses to *Alectra* indicate that the genes controlling these parasites are non-allelic and independent of one another.

Some of the cowpea lines and varieties supported many *Alectra* shoots per pot and some supported less. Resistant genotypes had zero emerged *Alectra* plants of a particular isolate and variable numbers of un-emerged parasite shoots indicating that these genotypes can prevent the parasite from successful establishment of a parasite to a particular isolate. Two of the studied cowpeas accessions (B301 and IT99K-7-21-7-2-1) demonstrated resistance to *Alectra* infestation. The studies have been done on *Striga spp*, Roots of a *Striga*-resistant sorghum variety impaired the establishment of young parasite plants by partially inhibiting developing haustorium, reducing nutrient translocation, and the accumulation of phenolic compounds (Arnaud *et al*., 1999). The B301 and IT99K-7-21-7-2-1 behaved as *striga* resistance sorghum, further studies of these cowpeas are need.

Riches (1987) also reported that parasitic shoots are smaller and usually not emerging on low susceptible cowpea genotypes. The resistance of local variety B301 and IT99K-7-21-2-2-1 line is because of its low production of stimulants for the germination of *A.vogelii* seeds as well as attachment and prevention of haustorial formation and subsequent development of the seedling of the parasite through antibiosis (Lane and Bailey, 1989).

In 2008, Dr. Mbwaga and Dr. Mligo through McKnight project, tested B301 with *Alectra* collected from Msungua Ikhanoda Kikombo Mkungugu Mangalali, no emergence was observed. In this study, A. *vogelii* from utegi and Mayale were able to parasitize B301 cowpea local variety, while Initially B301 identified for resistance to *A. vogelii* in Botswana has been observed to exhibit multigenic resistance to both strains of *Striga* and *Alectra* at various locations in West and Central Africa, including Nigeria (Riches, 1987; Emechebe & Singh, 1991) which is contrary to *Alectra* from Tanzania. The IT99K-7-21-2-2-1 was inoculated the *A. vogelii* from Ismani in Iringa region; Bihawana Farmer Training Centre and Hombolo Research station in Dodoma, the results showed late emergence of *Alectra* (Mbwaga *et al*., 2008). The same was observed on this experiment.

The variety, Vuli 1, Vuli 2 and Tumaini cowpea varieties, IT00K-1263 and IT82E-16 cowpea lines shows high susceptibility to *A.vogelii* isolates collected from both Tanzania and Malawi sites. Very few *A.vogelii* isolates was not able to emerge on these cowpeas, confirming that these varieties are highly susceptible to *A.vogelii* isolates collected. Also the same experiment was done in Tanzania at ARI- Ilonga, high emergence of *A.vogelii* collected from Msungua, Ilonga and Ismani after inoculation in Vuli 1, Vuli 2 and Tumaini (Mbwaga *et al*., 2007).

The results, the proved Vuli 1, Vuli 2 and Tumaini varieties are highly affected by *A.vogelii* isolates as a sign of susceptibility to the parasite.

Also these cowpea varieties Vuli 1, Vuli 2 and Tumaini supports early emergence of *A.vogelii* (at 6th weeks) compared to other varieties and lines.

The susceptibility of these varieties is due to high production of chemicals which facilitate parasite emergence, the studies conducted showed that *A.vogelii* seed germination occurs in response to the root exudates of potential host and a hemi–parasitic phase after emergence follows the holo-parasitic development of the plant on the host roots (Botha, 1984). In case of IT82K-16, its experiment was done by Mbwaga *et al*.,(2008), the *A.vogelii* from Msungua, Malawi and Bihawana was inoculated to this line, both of them showed high number of emergence as parallel to our experiment.

Out of 23 *A. vogelii* isolates collected from Tanzania and Malawi, half of isolate were able to parasitize TZA 263, IT99K-1122, Bunda 1 and IT99K-573-1 Varieties as shown on the results. Bunda 1 cowpea variety was thought to be resistant to *A.vogelii* as it was tested in the screen house at Bunda College; no *A. Vogelii* emerged on Bunda 1 cowpea variety (Mbwaga *et al*., 2008). These results were contrary to our results, whereby half of *A.vogelii* collected both in Tanzania and Malawi were reactive to Bunda 1. TZA 263 was highly susceptible to *A.vogelii* collected from Tanzania than that collected from Malawi, some of *A.vogelii* emerged early and others emerged late as results suggest.

Alectra resistance and earliness of selected cowpea lines including TZA 263 were being increased at Ilonga Research Institute in 2009 under the McKnight project, these were tested only with *Alectra* from Ismani, Bihawana and Hombolo, Results showed it was resistant to *A.vogelii*, where by this study results shows, TZA 263 is susceptible to *A. vogelii*, half of 23 isolate grow on it.

The *A. vogelii* showed to be more reactive on IT99K-1122 as explained on results, for this reason IT99K-1122 is susceptible to *A.vogelii* and facilitate early emergence of *A.vogelii*. The same experiment was done under McKnight project during 2008, the inoculums from Ilonga and Ismani was tested on this line, the results showed no emergence of *Alectra*. But in our results half of isolates supported the emergence of *A. vogelii*. This proves that IT99K-1122 is not resistant to *A. vogelii*.

Late emergence of *A. vogelii* was observed on IT99K-573-1, previous IT99K-573-1 was proved to be resistance to *A.vogelii*, when the *Alectra* collected from Bihawana, Ilonga and Msungua was used as inoculums, no *Alectra* emergence was observed (Mbwaga *et al*., 2008). Also in this experiment, *Alectra* collected from Bihawana, Ilonga and Msungua did not emerge on IT99K-573-1, but that of Bihawana showed late attachment. IT99K-573-1 is susceptible to some of *A.vogelii* collected from Tanzania and Malawi out of these three tested under McKnight project. IT00K-1263 supported early emergence of *A.vogelii*, although the number of *A. vogelii* emerged was not high, but it was highly susceptible to *A.vogelii*.

In general results show parasites did grow differently on different cowpea varieties or lines; the reason can be due to different condition of cowpeas and climatical conditions that facilitate the *A.vogelii* germination. *A.vogelli* seeds can germinate immediately when germination requirements are met (Riches, 1989).

Seed germination occurs when ripened seeds are preconditioned by exposure to warm moist conditions for several days followed by exogenous chemical signals produced by host roots and some non-hosts germination stimulant (Worsham, 1987).

Some of host seeds will not stimulate the emergence of *A.vogelii*, not because the host is resistant to *A.vogelii* but it is because the stimulant did not reach the host. According to Maass (2001) Most of the seeds in the soil will not be reached by the stimulant, but will remain viable for up to 15 years, forming a seed reservoir for the next cropping seasons.
Some of *A.vogelii* collected differs on virulence, where by other supported high number of *A.vogelii* and some of them did not support high number of *A. vogelii* emergence. Mostly *A. vogelii* from Tanzania seems to be more aggressive than that of Malawi. Many authors have worked on parasitic plant and discover that resistance could depend on differences in virulence of *A. vogelii* and *Striga* strains (Parker and Reid, 1979, Ramaiah, 1987). Working on the related taxon *Alectra,* others found variability in virulence on different cultivars of cowpea (*Vigna unguiculata*) (Riches *et al*, 1992).

A recent report showed the occurrence of distinct varietal specificity among races of *S. gesnerioides*, indicating the existence of significant inter-population genetic divergence (Toure *et al*., 1998). This finding has prompted to conclude that varietal resistance could be overcome by some races of the parasitic weed emphasizing the necessity to perform cowpea selection for resistance to various *A. vogelii*. Availability of suitable sources of resistance is a basic prerequisite for successful resistance Breeding.

Breeding and deployment of cultivars carrying genes that condition resistance to *Alectra* could play a major role in controlling the parasite. Similarly, improvement of genotypes that had only underground-attached *Alectra* shoots for yield and other agronomic traits and use may help in reducing the parasite seed bank through stimulating germination and allowing attachment but without having a significant effect on the host plant.

In this study, observed considerable numbers of *Alectra* resistant genotypes for further testing and utilization in our cowpea improvement programme

In objective three, the selected Pulse crops (groundnuts, bambara groundnuts and soyabean), differed in stimulating *Alectra* seed germination. All of them showed late emergence of *A.vogelii* and some of them did not support the growth of *A.vogelii* weed. Soyabean, groundnuts and bambara groundnut allow the growth of different number the weed, demonstrating presence of genetic divergence among collection of *A.vogelii*. Resistant of pulse crop to a particular isolate had zero emerged *Alectra* plants while the susceptible ones showed the attachment. Few *A.vogelii* isolates were supported to grow and variable numbers of un-emerged parasite shoots indicating these pulse crops can prevent the parasite from successful establishment.

All isolates inoculated on soyabean, groundnuts and bambara groundnuts showed late growth of *A.vogelii*, this could be due to several reasons, may be the *A.vogelii* seeds were not reached with the stimulant. According to Botha (1984) the seeds, which receive a chemical stimulant, produced by the host roots will germinate. The pre requisite of such a signal ensures that a host is available and near enough to be reached by a germ tube which grows in direction of the origin of the signal (chemotropism). The germination stimulant is exuded 3-6 mm behind the root tip of the host plant. No stimulant is produced in older root cells; also the late emergence could be due to resistance of the pulse crops to parasitic plants.

Previous research had done shows that, varying levels of resistance have been identified and exploited in the breeding programmes of several crops (Rispail *et al.*, 2007).

Resistance is mainly determined by the coexistence of several mechanisms controlled by multigenic interaction and their associated resistance mechanisms at the histological, genetic and molecular levels (Rispail *et al*., 2007).

According to result, the *A.vogelii* collected from bambara groundnuts is different from cowpea and groundnuts. Most of isolates collected from cowpea and groundnuts grow on bambara groundnuts while that collected from bambara groundnut was not able to emerge on cowpea and groundnuts, apart from results the number of un-emerged *A.vogelii* observed differs in number, others had high numbers while others showed to have lower number of *A.vogelii*.

Menkir (2006) also found a broad range of numbers of *Striga* attached to maize roots and variation in the establishment of the parasite on host roots. This could be also due to the parasitic shoots are smaller and usually do not emerge on low susceptible cowpea genotypes (Riches, 1987). The same experiment was done in Nigeria on soyabean, response of Soybean Genotypes to *A. vogelii* Infestation under Natural Field conditions in 1995 and 1996, Analysis showed significant differences amongst genotypes for number of *Alectra* plants emerged at 9 and 10 Week, days to first *Alectra* emergence (Kureh *et al*., 2005).
The experiment was done by Riches in 1992, the host preference of 9 seed samples of *A. vogelii* from eastern, western and southern Africa and of 2 samples of *A. picta* from Cameroon and Ethiopia, to cultivars of cowpeas, groundnuts, bambara (*V. subterranea*) and mung bean (*Vigna radiata*), was assessed.

A susceptible cowpea cultivar, cv. blackeye, and 4 cultivars of groundnut were attacked by all samples of both parasitic species, regardless of whether the original host was cowpeas, groundnuts or bambara (Riches *et al*, 1992).

According to data, all the host variants (bambara groundnuts, groundnuts and soybean) showed late emergence, which is a good sign that they can mature before the *A.vogelii* has emerged. This shows that, they have some of resistance whereby they can be grown by the farmers without yield loss.

Alectra - resistant soybean, bambara groundnuts and groundnuts genotypes can provide an economic means of *Alectra* control and could be important components of integrated *Alectra* management strategies. Information concerning the performance of soybean, bambara groundnuts and groundnuts genotypes under *Alectra* infestation would be valuable to soybean, bambara groundnuts and groundnuts breeders' in planning future of soybean, bambara groundnuts, groundnuts selection and development programmes aimed at increasing their yield.

CHAPTER SIX

6.0 GENERAL CONCLUSION AND RECOMMENDATIONS

In conclusion, few rice bean and *Striga hermothica* markers were able to indicate the geographical distribution of *A.vogelii* in Tanzania and Malawi, but the ISSR, Mitochondria and *S. gesnerioides* markers were not useful to detect more polymorphisms. Inconsistency of ISSR markers, poor amplification of *S. gesnerioides* markers and no polymorphism on mitochondria markers was observed. These are not recommended as good markers for genetic diversity of *A.vogelii*.

Since characterization of *A.vogeli* (witch weed) using molecular marker has not been previous published and no evidence of genomic sequence of *A.vogelii* that has been developed, the characterization of *A.vogelii* became a difficult task that involved screening of different primers generated for specific crops. The suggestion was to develop the genomic sequence of *A.vogelii*, so that the specific primers to *A.vogelii* can be developed.

Also objective two results suggest that, the IT99K-7-21-2-2-1 cowpea line is recommended to be grown in wider range of environment due to its resistance. Also IT99K-1121, TZA 263, IT99K-573-1 and Bunda 1 are recommended to be grown on specific areas where zero emergence of *Alectra* was observed. Vuli 1, Vuli 2, Tumaini and Fahari cowpea varieties, IT00K-1263 and IT82E-16 cowpea lines are not recommended as resistant materials to *A. vogelii.*

Based on the foregoing, it is clear that legume production is threatened by *Alectra* and it is important to screen for *Alectra* resistance among existing legume crops or varieties in order to avoid working with the most susceptible varieties. This would ensure that farmers are not discouraged from using legumes.

There is a need to prove the resistance of B301 (local landrace and used as parental material as it has been proved to be resistant to *A. vogelii* isolate. But the results prove that *A. vogelii* collected from Mayale and Utegi in Tanzania can infest this local landrace. This local landrance is used as parental material due to its resistance to *A. vogelii* in West Africa; still there is need for more investigation of B301 especilly in Tanzania.This will help in finding the cowpea resistance material specifically to Tanzania.

More investigation of *A. vogelii* collected from sunflower at Ikhanoda village in Singida is encouraged due to it high differences with the other *A. vogelii* isolates. The work should focus on it speciality as well as the yield losses in especially on sunflower.

Also breeding for *A.vogelii* resistance to drought tolerant lines (IT00K-1263 and IT99K-1122) is highly recommended as the line are most preferred by farmers due to early maturing.

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