RESPONSE OF IMPROVED COWPEA GENOTYPES TO ALECTRA VOGELII STRAINS FROM SELECTED AREAS OF TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

MOROGORO, TANZANIA.

2018

EXTENDED ABSTRACT

Copwpea, (*Vigna unguiculata* L. Walp), belonging to the family Fabaceae, is one of the most important food legumes in semi-arid areas. It is a multipurpose crop with immense nutritional value and has significant potential to address malnutrition. However, the parasitic weed *Alectra vogelii* poses major threat to cowpea productivity throughout tropical and sub-tropical Africa. *A. vogelii* has developed strains specific to cowpea. Effective control of damage caused by *A. vogelli* in cowpea fields can be done through incorporation of resistance in cowpea varieties. The current study focused on determining existing genetic variability amongst strains of *A. vogelli* and response of improved cowpea genotypes. A total of 23 simple sequence repeats (SSR) markers were used to assess the genetic variability. The polymorphic information content (PIC) value computed was 0.929 with a gene diversity of 0.7913. Cluster analysis revealed existence of four distinct clusters amongst assessed populations of *A. vogelli* collected from different locations. This highlights the importance of developing and testing cowpea genotypes resistant to *A. vogelii* in multiple locations. The ability of improved cowpea varieties to thrive alongside *A. vogelli* was evaluated in pots in a screenhouse arranged in a split plot manner with treatments in randomized complete block design (RCBD). The results revealed that *A. vogelii* had negative significant effect on cowpea genotypes. Cowpea genotype B 301 had the highest yield compared to the rest of the improved varieties followed by vuli-1. Results from this study also indicated existence of high diversity of *A. vogelli.*

Key words: *Alectra vogelii*, genetic variability, host resistance, cowpea

DECLARATION

I, FRENK REUBEN, do hereby declare to the Senate of Sokoine University of Agriculture, that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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ACKNOWLEGMENT

I can’t start without praising God for keeping me inspired and courageous to go through my studies. I am extremely grateful to my supervisors Dr. A. B. Kudra and Dr. G. M. Tryphone. Personally, I lack words to express my profound gratitude to them. Their advice, constructive criticism, corrections right from research proposal to the final stage of writing this report, moral support and sometimes material support helped a lot to build up this work. I am grateful to them for taking time to go through my dissertation, to correct spellings, missing comas and insisting on making corrections. Without the professional expertise, insight and encouragement of my supervisors, the completion of this work would not have been possible. Their supervision is always of smiles and happiness which makes them be people of friendliness, readiness and willingness to train a student. I am praying for our Almighty God to make them live longer and better.

My deepest sincere gratitude goes to the Head of Department Crop Science and Horticulture, Prof. Maulid W. Mwatawala and his entire academic staff. I extend my sincere heartfelt thanks to Dr Luseko Amos Chilagane, Prof Paul Mbogo Kusolwa, Prof Cornel L. Rweyemamu for their their cordial adive and encouragement for this research work. I am truly grateful to Methusela Obedy Ntiyakunze, the technician at TARI Ilonga.

I am highly indebted to Dr. Ernest R. Mbega, for trusting and accepting me to be part of McKnight project that allowed me to pursue this postgraduate study. I am pleased to direct my sincere thanks to Sylivia Mlemba and Catherine Sangu for their worth advice.

I find neither words nor equivalent payback to express my deep sense of gratitude and heart felt respect to Mr. Faraja Makwinja, my friend and my classmate for his highly acknowledged support. You all inspired me to become a plant breeder.

Most importantly, I am grateful to my beloved wife Lydia Richard and my daughter Faith Frank. I also feel obliged to thank specifically my wife for allowing me to use most of the family's time for this research. My wife's support is of great appreciation for she was parenting our daughter all the time in my absence. Her cares to the family was of great importance. Her undoubted appreciation cannot be quantified and therefore remains a treasure of my heart.

Last, but certainly not least, I would like to forward the largest and loving thanks to my parents for their never-ending concern, support and continuous encouragement. I don't have a word to thank you for being there for me in all my needs and helped me out in every aspect of my life. Thank you is the least I can say.

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DEDICATION

This piece of work is dedicated to my beloved daughter Faith Frank.

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LIST OF ABREVIATION AND SYMBOLS

AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ARI	Agricultural Research Institute
C.V	Coefficient of variation
DAP	Days after planting
DNA	Deoxyribonucleic Acid
g	Gram
GM	Grand mean
m.a.s.l.	Metre above sea level
P<0.05	Significant at less than 5 % level
PCR	Polymerase Chain Reaction
RCBD	Randomised Complete Block Design
SE	Standard error
SSRs	Simple Sequence Reapeats
SUA	Sokoine University of Agriculture
TBE	Tris borate EDTA (buffer)
TE	Tris EDTA (buffer)
v/v	volume by volume
WAS	Weeks after sowing
%	percent
µl	Microlitre

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Cowpea (*Vigna unguiculata* (L.) Walp), is a grain legume indigenous to Africa. It is a primary source of protein and is extensively grown throughout Sub-Saharan Africa (Omoigui *et al*., 2015; Lado *et al*., 2016; Okeyo-Ikawa *et al.,* 2016). Mature dry cowpeas are important in the diets of many population groups around the world, as they are popular, inexpensive and readily available sources of food protein (Mamiro *et al*., 2011). Cowpea is one of a multifunctional crop of vital importance, similar in appearance to common bean (Karanja *et al.,* 2012; Gogile *et al*., 2013). The crop provides food for humans, feed for livestock, cash, soil ameliorant and thus contributes to food security, income generation and maintenance of the environment for a large number of resource-poor farmers (Olufajo, 2012; Gogile *et al*., 2013; Osunbitan *et al*., 2016; Thio *et al*., 2016; Olasupo *et al*., 2016). Its roots consist of nodules that harbor soil bacteria called Rhizobia for nitrogen fixation.

Cowpea grain is nutritious and inexpensive, provides major low-cost dietary protein for millions of smallholder farmers and consumers, who cannot afford high protein foods, such as fish and meat (Timko and Singh, 2008; Ali *et al.,* 2015; Osunbitan, *et al.,* 2016). Cowpea provides the ground cover and plant residues, which minimize erosion and subsequent land deterioration. The deep root systems of cowpea help to stabilize soil, and the ground cover provided by cowpea preserves moisture; these traits are particularly important in the semi-arid areas where moisture is always needed, soil is fragile and subject to erosion. Thus, cowpea offers multiple benefits to smallholder farmers in terms of food, cash income, and livestock feed, and improved soil fertility.

Because of its multipurpose and multifunctional importance, each year, 45 countries in the world produces 7.56 million tons of cowpea, by cultivating 12.76 million to 14.5 million hectares on average, with 84%-95% of the total area being contributed by major African cowpea producing countries (Abate *et al.,* 2012). Asia is the second producing continent, representing less than 3% in average of the global production. Nigeria produces cowpea accounting for 61 percent of production in Africa and 45 to 58% worldwide by cultivating on 4 million hectares (Abate *et al*., 2012). The acreage for cowpea production in Tanzania has increased from 145 000 hectares up to 158 000 ha.

However, despite the potential of the crop in ensuring food security, the production of this important crop is threatened by biotic and abiotic constraints which generally lead to low yields compared to potential yield (Mbwando *et al*., 2016). A major biotic pest of concerns in lowering cowpea yields in Africa is a parasitic weed *Alectra vogelii* (Benth) an obligate root-parasitic flowering plant of the family Scrophulariaceae (Karanja *et al.,* 2013). Unexpected occurrence of the parasitic weed *Alectra vogelii* leads to the disqualification of some of the seed plots (Sibuga *et al*., 2010). Due to wide geographical distribution, the *A. vogelii* includes strains that are considered to be harmful to cowpea crops (Timko *et al.,* 2007). In alleviating the *A. vogelii* problem, chemical, mechanical and cultural controls have proved difficult. The difficulty in controlling *A. vogelii* is due in large part to the highly specialized life cycle of parasitic plants.

The extent of damage to cowpea by *A. vogelii* infection is related to the close parasitic association between the host itself and the parasitic weed (Asare *et al.,* 2013). The underground location of the parasites, their physical attachment to host roots, and their synchrony of growth with the host complicate control by mechanical or chemical approaches.

The measures for *A. vogelii* and other parasitic weed management are hindered by a unique survival strategy of the weed, whereby it produces large amount of viable seeds, which remain dormant for many years, only germinating in the presence of potential host species that exude specific chemical signals (Westwood *et al*., 2012, Kudra *et al*., 2014). Therefore, it is essential to prevent further buildup of the seed bank of parasitic weeds (Kudra *et al.,* 2014). *A. vogelii* poses a serious threat to cowpea production by inducing the flux of nutrients and water from the host to itself. The extent of yield loss is related to the incidence and severity of attack, the host's susceptibility to *A. vogelii* environmental factors (edaphic and climatic) and the management level at which the crop is produced.

The *A. vogelii* to cowpea causes damage by competition for carbon and nutrients and through metabolic processes and physiological interactions. Its effects on crops range from stunted growth, through wilting, yellowing, and scorching of leaves, to lowered yields and death of many affected plants. The weed, lack chlorophyll and therefore rely on host photosynthate and other nutrients for survival. They must attach to a suitable host soon after germination to survive. Grain yield reduction caused by *Alectra* infestation is attributed by reduced root nodulation, shoot-root ratio and leaf area. *Alectra* infestation causes delayed onset of flowering and reduced number of flowers as well as reduced number of pods per plant, individual seed weight and grain total soluble carbohydrate (Olufajo, 2012).

Control methods used for the management of *A. vogelii* include, hoeing, deep cultivation and herbicides, destroying crop residue after harvest, and crop rotation, but these methods generally were found to be not effective (Shinggu, 2015). Hand hoeing is not effective due to nature of the weed and deep cultivation is too expensive for the resource poor farmers. Herbicides are not widely used because of high cost and unavailability of the

chemicals and application equipments. Effective control of parasitic weeds through conventional agronomic practices has been difficult to achieve since the parasite exerts its greatest damage before its emergence above ground (Geleta, 2010). The seeds germinate in response to exude specific chemical signals produced by the host plant. The parasite seedling must then attach to the host and form vascular connections to access water and other resources required for growth. Each of these steps involves exquisite communication between parasite and host that represent both fascinating biological adaptations and potential points of weakness that can be targeted for parasite control (Westwood *et al.,* 2012).

Breeding for resistance is one of the main approaches to decreased yield losses caused by *A. vogelii* infestation and is said to be the main hope to control *A. vogelii*. Therefore, breeding for improved cowpea varieties resistant to *A. vogelii* is the most potentially promising economic control measure since it is more affordable and cost-effective to farmers and resistant varieties can be grown without additional inputs (Teka, 2014). The method will not only eliminate the need of application equipment, but will also reduce the cost of production, reduce *A. vogelii* infestation and increase cowpea yields. However, it is a matter of concern that the widespread use of only a few resistance genes might accelerate the evolution of new *A. vogelii* strains (races) leading to serious losses in cowpea production.

1.2 Justification

A. vogelii is variable in its parasitic abilities (Omoigui *et al*., 2015) and it has physiological strains (Mbwaga *et al*., 2007). The occurrence of susceptibility of improved cowpea genotypes, among *A. vogelii* highlights the risk of specific strains overcoming the resistance of the improved cowpea genotypes. The major constraint to cowpea resistance

breeding is the complicated ability of the *A. vogelii* to overcome resistance, due to obligate crossing over behavior, which results in high levels of genetic variation. The genetic variability enables the *A. vogelii* to adapt to new resistance alleles.

The current situation shows that, in some hot spots of the country, there is *A. vogelii* infestation on the new improved resistant varieties. This infestation, suggests a breakdown of the *A. vogelii* resistance. Such breakdown of resistance is observed in the cowpea varieties when are grown in different locations in Tanzania. The major reason of this resistance breakdown is development of new *A. vogelii* strains or an increase in the aggressiveness of the current *A. vogelii* strains. This means that, presence of strains of *A. vogelii* is thought to be responsible for the breakdown of resistance in cowpea. The development of specific strains could be attributed due to evolutionary changes presumably encouraged by geographical isolation over a number of years. This confirms that, a number of *A. vogelii* strains exist and thus infest improved cowpea varieties in Tanzania. Therefore, different sources of cowpea genetic resistance for breeding of improved cowpea resistant to each *A. vogelii* strain in different areas with other incorporated desirable grain qualities are required to be employed in cowpea breeding as donor parents of resistant genes (Afiukwa *et al*., 2013). For effective resistance breeding, knowledge about the genetic variability of the *A. vogelii* is essential. Though a lot have been done to give the evidence on variability of *A. vogelii*, but very scanty information is available on the impact this variability has on the breeding programmes and on the stability of resistant genotypes. Identification of strain-specific responses in cowpea is relevant for the development of target resistant genotypes (Asare *et al.*, 2013). It is therefore important to have a detailed understanding on the genetic variability of the *A. vogelii,* so as to breed elite resistant cowpea varieties with both broad spectrum and durable resistance while targeting to specific *A. vogelii* strains in particular location for

different geographical areas. It is possible that, understanding genetic variability in *A. vogelii* may help to target appropriate sources of cowpea resistance against particular strain.

Identification of differences found in cowpeas, Bambara groundnuts and sunflower from Malawi and Tanzania was conducted to study the phylogenetic relationships among populations of *A. vogelii* growing on different hosts (Mwaipopo, 2014). Therefore, there is a need to provide the important insights into the evolutionary processes that influence the structure of genetic variation within and among populations of *A. vogelii*.

Information on genetic variation has implication on an indication of variability for virulence and control of the weeds and helps to target the areas and appropriate sources of resistance for identified strains. This knowledge on genetic variability of *A. vogelii* gives potential opportunity in breeding for resistamce by pyramiding genes for a broader resistance to *A. vogelii*. The genetic variability within and among these strains should be identified at molecular level for cowpea variety deployment. A better understanding of the genetic variability of *A. vogelii* and of cowpea-*Alectra* interactions is essential for more efficient *Alectra* resistance breeding and deployment of the resistant cowpea varieties in *A. vogelii* control. Based on this fact, there is therefore a need to understand the genetic variability of *A. vogelii* and the response of resistant cowpeas on it, so as to deploy suitable and appropriate breeding techniques that will make varieties remain effectively resistant to *A. vogelii* over time and across different locations.

1.3 Objectives

1.3.1 Overall objective

Establishment of *Alectra vogelii* strains existing in selected areas of Tanzania for developing an appropriate breeding strategy.

1.3 2 Specific objectives

i) To identify genetic variability of *Alectra vogelii* strains from different geographical locations.

ii) To determine the response of improved cowpea genotypes to *Alectra vogelii* strains collected from different locations.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin, Domestication and Global Distribution of Cowpeas

Cowpea is the self- pollinating crop which belongs to family Fabaceae. The name cowpea originated from the fact that the plant was an important source of hay for cows in US and in other parts of the world (Timko *et al.*, 2007). Cowpea which is cultivated in the tropical and subtropical regions of the world has been cultivated since Neolithic times and is now covering sixty-five countries in Asia, Middle East, Southern Europe, Africa, southern USA and Central and South America (Timko *et al.*, 2007). Southern and West Africa are the most probable center of cowpea domestication and ancient of wild cowpea occurs in Namibia, Botswana, Zambia, Zimbabwe, Mozambique, and South Africa (Angessa, 2006; Timko and Singh, 2008). Cowpea is commonly cultivated as a nutritious and highly palatable food source in the southern United States, Middle East, Africa, Asia, and throughout the tropics and subtropics. Cowpea was introduced in USA from West Africa early in 18th century by slaves during colonial era while India, Europe and Asia received cowpea from Africa between 3500 and 1700 BC years ago (Tosti and Negri, 2002; Fang *et al.*, 2007).

2.2 Economic Importance of Cowpea

2.2.1 Soil improvement

Cowpea is a fast-growing crop with bushy growth that provides ground cover against soil erosion. The crop grows well in sandy, poor, acidic soils but due to its high nitrogen fixing capability restores soil fertility. In the soils with sandy loam texture, moderate to low natural fertility and low external inputs, cowpea is grown. It fixes atmospheric N using soil fixing bacteria in its nodules of the roots and its soil residues ameliorate soil

fertility. The crop tolerates drought, poor soil fertility and extensive range of soil pH due to its nitrogen fixation ability and effective symbiosis with mycorrhizae and the crop acts as a soil cover crop, reduces soil erosion, and suppresses the weeds (Tarawali *et al.,* 2002). Cowpea fixes about 240 kg per ha of atmospheric nitrogen and makes about 60-70 kg per ha nitrogen be available in the soil (Aikins and Afiukwa, 2008; Abayomi *et al.,* 2008).

2.2.2 Human food and animal feed

Cowpea a nutritious crop plays a critical role in the lives of millions of people in Africa and other parts of developing world (Timko *et al.,* 2007). The seeds contain 23% protein, 57% carbohydrates, 50-67 % starch, and 27-34% protein is in leaves (Ghaly and Alkoaik, 2010; Cisse and Hall, 2010). Cowpea also contains vitamins and essential micro nutrients such as iron, calcium and zinc. Its roots consist of nodules that harbor soil bacteria called Rhizobia for nitrogen fixation. As the result of high protein, cowpea is customary referred to as poor man's meat. This makes a crop be a cheap source of protein for the resource-poor farmers in Sub-Sharan Africa. Immature seeds, pods and leaves of cowpea are eaten as vegetables because they contain the amino acid contents which can meet the amount required by humans (Akpan and Mbah, 2016). Cowpeas can be sold at the market, and consumed at household mainly during hunger months before harvesting grain and the crop is perceived as a 'blood giving' crop through preventing iron deficiency to growing children (Ishiyaku, 2012). Cowpea leaves contain high amount of protein and minerals, such as calcium, phosphorus and vitamin. Thus, the crop is comparatively a cheap source of protein, phosphorus, iron, vitamins and excellent substitute of meat, egg, and other protein rich foods. Apart from human consumption, cowpea leaves and stems are also an important source of high-quality hay for livestock feed (Tarawali *et al*., 2002). Cowpea provides the first food from the current harvest sooner than any other crop as it matures

earlier particularly in as few as 55 days after planting, thereby shortening the hungry period (Timko *et al.,* 2007)

2.3 Biotic and Abiotic Constraints for Cowpea Production

The current agronomic practices such as date of planting, planting populations, maintenance of the soil's physical properties and fertility, weed control and cropping patterns strongly influence yields of cowpeas. Cowpeas are host to a range of insect pests, a wide range of bacterial diseases, fungal disease, viral diseases, nematodes, and parasitic flowering plants like *Striga gesnerioides* and *Alectra vogelii* are biotic constraints to cowpea production (Emechebe and Lagoke, 2002; Timko *et al.,* 2007; Mbwaga *et al.,* 2010). These may affect the whole plant, the flower or the pods. Poor soil fertility, drought, heat, acidity, and inadequate amount of rainfall, all are abiotic factors hindering cowpea production (Timko *et al*., 2007). Phosphorus deficiency is the most limiting nutrient for cowpea production (Magani *et al.,* 2009). Phosphorus, although not required in large quantities, is critical to cowpea yield (particularly for improved photoperiod-insensitive cultivars) because of its multiple effects on nutrition. Other nutrients like K, S and Ca are of little importance and need only to be supplied where soils are particularly deficient, for instance in highly leached and eroded soils.

Among the numerous pathogens affecting cowpea, viruses are known to infect cowpea either at one stage or throughout the life of the plant. The major viral diseases include bean common virus (Degri *et al.,* 2012), cowpea binding mosaic, chlorotic spot, aphid-borne mosaic, cowpea necrosis and cowpea yellow fleck. Fungal diseases are seed and seedling rot, root rot, wilt, *Phytopthira* blight, web blight, antracnose, powdery mildew, *Cercospora* leaf spot and rust, whereas the major bacterial disease is bacterial blight.

Economic losses also occur from nematode diseases such as root knot and root lesion. The only important mycoplasm disease is phyllody.

Cowpea is attacked by many insect pests throughout its geographical range, although the number and their status vary from one region to another (Sule, 2013). These pests include aphids, beanfly, leafhoppers, thrips, pod borers, pod-sucking bugs, cowpea curculio and the storage beetle. Insects covering the main phytophagous taxa attack the crop from seedling to harvest and can cause economic damage at all stages of plant growth. Adults feed on above ground of cowpea such as pods, stems, and leaves. Any major insect pest has effect on cowpea at a certain stage in the life cycle (Asiwe, 2009). For example, cowpea is attacked by aphids, flower thrips, pod borer, pod sucking bugs and the weevil in the seedling stage, at flowering, at flowering pod formation, at pod development, and during seed storage respectively. Red spider mite and greasy cutworms affect cowpea just after seedling emergence (Uddin *et al*., 2013). Insect pests affect yield by causing quantitative and qualitative losses. A realistic method of control appears to be cultivation of insect resistant varieties in combination with applications of insecticides in minimal amounts and use of cultural control methods.

Weeds directly damage cowpeas, competing for light, moisture, space and nutrients. Weeds also indirectly damage cowpeas by harboring insect pests or intercepting insecticides and reducing their effectiveness. Parasitic weeds like *Striga gesneriodes* and *A. vogelii* sporadically cause damage to cowpeas. *S. gesneriodes*is more prevalent in dry and hot areas and *A. vogelii* is mostly found in relatively dry and cooler areas. The occurrence of these weeds is generally associated with continuous cropping of cowpeas.

2.4 Botany and Geographical Distribution of *Alectra vogelii*

Alectra vogelii Benth belongs to a family Scrophulariaceae (Timko *et al.,* 2007), which is currently known as Orabanchaceae. The Orabanchaceae consists of almost all parasitic

plants due to breaking up and reorganization of the Scophulariaceae basing on several recent phylogenetic analyses. It is found in subtropical of Asia and South America, tropical Africa and subtropical southern Africa, west and central Africa, eastern and southern Africa (Timko *et al.*, 2007). This parasitic weed has spread in Tanzania, Kenya, Uganda, Botswana, Congo, Ghana, Guinea, Malawi, Mozambique, Nigeria, Zambia, Zimbabwe (Ghazanfar *et al.*, 2008).

A. vogelii is most commonly found in areas of mono-modal rainfall with a long dry season but it is also found in bimodal rainfall areas. In Tanzania, *A. vogelii* was reported for the first time in Hombolo and Naliendele in 1988/1989, but today *Alectra* is found in wide geographical locations of the country, infesting all cowpea varieties released in Tanzania such as Tumaini, Fahari, Vuli-1, and Vuli-2 (Mbwaga *et al.*, 2007). In the country, *A. vogelii* is distributed in Mwanza, Shinyanga, Dodoma (Kongwa, Dodoma Rural, and Dodoma Urban), Singida (Singida Rural, Manyoni, Mkalama), Iringa, Njombe, Tanga (Korogwe), Morogoro (Kilosa), Ruvuma.

2.5 Effect of *Alectra* on Cowpea Yield Losses

In the country, cowpea productivity is affected by existence of *A. vogelii* which cause up to 50% yield loss (Karanja *et al*., 2013; Kabambe *et al.*, 2013; Mbwaga *et al*., 2013). By average, low cowpea yield was reported in Tanzania as 317 kg/ha (Singh *et al.*, 2001), 319 kg/ha (Mbwaga *et al.*, 2010), and 225.7 kg/ha (ICRISAT, 2011). These reported average yields are low compared to a potential cowpea yield of 2000 to 3000 kg/ha in Tanzania (Mbwaga *et al.*, 2013). On the efforts of controlling this *Alectra*, improved *Alectra* resistant cowpea genotypes were developed and released in Tanzania (Kabambe *et al.*, 2008; Mbwaga *et al.*, 2013).

Lacking functional roots and a photosynthetic system, the *Alectra* develops special intrusive organs (haustorium) that directly connect to the vascular system of the host crop.by developing a metabolic sink stronger than that of the host; the *Alectra* channels the flow of water and nutrients from the host crop, thereby demanding crop development. In short, the roots of cowpea together with that of growing *Alectra* permit transfer of water and nutrients from cowpea to parasitic *Alectra* by creating a sink demand that its consequence results into reduction in cowpea shoot biomass and eventually cause the reduction in pod formation resulting into poor cowpea yield (Timko *et al.,* 2007). So, A. *vogelii* has effect on root biomass, stem thickness, root nodulation, reduction on shoot biomass and consequently cowpea yield loss (Omoigui *et al.,* 2012; Kutama *et al*., 2014; Karanja *et al.*, 2013). Before *A. vogelii* emergence above the ground, affected cowpea crop may show wilting, delayed flowering, and may show reduced number of flowers and pods which contribute to yield loss. But, the yield loss depends on the genetic potential of the cultivar.

A. vogelii infestation remains as a major constraint to cowpea production in Sub Saharan Africa. Infestation by *A. vogelii* is by diverse *Alectra* strains that attack cowpea and has resulted into abandoning of several arable lands (Emechebe *et al.,* 2002; Magani *et al.,* 2009). Crop yield losses resulting from *A. vogelii* infestation cause crop loss in highly susceptible cultivars, sometimes can be low loss depending on the type of crop or cowpea variety attacked. Typical yield losses of 50 % - 100 % due to *A. vogelii* has been observed in heavily infested fields, depending on the infestation level and susceptibility of the cultivar (Mbwaga *et al.,* 2000; Kabambe *et al.,* 2013; Mbwaga *et al.,* 2010; Karanja *et al*., 2013). In cowpea growing areas where there is no high infestation the crop is either resistant to the *A. vogelii* strain or there is less virulent strain (Hussien *et al.,* 2006). Variability for *A. vogelii* resistance has been widely reported (Riches, 2001) and it is

recommended that routine screening against *Alectra* strains is conducted so that choices of varieties to farmers may include *A. vogelii* resistance. However, among other factors, cowpea yields in Tanzania remain low as compared to potential yields mainly due to *Alectra* strain that has genetic diversity which is broadly distributed across *A. vogelii.*

2.6 Control Strategies of *Alectra vogelii*

Many control strategies of *A. vogelii* have been proposed and developed. These strategies include crop rotation, trap-cropping, mixed cropping, herbicide application, fumigation of the soil, nitrogen and phosphorus fertilization all which have certain level of efficacy for controlling the *A. vogelii*, but are beyond the reach of the average resource- limited farmers in Sub Saharan Africa (Gressel *et al.,* 2004). Thus, the proposed strategies are unfordable by small scale farmers, who make up to 75-80% of the farmers in Sub-Saharan Africa (Gressel *et al.,* 2004). Breeding for resistance is a well-established way of controlling and reducing the level of infestation (Koyoma *et al*., 2000). The tolerant and resistant varieties if are integrated with other appropriate methods, they bring potential to deplete seed bank and to prevent Alectra reproduction. For the plants with degree of resistance, there is breakdown of host resistance if new, more virulent, strains of *Alectra* occur. Therefore, it is of great importance to take measures that ensure the virulent strains are controlled by breeding for the durable resistance with broad spectrum of the improved genotypes. Sometimes, if the seeds are contaminated by *Alectra* seeds, through exchange of the seeds, there is high possibility that the resistance breakdown in one area could spread into another geographical area. Because of this, there is a need to understand the genetics of resistance of cowpea to *A. vogelii* and understand the molecular genetic bases of *Alectra* virulence (Mohammed *et al*., 2007)

2.7 Genetics of Resistance of Cowpea to *Alectra vogelii*

Cowpea is a diploid (2n = 2x = 22) crop having 22 chromosomes and nuclear genome which is estimated to 620 million base pairs (Timko and Singh, 2008, Timko *et al.,* 2007). The genetics of cowpea *Alectra*-resistance varies according to the biotype (strains/races) of the parasite and varieties, and it is inherited mainly as a single, nuclear, dominant gene (Carsky *et al*., 2003). However, the resistance is conferred by two independent dominant genes or a recessive single gene. In cowpea, resistance depends on *Alectra* strains and results from one or a combination of several recognized mechanisms that influence the development of the parasite. In addition, it is noteworthy that low emergence of *Alectra* under infestation is sufficient to confer field resistance *Alectra,* independent of other resistance mechanisms. New cowpea varieties are built with a single resistant gene that makes *Alectra* strain arise and overcome the stability of the varieties in the field. This is because *A. vogelii* has distinct strains that differentially parasitize cowpea varieties (Timko *et al.,* 2007). 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. The same landrace B301 is having high resistance against *A. vogelii* in some areas of Tanzania where it has been tested (Hella *et al.,* 2013). The cowpea breeding line IT81D-994 is resistant to *A. vogelii* in Nigeria, but susceptible to isolates from Malawi. The resistance, which is based on a combination of several resistance mechanisms, is more likely to last longer than resistances that are based on a single gene (Rubiales *et al.,* 2006). A line is a plant or progeny still under breeding process like B301. A variety is a group of plants within a species or subspecies which share similar characteristics but differ in respect of those characteristics from other groups or varieties within the species.

Breeding work has been done on resistant cowpeas to *A. vogelii*. Resistant sources were identified under field screening trials using different cowpea accessions, followed by

evaluation at many locations hence identification of new source of resistant genotypes which showed stable resistance to *A. vogelii*. This means that, a number of other lines and varieties were identified. These lines and varieties include, IT82D-849 (an improved breeding line from IITA), B301 (a landrace from Botswana), IT86D-534, IT86D-371, IT84D-666, and Suvita-2. However, there are some challenges on these genotypes. For example, IT82D-849 found to be resistant to *Striga* but susceptible to *Alectra*, whereas IT86D-534, IT86D-371 and IT84D-666 are moderate resistant to *Striga* and highly resistant to *Alectra*. B301 is highly resistant to both *Striga* and *A. vogelii.* Cowpea genotype B 301 is resistant to *Alectra*, because its genetics of resistance to *Alectra* is conditioned by duplicate dominant genes. Suvita-2 is resistant to the Striga, but susceptible to *A. vogelii.*

Since 2006, in Tanzania, various series of screening trials were conducted to identify genotypes of cowpea for resistance to *A. vogelii*, under cowpea-*Alectra* project in McKnight Foundation Collaborative Crops Research Programme. This has been revealed on the existence of cowpea accessions which are resistant to *A. vogelii* (Mbwaga *et al*., 2007; 2010). This project also works in Malawi where -IT99K-494-6 was released as Mkanakaufiti a variety resistant to *A. vogelii* (Hella *et al.,* 2013; Mbwaga *et al.,* 2013; Kabambe *et al.,* 2014). Various cowpea accessions from the research institutions in Tanzania, National Plant Genetic Research Centre (NPGRC), International Institute of Tropical Agriculture (IITA) and other seeds from farmers were assembled and screened for *Alectra* resistance in various locations with high infestation of *Alectra.* Some few lines showed resistance to the growth of *A. vogelii*. These include B301, IT97K-499-38, IT81D-994, IT99K-573-1-1, IT97K-499-8, IT97K-818-35, IT97K-819-118, IT99K-7-21-2-2, IT99K-494-6 and TZA 263 on station and on farm trials *(*Mbwaga *et al*., 2010; Hella

et al., 2013). Out of these, the lines IT99K-573-1-1 and IT99K-7- 21-2-2 were released as Vuli-AR1 and Vuli-AR2 in Tanzania (Hella *et al.,* 2013; Mbwaga *et al.,* 2013).

The genetic diversity in *A. vogelii* strains is due to increased use of cowpea cultivars with improved resistance and hybridization in some countries, persistent *Alectra* seed bank for various generations of populations, long distance dispersal, wide geographical distribution, wide host range, local host preference and adaptation (Mohamed *et al.,* 2007). Timko *et al.,* (2007), reported on geographic variation in host preference observed in *A. vogelii* on the sense that, *A. vogelii* populations from West Africa and Cameroon attack cowpea and groundnut. 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 Bambaranuts. "Studies in Tanzania, which was undertaken in pot experiment indicated that at a species level, there are 3 strains of *Alectra* by host range for example, *Alectra* from Singida attaches and emerges on cowpea and groundnut but not on mung or common bean, *Alectra* from Bihawana and Ismani attaches and emerges on cowpea, groundnut, common bean but not on mung bea and *Alectra* from Malawi sites attaches and emerges on cowpea, groundnut, common bean and mung bean" (Mbwaga *et al.,* 2008).

Alectra vogelii is well known for its impressive abilities to adapt to different habitats and agro-ecosystems by developing host specific strains and ecotypes across their ranges, hence, geographical distribution appears to play a role in genetic diversity of *A. vogelii* (Aigbokhan *et al.,* 2000). This makes *A. vogelii* to be wide spread in cowpea growing areas of Tanzania and Sub-Sahara African countries at large. The virulence variability within *A. vogelii* due to diverse *A. vogelii* strains makes breeding programs for resistant

cowpea varieties be more complicated. This therefore, calls for an intensive molecular study on the level of genetic variability within *A. vogelii* strains for effective breeding programs.

2.8 Molecular Markers Used in Diversity Studies of *Alectra vogelii*

Genetic characterization using molecular markers offers a more powerful technique in detection of the genetic diversity of parasitic plants. The differences revealed by phenotypic observation are at the level of protein or gene expression, hence to study this requires the molecular markers. Molecular markers are DNA sequences associated with certain parts of the genome which are used to assess genetic diversity and to establish phylogenetic relationship among the plant species (Koyama, 2000; Rasha *et al.,* 2009). The molecular methods reveal differences in genotypes variation embodied by DNA sequencing of organisms which is not influenced by the environmental factors. Molecular markers are more reliable for genetic studies than morphological characteristics because the environment does not affect them.

The morphological markers, biochemical markers and the DNA markers are markers, with important application in studying the genetic diversity of parasitic weeds like Striga and *A. vogelii*. Morphological markers are visible phenotypic markers such as flower color, seed shape, growth habit or pigmentation used to study the variations of visible traits between individuals (Semagn *et al.,* 2006). Biochemical markers are allelic variants of genes encoded proteins and enzymes called isozyme (Linda *et al.,* 2009). DNA markers are more efficient and reliable as compared to morphological and biochemical markers. DNA markers can be grouped in two generations. The first generation is that of DNA markers that employ Southern Blot Technology. Examples of such markers include the Restriction Fragment Length Polymorphism (RFLP) and Variable Number Tandem

Repeats (Yong *et al.,* 2009). The second generation of DNA markers includes those that employ Polymerase Chain Reaction (PCR) Technology (Yong *et al.,* 2009). The advantage of using the PCR technology in genetic diversity studies is that, it requires only a small amount of DNA to allow analysis and it is inexpensive. A variety of PCR based techniques have been applied in investigations of genetic diversity in Striga and *A. vogelii* which are Random Amplified Polymorphic DNA, Amplified Fragment Length Polymorphism and Simple Sequence Repeats.

2.8.1 Simple sequence repeats

Simple sequence repeats (SSR) markers are one of the most frequently used markers in the genetic diversity analysis of plant species (Asare *et al*., 2010; Badiane *et al.,* 2012). Simple Sequence Repeats markers are abundantly distributed in genomes and they are preferentially associated with non- repetitive DNA. The SSR are very polymorphic due to high mutation rates affecting the number of repeat units. The repeating sequence is small, consisting of 2, 3 or 4 nucleotides and can be repeated 3 to 100 times with the longer loci generally having more alleles due to greater potential for slippage (Wang *et al.,* 2008). The SSR markers are effective in determining genetic diversity among *A. vogelii* and Striga species (Yoshida *et al.,* 2010). The molecular markers linked to Striga resistance gene have been identified (Timko *et al.,* 2007). SSR marker which is linked to resistance to *Striga* has been the most widely applied in the Marker Assisted Selection and Marker Assisted Breeding for cowpea varieties.

Two main protocols are used to develop the Simple Sequence Repeats, and these protocols are, first, classical method which involves isolating SSRs from partial genomic libraries containing small size inserts by colony hybridization with probes that contain SSR sequence motifs. However, this technique is inefficient and a laborious task in cases

where species have large genomes (Varshney *et al.,* 2009). Though recent techniques are using oligonucleotide sequences which consist of repeats that are complimentary to repeats in the microsatellite to „enrich" the DNA extracted. Secondly, SSRs can be developed by mining data stored in the databank library. In the databank, genome scale molecular resources are deposited after sequencing the genome of plants. The sequences can be accessed and used by anyone for molecular diagnosis, for biotyping and for investigating genetic diversity and population structure of subject organisms. It begins by construction of a full length enriched complimentary DNA library and generation of a large scale Expressed Sequence Tags dataset by reading the sequences of individual clones. The SSRs markers developed from the cDNA clones stored in the databank are referred to as ESTs. ESTs are a less expensive alternative for gaining information about the expressed genes of an organism (Rudd, 2003).

The PCR process is used to test for polymorphism between individuals in a population or between populations in a species. Primer sets to be used in Polymerase Chain Reaction (PCR) are designed using sequences that are flanking the EST-SSR markers. EST-SSR markers seem to be effective in determining genetic diversity among *Alectra vogelii* and Striga species. EST means Expressed Sequence Tags which is a short sub-sequence of a cDNA sequence. The ESTs are instrumental in identifying gene transcripts, for gene discovery, gene sequence determination and in phylogenetics. EST from a full-length enriched cDNA library provides the complete sequences of functional proteins (Sarukai *et al.,* 2007). EST results from one short sequence of a cloned cDNA. The cDNA used for EST generation are typically individual clones from a cDNA library.

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CHAPTER THREE

3.0 IDENTIFICATION OF GENETIC VARIABILITY AMONG ALECTRA VOGELII STRAINS FROM DIFFERENT GEOGRAPHICAL LOCATIONS

Abstract

Alectra vogelii is a parasitic weed that poses a serious threat to the production of economically important cowpea crop in Sub-Saharan Africa. The weed presents a challenge to the successful development and deployment of effective control strategies against this parasitic weed. The information on magnitude and type of genetic variability of the *A. vogelii* populations facilitates the design and deployment of appropriate breeding strategy for *A. vogelii* resistance and other effective methods to control this parasite. In the present study, the genetic variability of the populations was analysed using 23 simple sequence repeats (SSR) markers. SSR marker analysis showed significant level of genetic variation within the populations of *A. vogelii* as revealed by high level of genetic variation within the populations. The values for observed number of alleles (Na), effective number of alleles (Ne), expected heterozygosity (He) and Shannon's Information index (I) for populations showed a relatively high level of genetic variability. Average variability was presented by Na value of 8 (3-11), Ne value of 1.5648 (1.264 - 1.7572), He value of 0.648 (0.532 - 0.877) and I spanned with value of 0.5169 (0.362 - 0.6197) with an average value of 0.5169. The mean PIC value was 0.8301. Genetic differentiation among the populations (Fst) was 0.2986 leaving 70.14 % of genetic variation exhibited within the populations. The high genetic differentiation among populations was coupled with existence of significantly high genetic diversity. A dendogram generated using NTSYS -pc (UPGMA) formed 4 clusters. The results suggest indicated diversity of A. vogelii in both same and different locations, hence breeding program should take care of

different strains. More information was generated on genetic variability among the existing *Alectra* strains to be used as a guide in broadening the gene pool of the cowpea crop for selection and development of resistant genotypes.

Keywords: *A. vogelii*, polymorphic information content, SSR markers, genetic variability

3.1 Introduction

Parasitic weeds of the Orabanchaceae are the devastating, destructive and affect many developing countries. They have potential to greatly decrease yield and quality of the host crops thus inflict on food and fodder plants (Westwood *et al*., 2012). *Alectra vogelii* (Benth) is a parasitic plant known to cause substantial losses in cowpea and other leguminous crops across countries in Sub Saharan Africa (Mbega *et al*., 2016; Mbwando *et al*., 2016; Njekete *al.*, 2017; Mbwando *et al.,* 2017). In Tanzania it is widespread in Mwanza, Shinyanga, Dodoma, Singida, Iringa, Njombe, and Ruvuma regions where cowpea yield losses of up to 50% has been reported (Mbwaga *et al*., 2000). Many cowpea fields have been abandoned because of high rates of *A. vogelii.* This weed is well known as it qualifies to be considered as a potential threat to crops because of its ability to adapt to different environmental conditions by developing host-specific strains (Mbega *et al*., 2016; Njekete *et al.*, 2017).

Control measures used for *A. vogelii* include, hoeing, deep cultivation and herbicides, destroying crop residue after harvest, crop rotation, the use of unimproved low yielding *Alectra* tolerant cultivars, with little effect, but these methods were generally found to be not effective (Shinggu, 2015). This is because hand hoeing is labour intensive and deep cultivation is too expensive for the resource poor farmers. Herbicides are not widely used because of high cost and unavailability of the chemicals and application equipment. The control of the weed through conventional agronomic practices has been difficult to

achieve since the parasite exerts its greatest damage before its emergence above ground (Geleta, 2010). Breeding for resistance is one of the main approaches to decrease yield losses caused by *A. vogelii* infestation and is said to be the main hope to control *A. vogelii*. So, breeding for improved cowpea varieties resistant to *A. vogelii* is the most potentially promising economic control measure since it is more affordable and cost-effective to farmers and resistant varieties can be grown without additional inputs (Teka, 2014). The method will not only eliminate the need of application equipment, but will also reduce the cost of production, reduce *A. vogelii* infestation and increase cowpea yields.

In different cowpea fields, locally adapted *Alectra* strains which are diverse at the intraspecific level have been observed being quickly adapting to the host (Welsh and Mohammed, 2011; Atera *et al.*, 2013). They cause successive breakdown in host resistance. A recurring problem in breeding for resistance to *A. vogelii* strains is that, the crop resistance developed through breeding at one location may not hold up when the crop is moved to new areas with different parasite populations (Westwood *et al.*, 2012). Practically, presence of *Alectra* strains in the country bring difficulty, complicate and frustrate efforts to develop universally sustainable resistance, thus undermining the struggle to attain food security, and so their control must be addressed by any means (Mbwaga *et al.*, 2007; Westwood *et al.*, 2012; Atera *et al.*, 2013).

Identification of genetic variability of *A. vogelii* is one of the important cornerstone of crop improvement because it gives an understanding of the extent, distribution and patterns of genetic variation of *A. vogelii*. Having the knowledge on genetic variability of *Alectra* weed provides vital information for the development of innovative control options (Slotta, 2008). To get knowledge on genetic variability, DNA based molecular

markers such as SSR are used. SSR markers are easy to use, co-dominant, locus-specific, highly polymorphic, abundant and, dispersed throughout the genome. This enables powerful comparative genetic and genomic analysis (Appleby *et al.,* 2009; Yoshida *et al*., 2010; Estep *et al*., 2011). They have been used to determine gene flow and mating system in parasitic species of the Orobanchaceae (Appleby *et al*., 2009; Yoshida *et al*., 2010; Estep *et al.,* 2011).

In overcoming *Alectra* strain problems in cowpea, the most reliable method is to identify polymorphic gene loci across the genome of *A. vogelli* linked to cowpea specific parasitism using molecular markers (Mbwaga *et al*., 2009; Westood *et al*., 2012). The information obtained can further be used in designing a breeding scheme for cowpea genotypes with resistance specific to each *Alectra* strain. The objective of the study was to determine the genetic relationships among *A. vogelii* populations from different locations using SSR analysis.

3.2 Materials and Methods

3.2.1 Study area

The study was conducted at Sokoine University of Agriculture (SUA), Morogoro, Tanzania. The University is at, latitude of $6^0$5'S, longitude of $37^0$39'E and an altitude of 524 m a.s.l. The young leaf samples of *A. vogelii* were collected directly from the infested cowpea fields across the selected locations (Table 1 and Fig 1). All these populations of *A. vogelii* were collected from five different administrative regions between altitudes of 450- 1900 m.a.s.l, (Table 1) which are low, mid to high altitude areas.

3.2.2 Collection and preservation of leaf samples

The samples of young leaves were randomly collected with at each location. Young leaves have lower content of polyphenols, polysaccharides and other secondary

metabolites which coprecipitate with DNA in the extraction procedure (Zhang and McStewart, 2000). The leaf samples were preserved at -20°C in 1.5 mls eppendorf tubes for two weeks to freeze the tissue before DNA extraction was carried out.

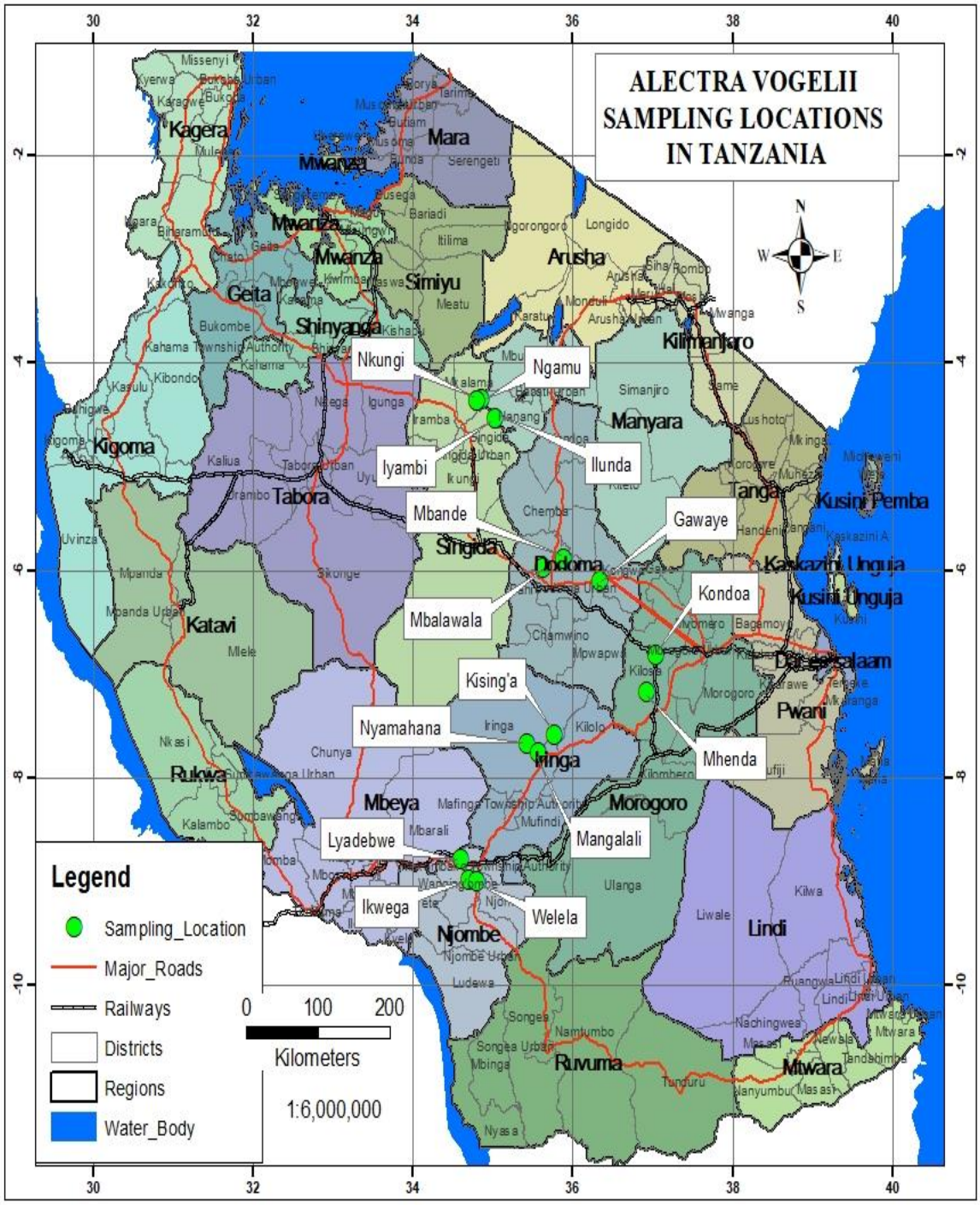

Figure 3.1: Map of Tanzania highlighting *Alectra vogelii* sampling locations

Table 3.1: *A. vogelii* collection sites from selected areas of Tanzania

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

3.2.3 DNA Extraction

The DNA was extracted using Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (Doyle and Doyle 1990). A total volume of 50 mls CTAB buffer (CTAB 1 g, Tris base 0.605 g, EDTA 0. 37 g, NaCl 4.1 g) was prepared. In the fume hood, 40 µl β-mercaptoethanol was added in the cooled CTAB buffer. Approximately 300 milligrams of the leaf materials used for DNA extraction were ground in 600 µl CTAB using cleaned and autoclaved mortar and pestle. The homogenate ground DNA sample was transferred into 1.5 ml tube and incubated at 65 ºc for 30 minutes in a water bath followed by cooling under room temperature. Then, 600 µl of chloroform: isoamyl alcohol (24:1) was added to the sample equally and placed for vortex until white. Centrifugation was done for 10 minutes at 14 000 rpm.

After centrifugation and before adding the isopropanol, there was aliquot of the supernatant to a new tube. In order for the DNA in the solution to aggregate and precipitate out, 600 µl isopropanol was added followed by 60 µl of 0.75 M ammonium acetate. The aqueous was gently mixed. Ammonium acetate was added to remove cellular and histone proteins bound to the DNA. It was followed by freezing at 30 minutes, and centrifugation was done for 10 minutes at 14 000 rpm. The supernatant was discarded. The DNA pellet was washed with 800 µl of cold 70% ethanol, followed by incubation at -20 ºc for 10 minutes, and centrifugation at 13 000 rpm for 5 minutes. The pellets were diluted by 50 µl TE (pH 7.4, 10 mM Tris, 1 mM EDTA) for PCR after re- suspension.

3.2.4 Evaluation of Genomic DNA

Genomic DNA of the samples was analysed by agarose gel electrophoresis. DNA concentration of each *Alectra* sample was estimated by running samples in 1 % agarose gels. The gels were prepared in advance, using 1 g of agarose diluted in 1X 100 mL TBE

buffer (0.89 M Tris base, 0.89 Boric acid, 20 mM EDTA, pH 8.0) and stained with 10 µL Ethidium bromide. Four microliters of extracted genomic DNA was mixed with 6 µl of loading dye (New England Bio Labs Inc) (NEB). The DNA was run alongside 6 µl of 50 kb genomic DNA ladder (New England Bio Labs Inc) (NEB). The gel was run at 120 volts for 1 hour and then visualized using an ultra-violet transilluminator. A photo of the resulting gel was documented using a digital camera.

3.2.5 Polymerase Chain Reaction (PCR)

A total of 23 SSR pair of primers developed specifically for *Striga hermonthica* and *Striga asiatica* (Yoshinda *et al.*, 2010; Estep *et al*., 2012) as amplifying more numbers and clear diversity bands were selected to be used in this study. These primers were used to identify DNA fragments from 15 *Alectra* populations. To determine the genetic variation, the SSR primers (Integrated DNA Technologies) and a master mix (New England Bio Labs Inc) (NEB) were used. The PCR reactions contained of 12.5 µl of 2X master mix with standard buffer, 0.5 µl each of forward and reverse primer, 9.5 µl of nuclease- free water and 2 µl of template DNA in a final volume of 25 µl PCR reaction mixture. The polymerase chain reactions were performed in a master cycler machine. The reactions involved initial denaturation at 94 ºC for 1 min, annealing 45 ºC for 30 seconds, extension 72 ºC for 1 min. The PCR consisted of 35 cycles with a final elongation of 10 min at 72 ºC then stored at 4°C (∞). The PCR product was stored in a refrigerator at 4 ºC until analyzed.

3.2.6 Gel Electrophoresis

The PCR products were separated using 3% agarose gel. Electrophoresis was done using 1X TBE buffer. The gel was pre-stained using Ethidium Bromide (EtBr). Fragments were separated by horizontal electrophoresis apparatus at 120 V for 1 hour. The gels were photographed using a digital camera under UV transilluminator.

3.3 Statistical Analysis

Amplified bands of each marker were scored for their sizes. The single-population descriptive genetic statistics including number of alleles per locus (Na), number of effective alleles per locus (Ne), diversity (richness), effective allelic diversity (evenness), expected heterozygosity (gene diversity), observed heterozygosity and Shannon's Information index were calculated by using GenAIEx 6.1, and Popgene software version 1.32 (Peakall and Smouse, 2006). In order to measure the efficiency of polymorphic loci of 8 primer combinations for detecting the genetic diversity among the studied populations, the polymorphic information content (PIC) values were calculated. The values were obtained basing on allele frequency (P). The PIC values were calculated using the formula PIC value = $1 - \sum_i^n 1Pi^2$ where, *pi* is the frequency of the *i*th allele (Smith *et al.,* 1997). The additional information about genetic variation of the *A. vogelii* populations were studied on the mean descriptive population genetic statistics for all the 15 populations. The fixation index per population was calculated.

Analysis of Molecular Variance (AMOVA) was performed to determine the variance among and within the population by using GenAIEx 6.1 software based on 1000 permutations. The AMOVA was estimated and partitioned into the total molecular variance within and between populations and then tested the significance of partitioned variance components using permutation testing procedures. Genetic relatedness among the populations was studied by UPGMA (Unweighted Pair Group Method with Arithmetic Average) cluster analysis using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) Version 2.1 (Rohif, 1998).

The F-statistics (Fst) (Wright, 1978) were computed for polymorphic loci to test for the departure from Hardy- Weinberg equilibrium and to estimate genetic differentiation

among *A. vogelii* populations. The outcrossing rate $t = (1 - Fst)/(1 + Fst)$ was calculated on bases of Fst values to estimate indirect mating pattern of *A. vogelii* (Wright, 1978). The genetic distance matrix was then used in the subsequent Principal Coordinates Analysis (PCoA) and Mantle's test. To assess and understand further the genetic relationships of *A. vogelii,* a principal coordinate analysis (PCoA) was conducted based on the SSR data matrix of the 15 *A. vogelii* populations. The correlation matrix was selected to calculate coefficients of principal coordinate analysis. The Mantel's test was performed to examine the correlation between genetic distance and geographic distance among 15 populations. The ArcGIS software was used to generate the map and geographic distance matrix from GPS cordinates. The number of migrants per generation was calculated from Fst value using the equation Nm $=(1 - Fst)/(4Fst)$ to determine the gene flow among the populations.

3.4 Results

3.4.1 Variation in efficiency and polymorphism of SSR Markers

Among twenty-three primer pairs tested, only eight (SH 1007, SH1008, SH 1016, SH 1029, SH 3031, SH1032, SH1042 and SH1061) generated reproducible amplification products (Table 3.2).

Markers had the mean effective number of alleles (Ne) of 1.3893, however, SH1008, SH1016, SH3031 gave highest effective number of allele values whereas SH 1061 gave the lowest value (Table 3.2). All loci had almost equal effective alleles which means, the highest diversity. The loci also had the highest allelic diversity (richness) as well as effective allelic diversity (evenness) in the same order, SH 1008 had highest values and SH 1061 with lowest values. The diversity analysis of the markers using Shannon's index (I) as well as the expected heterozygosity (He) ranked the markers with an average of 0.4238 (I) and 0.7913 (He) respectively. The (I) ranged from 0.2512 to 0.6563. The markers SH1008, SH1016 and SH3031 gave the highest Shannon's index (I) value

whereas SH 1061 gave the lowest Shannon's index value. Average observed heterozygosity (Ho) was 0.7015 ranging from 0.3132 to 0.8194 while average gene diversity or mean expected heterozygosity (He) was 0.7913 ranged from 0.3451 to 0.9309. The eight primers all had PIC above 0.5 which means that, they are highly informative. The average polymorphic information content (PIC) was 0.929. The lowest PIC value was 0.5969 for primer SH1008 and the highest was 0.9952 for primer SH1061. The SSR marker analysis of the fifteen *A. vogelii* populations revealed a high level of genetic variation of eight loci. For all primers used, the amplification was inefficient because 35% of primers were amplified.

Table 3.2: Population genetic structure data of SSR loci linked to *A. vogelii* strains

Locus	Na	Ne	Aa	Ae	I	Ho	He	PIC
SH1007	3	1.2328	8.0328	4.8189	0.3372	0.8194	0.8944	0.9888
SH1008	13	1.8644	8.6644	7.6306	0.6563	0.4183	0.3451	0.5969
SH1016	7	1.6500	8.4500	6.9391	0.583	0.3132	0.7189	0.9276
SH1029	3	1.2328	8.0328	4.8819	0.3372	0.8127	0.8944	0.9888
SH3031	5	1.4279	8.2279	4.9973	0.4767	0.8152	0.8101	0.9663
SH1032	3	1.2328	8.0328	4.8819	0.3372	0.8127	0.8944	0.9888
SH1042	4	1.3263	8.1263	6.462	0.4115	0.8101	0.8423	0.9794
SH1061	2	1.1475	7.9475	4.2186	0.2512	0.8107	0.9309	0.9952
Mean	5	1.3893	8.1893	5.6038	0.4238	0.7015	0.7913	0.929

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

The additional information about genetic variation of the *A. vogelii* populations indicated high level of diversity within the populations (Table 3.3). Populations from Ilunda and Iyambi exhibited highest alleles, whereas Kondoa had fewest alleles. The diversity analysis using Ne, Aa, Ae, I, Ho, He, PIC, F and Nm, ranked the populations from the most diverse to the least diverse. The populations with similar values of descriptive genetic statistics were ranked together. The fixation index (F) also called the inbreeding

coefficients, exhibited values between -1 and +1 which are the limit values for the coefficients (Table 3.3).

Table 3.3: Descriptive population genetic statistics for all *A. vogelii* populations

Populations	Na	Ne	Aa	Ae	I	Ho	He	PIC	F
Lyadebwe	8	1.5003	8.3003	6.1822	0.4929	0.6339	0.7222	0.8918	0.1222
Ikwega	10	1.6462	8.4462	6.3140	0.5665	0.6168	0.5776	0.7816	-0.0679
Welela	8	1.6474	8.4474	6.5223	0.5720	0.5156	0.6247	0.8303	0.1746
Nyamahana	9	1.6462	8.4462	6.314	0.5665	0.6168	0.5776	0.7816	-0.0679
Mangalali	4	1.3434	8.1434	5.3255	0.3905	0.6478	0.8481	0.9705	0.2362
Kising'a	8	1.5486	8.3486	6.2563	0.4968	0.6155	0.6198	0.7929	0.0069
Gawaye	8	1.5486	8.3486	6.2248	0.4968	0.6189	0.6198	0.7929	0.0015
Mbalawala	8	1.6474	8.4474	6.5223	0.572	0.5156	0.6247	0.8303	0.1746
Mbande	6	1.4255	8.2255	5.6978	0.433	0.6349	0.7567	0.8995	0.1610
Ngamu	9	1.5954	8.3954	7.0463	0.5339	0.6142	0.5937	0.7882	-0.0345
Nkungi	9	1.5954	8.3954	7.0463	0.5339	0.6142	0.5937	0.7882	-0.0345
Iyambi	11	1.7572	8.5572	7.2849	0.6197	0.3658	0.532	0.7623	0.3124
Ilunda	11	1.7572	8.5572	7.2849	0.6197	0.3658	0.532	0.7623	0.3124
Mhenda	8	1.5486	8.3486	6.2563	0.4968	0.6155	0.6198	0.7929	0.0069
Kondoa	3	1.2640	8.064	5.4086	0.3620	0.8118	0.877	0.9857	0.0743
Mean	8	1.5648	8.3648	6.3791	0.5169	0.5869	0.648	0.8301	0.0919

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

3.4.2 Genetic diversity

The genetic relationship among the 15 *A. vogelii* populations was revealed by Nei‟s genetic distance values that ranged from 0.2027 to 1.3540, the smaller values indicating a closer relationship (Table 3.4). The highest similarity (0.2027) was observed between Ikwega and Welela, Ikwega and Mbalawala, Welela and Nyamahana, Welela and Iyambi, Welela and Ilunda, Nyamahana and Mbalawala *A. vogelii* populations. The lowest similarity (most diversified) was observed between *A. vogelii* populations from Lyadebwe and Mangalali, Mbande and Kondoa (1.354), revealing high genetic variability between these populations.

Table 3.4: Pairwise population matrix of Nei‟s genetic distance (1972) for the 15 *A. vogelii* populations

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

The AMOVA which was obtained by the genetic distance matrix gave highest genetic differentiation (Fst) and all populations were significantly ($P < 0.01$) different (Table 3. 5). AMOVA analysis showed that a significant genetic variation was observed at the level 1% when the observed value was greater than the permutated value at 99%. Internal variation was observed within *A. vogelii* populations. Furthermore, significant divergence (29.86 %; *Fst* = 0.2986; p – value = 0.001) among the fifteen *A. vogelii* populations was also detected. The genetic differentiation in AMOVA (Fst = 0.2986) using stepwise mutation gave the number of migrants per generation, thus the gene flow among the populations was 0.5872

Table 3.5: Analysis of molecular variance (AMOVA) for 15 *A. vogelii* populations

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

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

3.4.3 Cluster analysis

The genetic distance from one cluster to another gave the genetic relationship among clusters (Fig. 3.2). The smaller values of genetic distance indicated a closer relationship with populations of highest similarity. The genetic distance values had mean genetic identity with a value of 0.43 for all clusters. The genetic distance at less than 0.43 revealed biologically meaningful numbers of clusters. Thus, at 22 % of the variations observed, the dendogram grouped the populations into four clusters. The first cluster encompassed of seven populations from Lyadebwe, Ikwega, Welela, Mbalawala, Kondoa,

Iyambi and Ilunda, meaning that they are related. The second cluster consisted of four populations from Nyamahana, Mangalali, Kisng'a and Ngamu, which are closely related. The third cluster included two populations fom Gawaye and Mbande. The rest two populations from Nkungi and Mhenda formed the fourth cluster which showed distant relationship with the first, second, and third.

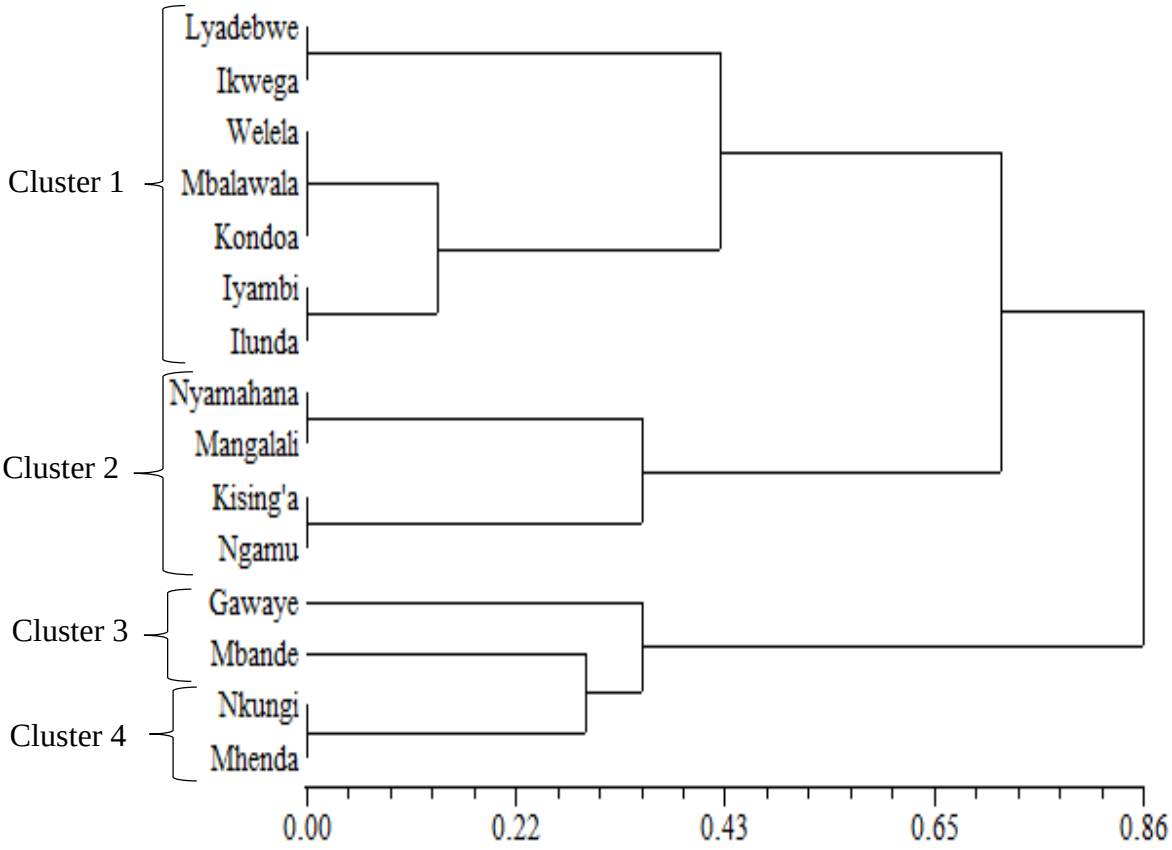

Figure 3.2: Phylogenetic relationship among the *A. vogelii* populations. Shown is a UPGMA dendrogram constructed based on Nei‟s (1972) genetic distance with NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) Version 2.1.

3.4.4 Principal Coordinate Analysis (PCoA)

Principal coordinate analysis explains the variation of populations and splits the geographical locations into two distinct groups in three-dimensional space (Fig. 3.3). This

was conducted using pairwise comparisons of Nei's standard genetic distance to identify major patterns within the data set and possible differences between populations. Group one (on the left) contains populations of Kondoa, Gawaye, Nkungi, Ngamu, Kising'a, Mhenda, Mbande and Mangalali and group two (on the right) contains populations from Lyadebwe, Iyambi, Ilunda, Nyamahana, Ikwega, Mbalawala and Welela. The populations from Nkungi, Ngamu, Mhenda and Kising'a, were close to each other, whereas other populations which were close are Mbalawala and Welela, Nyamahana and Ikwega as well as Iyambi and Ilunda. A relatively separated distribution of the populations within three dimensional spaces in the PCoA analysis futher revealed very high genetic differentiation among all populations of *A. vogelii*. The populations which were close to each other were significantly correlated, on orthogonal were not correlated and all populations on the opposite side of the center were significantly negatively correlated. The scatter plot of the first, second and third PCoA showed a clear genetic variation and differentiation pattern of the *A. vogelii.*

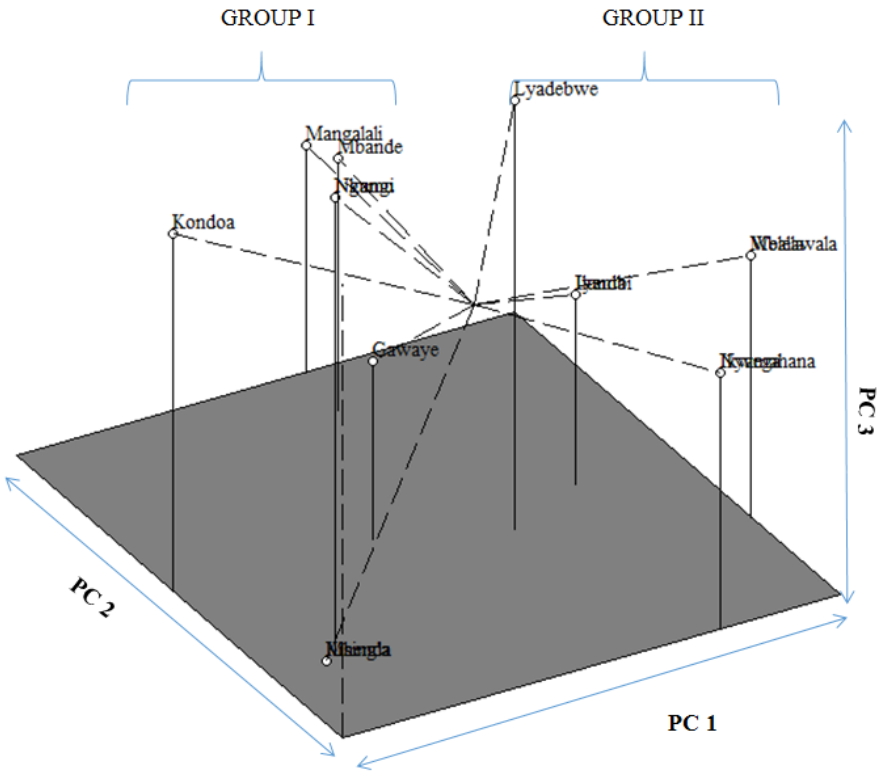

Figure 3.3: Principal Coordinate analysis of pairwise genetic distance between *A. vogelii* populations

3.4.5 The Mantel's test

Mantel's test provided the option and allowed to test the statistical relationships between the elements of two distance matrices between genetic distance (Table 3.4) and geographic distance (Table 3.6). The Mantel's test gave low isolation by distance (r = 0.14917). This was declared as not significant at level of 1% when observed value was less than the permuted values at 99%. Thus, this result indicated that, there was no significant correlation between the genetic and geographic distances.

Table 3.6: Geographical distance matrix (km) for the sampling locations

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

3.5 Discussion

All eight primer combinations were polymorphic and revealed high levels of genetic variability as indicated by high PIC values and Shannon's index. The PIC was used to assess the informative potential of SSR markers. In this study the obtained values of PIC were highly informative with the mean value of 0.929. This value on PIC, indicated that, these SSR markers are informative and they have ability to distinguish different populations. The result can be used to study the *A. vogelii* and other parasitic plants at molecular level. Loci polymorphism is divided into three levels based on information content. These levels are high, medium and slightly informative markers which are in the order of PIC value > 0.5, 0.5 > PIC value > 0.25, and PIC values < 0.25, respectively (Botstein *et al.*, 1980). All populations of *A. vogelii* used in this study exhibited PIC values greater than 0.5, with an average of 0.8301, expected heterozygosity (gene diversity) value and Shannon's index value of all populations were 0.648 and 0.5169 respectively. These values revealed that *A. vogelii* contain abundant genetic diversity.

The fixation index (F) also called the inbreeding coefficients, showed in which direction populations were trending out of Hardy-Weinberg proportions. Among 15 populations there were 11 populations appearing to have substantial excessive homozygosity and the remaining four populations indicating excess of heterozygosity due to negative assortive mating, or selection for heterozygosity in comparison to gene diversity. Departures from Hardy–Weinberg Equilibrium (HWE) due to heterozygosity excess were detected in four *Alectra* populations (Ikwega, Nyamahana, Ngamu and Nkungi), due to small reproductive population size, existence of heterosis and effect of gametophytic self-incompatible system. The small reproductive size causes heterozygote during non-random mating. The other 11 populations of 73.3% showed significant departure from HWE with heterozygote deficiencies thus excess homozygosity. An excess of homozygosity is due to the presence

of null alleles and inbreeding. Inbreeding is due to the limited dispersal of the *Alectra* mating with the siblings or close relatives. The populations from Nkungi, Ngamu and Gawaye showed the values with random mating whereby all values of genetic statistics revealed that *A. vogelii* contains abundant genetic diversity.

The genetic relationship among the 15 *A. vogelii* populations was revealed by Nei‟s genetic distance values, the smaller values indicating a closer relationship. The highest similarity was observed between Ikwega and Welela, Ikwega and Mbalawala, Welela and Nyamahana, Welela and Iyambi, Welela and Ilunda, Nyamahana and Mbalawala *A. vogelii* populations. This similarity was attributed by high probability of occurrence inbreeding within the populations driven by random genetic drift (Yang *et al.,* 2012). Therefore, low genetic diversity indicates that there has been inbreeding due to decrease in population size. The lowest similarity (most diversified) was observed between *A. vogelii* populations from Lyadebwe and Mangalali, Mbande and Kondoa, revealing high genetic variability between these populations. High genetic variability was attributed by very extensive and recurrent gene flow (Chiang *et al.,* 2006). Generally, the genetic variation of *A. vogelii* was influenced by dispersal of genes over long distance, history of introduction, genetic drift, population size and founder effects (Tremblay *et al.,* 2005; Barrett and Schluter, 2008; Begg *et al.,* 2012; Gaskin *et al.,* 2012). The study identified some populations from different geographical locations having very similar genetic diversity. These populations have genetically mixed in the fields, due to deliberate exchange of contaminated cowpeas seeds by farmers and trading activities from one location to another with uncontrolled genetic mixing of the *A. vogelii.* The other populations from the same geographical locations were identified to have different genetic diversity. This implies that these populations have not been markedly impacted by gene flow within the same locations, thus there is very low level of gene admixture. This

finding relates to Begg *et al. (*2012) who found that, the life history traits (time of flowering, fecundity and dormancy), genetic drift effects and selection to varying environmental conditions are the cause of genetic differentiation both between individuals and among accessions from different populations, of the same geographical locations.

The AMOVA gave significant divergence among the fifteen *A. vogelii* populations and all populations were significantly different ($P < 0.001$; Table 3.5). The genetic differentiation in AMOVA using stepwise mutation gave the number of migrants per generation, thus the gene flow among the populations which was 0.5872. The AMOVA further revealed the existence of high genetic diversity within the populations. The existence of high significant variations under the study may be due to sudden genetic variation in population size in short time spine. High genetic diversity ensures that *A. vogelii* is able to survive and adapt. If a new environmental pressure, come along all of the individuals of *A. vogelii*, chances are better that some individual plants will have a genetic makeup that allows them to survive. These individual plants will reproduce, and the population will survive. The observed coefficient of genetic differentiation among the populations demonstrated the presence of 29.86 % genetic variation among the populations and 70.14 % within the populations. The Fst value observed was 0.298 and according to Wright (1978) any value greater than 0.25 indicates that there was very high genetic differentiation within population. High variation was observed within the populations than among populations of *A. vogelii* thus*,* AMOVA of *A. vogelii* showed a high level of intra-population and low level of inter-population variation ($p < 0.001$). The very high level of genetic differentiation revealed by Fst indicated that, the populations had departed slightly from Hardy-Weinberg equilibrium (Liu *et al.,* 2006). This high magnitude of genetic differentiation was due to the influence of seed dispersal among the

populations, since the movement of genes of the populations is accomplished by the dispersal of the seeds (Hamrick and Loveless, 1986).

The genetic distance from one cluster to another gave the genetic relationship among clusters (Fig. 3.2). The smaller values of genetic distance indicated a closer relationship with populations of highest similarity. At 22 % of the variations observed, the dendogram grouped the populations into four clusters. The first cluster encompassed of seven populations from Lyadebwe, Ikwega, Welela, Mbalawala, Kondoa, Iyambi and Ilunda, meaning that they are related. The second cluster consisted of four populations from Nyamahana, Mangalali, Kisng'a and Ngamu, which are closely related. The third cluster included two populations fom Gawaye and Mbande. The rest two populations from Nkungi and Mhenda formed the fourth cluster which showed distant relationship with the first, second, and third. Such clustering revealed that, there was no significant relationship between geographical locations and genetic divergence of *A. vogelii.* The clusters formed provide essential information in the formulation of appropriate management strategies. The clusters obtained also are useful to screen and give information about selection of the promising cowpea genotypes resistant to *A. vogelii*. This finding corresponded with the findings by Welsh and Mohammed (2011) who reported that, there was no correlation between the genetic divergences of *Striga hermonthica* and their origin geographic distance. This is contributed mainly by seed dispersal of *A. vogelii.* The seeds of *A. vogelii* are dispersed by wind, animals, machinery and human (Rubiales and Aparicio, 2012). The genetic composition and structure of plant populations can be shaped by the patterns for seed dispersal (Hamrick *et al*., 1993; Tajdoost *et al.*, 2013). Therefore, seed dispersal influenced genetic variability and they contributed to the gene flow between the populations of *A. vogelii.* The genetic structure is affected by a number of evolutionary

factors including gene flow, seed dispersal, and mode of reproduction (Hamrick *et al.*, 1993; Duminil *et al*., 2007).

Most of the diversity can be explained by allelic variation within populations. This result also suggested that there was an amount of gene flow among populations, based on the number of migrants per generation (Nm= 0.5872), with the mating pattern of the populations at outcrossing rate 0.54. A small number of migrants per generation is enough gene flow to obscure or overcome the process of drift that causes populations to differentiate over time (Matt *et al.,* 2011). The *A. vogelii* had induced the seeds which were highly influencing the evolution. Thus, high gene flow was observed due to high dispersal of the seeds. The gene flow among the *A. vogelii* populations was caused by human mediated movement through active trading activities by entrepreneurs on the contaminated cowpeas, sharing of seeds among farmers themselves, dispersal by wind, water, use of machineries and forage animals hence affecting its diversity and variability (Matt *et al*., 2011). This leads to the gene flow among populations and produce overlapping and intermixing of *Alectra* populations.

A PCoA was conducted to identify possible differences between *Alectra* populations based on genetic distance and geographical distance. Unexpected results were observed, whereby out of 15 populations, 10 populations of *A. vogelii* including most geographically separated were grouped together as either a group of two or four populations. This situation is caused by the common restriction to gene flow among populations that reduce effective population size and different selection pressures among populations (Tremblay *et al.,* 2005). Thus, the populations ordinated closer to one another are more similar than those ordinated further away. *A. vogelii* is known to show diverse distribution pattern along different geographical locations. The high variation and high

diversity in the *A. vogelii,* were influenced by the long-term natural selection for the parasite to adapt to the environments of different geographical locations. The plants with high geographical ranges tend to have higher genetic diversity than geographically localized species. The genetic diversity within the populations is also influenced by many factors such as mating system, population size, extended time period with low number of individuals, genetic drift and gene flow.

The Mantel's test gave better understanding on what processes are differentiating the strains in the populations. Seed movement due to multiple events of introduction and casual dispersal events, mediated by human action contributed more to gene flow in most areas thus gave lack of significant correlation between genetic and geographic distances of *A. vogelii*. Therefore, the absence of significant correlation between genetic and geographic distances of *A. vogelii* populations suggested that, the spatial distribution of genetic diversity of this species was influenced by their reproductive system and history of colonization by seed dispersal, which was carried through long distances, since they are dispersed by wind. This result also reinforces that the *A. vogelii* populations may have been originated by different introduction events. Therefore, the results suggest that seed dispersal of *A. vogelii* is more efficient in dispersal due to colonization events of new areas.

3.6 Conclusion and Recommendation

In this study, the genetic variation was identified in the 15 *Alectra* pupultions based on SSR markers. Only 8 pairs of SSR markers provided effective and adequate information which was used to differentiate and identify genetic variability of *A. vogelii* populations. Furthermore, cluster analysis, and genetic structure analysis gave a clear differentiation among *A. vogelii* according to their genetic similarities and exhibited a high level of genetic diversity and variability. The results identified four groups of physiological

strains of *A. vogelii* which are adapted to cowpea crop. In this case, multisite screening trials during breeding programs should consider representation from each of these four clusters for the development of resistant/tolerant genotypes of cowpea. In order to understand and clarify on the strains of *A. vogelii* well enough for effective management, further studies on sequencing of the *A. vogelii* populations should be conducted in all *Alectra* infested areas. Studies that will include larger populations of different locations in the country is encouraged so as to understand widely on the genetic variability of each population.

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CHAPTER FOUR

4.0 RESPONSE OF IMPROVED COWPEA GENOTYPES TO ALECTRA VOGELII STRAINS COLLECTED FROM DIFFERENT LOCATIONS

Abstract

The parasitic weed *Alectra vogelii* (Benth) continually remains a great challenge for cowpea production in sub-Saharan Africa. Developing resistant, high and stable yielding genotypes in *Alectra* infested areas requires evaluation of available genetic resources. An experiment was conducted in the screen house at Tanzania Agricultural Research Institute (TARI)- Ilonga Centre, Morogoro, Tanzania. The experiment comprised of two factors which were cowpea genotypes and *A. vogelii* strains. A spilt plot was arranged in complete randomized block design with three replications. Results revealed significant differences observed amongst cowpea genotypes on days to first *Alectra* emergence and number of *Alectra* shoots emerged. The cowpea genotypes B 301, Mkanakaufiti, Vuli AR1, Vuli AR2 and Vuli-1 allowed *Alectra* emergence at 42.83, 37.25, 36.75, 37.42 and 33.17 days after planting respectively. There were varying number of *Alectra* shoots supported by genotypes, as 1.0, 5.0, 4.0, 5.0 and 14.0 for B301, Mkanakaufiti, Vuli AR1, Vuli AR2 and Vuli-1 respectively. The genotype B 301 recorded lowest number of *Alectra* shoots, highest seed weight, highest pod weight and highest number of pods per plant. The genotype Mkanakaufiti was latest genotype to attain 50% flowering and 95% physiological maturity. The genotype Vuli AR1 had highest Leaf Area Index and longest pods. The genotype Vuli AR 2 recorded highest mean value for 100 seed weight. The genotype Vuli-1 produced many numbers of seeds per pod and was earliest in 95% physiological maturity. Through this study, B 301 was identified to possess high level of resistance and would be useful in cowpea resistance breeding programs.

Keywords: *Alectra vogelii*, cowpea genotypes, varieties, strains, resistance, susceptibility

4.1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.), a member of the family Fabaceae, is an important food legume grown in tropical and subtropical regions of the world, primarily in Sub-Saharan Africa. Cowpea is predominantly a self-fertilizing crop (Shiringan and Shimelis, 2011). In sub-Sahara Africa, the crop is grown for forage, green pods and grains (Adeigbe *et al.,* 2011). The crop is a multi- harvest crop, chiefly a vegetable and grain legume which offers dietary protein and calories for human, and it provides a very safe fodder for livestock (Basavaraj *et al.,* 2013; Makanur *et al.,* 2013; Rugare *et al*., 2013; Animasaun *et al.,* 2015; Abdou *et al*., 2017).

Cowpea is both a delicacy and livelihood crop for many households in Sub Saharan Africa where it contributes to food security (Adeigbe *et al*., 2011). The crop has a tremendous potential to contribute to alleviation of malnutrition (Shiringan and Shimelis, 2011; Okonya and Maass, 2014; Ddungu *et al*., 2015. Millions of the poor people particularly in the developing world their health and livelihoods are improved by protein, starch, minerals and vitamin contents obtained from cowpea (Shiringan and Shimelis, 2011; Okonya and Maass., 2014). Due to high values for protein among the local and improved varieties of cowpea grown, the crop improves livelihoods in some regions of Tanzania (Mamiro *et al*., 2011). In harsh environments, the crop yields comparably higher than other food legumes (Shiringan and Shimelis, 2011).

The main important traits of this crop include the good protein quality with a high nutritional value, the nitrogen fixing ability, tolerant to drought and heat, quick growth, and rapid ground cover (Rugare *et al.,* 2013; Magashi *et al.,* 2014; Ddungu *et al*., 2015; Lado *et al.,* 2016). Some of these attributes have made the cowpea adaptable to harsh environments and withstand extreme temperatures, water limiting conditions and poor

soil fertility in marginal lands and drier areas, where rainfall is scanty, moisture is always needed, soil is sandy and subject to erosion, and soil has little organic matter and phosphorus (Shiringan and Shimelis, 2011; Tadele and Assefa, 2012; Magashi *et al.,* 2014; Ddungu *et al*., 2015). Thus, cowpea offers multiple benefits to smallholder farmers in terms of food, income, livestock feed, and improving and maintenance soil fertility (Adeigbe *et al.,* 2011; Olasupo *et al*., 2016; Thio *et al*., 2016; Lado *et al.,* 2016).

Though cowpea is a highly considerable nutritious crop (Kutama *et al*., 2014) yet the crop is faced with a number of biotic and abiotic constraints that result into low grain and fodder yield (Animasaun *et al.,* 2015; Horn *et al.,* 2015). *Alectra vogelii* Benth, is a principal parasitic weed belonging to the family Orobanchaceae, mainly attacks cowpea in semi-arid regions of Sub-Saharan Africa (Rugare *et al.,* 2013; Kutama *et al.,* 2014; Zitta *et al*., 2014; Horn *et al.,* 2015; Mbwando *et al.,* 2016; Njekete *et al*., 2017). *A. vogelii* is most abundant in dry, infertile soils in semi-arid areas and these areas have subsistence farmers who are not aware of the threat of this parasitic weed. The weed is becoming even more acute, particularly in areas of the marginal nutrient status of the soils (sandy soils), and unreliable rainfall (Kutama *et al*., 2014; Zitta *et al*., 2014). Failure to control *A. vogelii* before it flowers has often resulted in highly contamination of soils with *Alectra* seeds (Rugare *et al.,* 2013). As the seeds continue to increase, soil seed bank increases, seeds spread to new areas, of which the consequence is the effect to the land quality and food security (Atera *et al.,* 2013). A single *A. vogelii* plant produces more than hundred thousand viable seeds that add to the seed bank from previous years (Karanja *et al*., 2012). Combined with the fact that, there is complex host-parasite interactions, production of large number of seeds with prolonged viability of more than 15 years in the soil, plant breeders have a great challenge in developing resistant varieties (Rugare *et al*., 2013) as the resistant varieties offer an excellent approach to avoid yield

losses caused by *A. vogelii* in subsistence farmers' fields. Extensive longevity, together with ability to form "biotypes, races, strains" "ecotypes," and "crop-specific" its seeds dispersal has made farm abandonment; and farmers have migrated from location to location because of *A. vogelii.* Preventing buildup of new strains of *A. vogelii* to levels that overcome the resistance of a new variety is an absolute requirement.

A. vogelii causes tremendous damage to the host plants before it emerges from the soil (Kwaga, 2014). After emergence it grows by attaching itself to the roots of the cowpea. On the plants, the weed affects the growth and development of cowpea, destroys its vigour and weakens it causing substantial yield losses in susceptible varieties and hampers the efforts to improve cowpea yields (Mbwaga *et al.*, 2007; Karanja *et al.*, 2012., Hela *et al*., 2013; Kutama *et al.*, 2014; Kwaga, 2014). Although single exact value of economic yield losses due to *A. vogelii in* cowpeas have not been quantified, estimates and visual observations indicate 50-100% yield loss in severe infestations (Karanja *et al*., 2013; Kabambe *et al.*, 2013; Mbwaga *et al*., 2013). This parasitic weed, has greatest economic threat not only to cowpea but also represents a continuing danger to other crops (Kabambe *et al.*, 2013; Kwaga, 2014; Mbega *et al.*, 2016 and Njekete *et al*., 2017), like Bambara groundnut (*Vigna subterranea* (L.) Verdc.), peanut (*Arachis hypogaea*), common bean (*Phaseolus vulgaris*), soybean (*Glycine max*), and mung bean (*Vigna radiata*). The infestation by *A. vogelii* poses a big threat to the production of cowpea, alarming possibility of affecting the resource poor farmers in the semi-arid and drought prone areas of the country who solemnly depend on the crop for their protein source (Mbega *et al*., 2016).

A number of control measures have been developed but seem to be either not feasible economically or not successful (Rugare *et al*., 2013; Kwaga, 2014). The subsistence

farmers who populate the most threatened regions, are unable to afford expensive chemical treatments for control of the weed and often find it difficult to adopt new cultural practices. The development of high-yielding host cultivars with durable resistance is of utmost importance for reducing the agricultural and social impact of *A. vogelii* in these endemic regions. Destruction could be worse as resistant varieties of *A. vogelii* host offers the most sustainable and reliable control of *A. vogelii* infestation (Kutama *et al*., 2014).

Development and deployment of resistant cowpea varieties remain the most effective method to combat the menace presented by these parasitic weeds (Omoigui *et al*., 2012). This approach is successful, if the germplasm with resistance to *A. vogelii* parasitism is identified. Therefore, there is an imperative need for breeding high yielding cowpea genotypes that would withstand *Alectra*, to enhance food security among small holder farmers (Abdou, 2017). This enables the breeder to operate selection efficiently and subsequently develop appropriate breeding strategies to solve the problems of *Alectra* resistance, poor yield as well as improve the nutritive quality of the crop (Animasaun *et al.*, 2015; Horn *et al*., 2015).

Even though genetic resistance has been identified in cowpea, its actual value is limited because the parasite has shown an ability to overcome host resistance mechanisms. The resistant varieties are challenged by *A. vogelii* strains in some hotspots of the country. For example, some varieties which were reported to be resistant to *Alectra* in one area are found to be susceptible when grown in other areas which lead to speculation on the presence of the number of strains of *A. vogelii* across parasite endemic locations.

There are efforts to popularize cowpea, but as they are intensified, the incidence of *A. vogelii* appears to be on the increase (Kabambe *et al*., 2013). Preventing buildup of new strains of *A. vogelii* to levels that overcome the resistance of a new variety is an absolute requirement. The occurrence of new strains can complicate breeding varieties with stable resistance, unless varieties are to be developed with resistance to multiple strains. Resistance sources have been identified and used in breeding of few *Alectra* resistant varieties with widely effective field resistance to combat the parasite (Hela *et al*., 2013, Mbwaga *et al.,* 2013; Kabambe *et al.,* 2014; Mbega *et al.*, 2014). The aggressiveness and evolution of new virulence of strains of *A. vogelii*, call for studying the response of the improved varieties to *Alectra* strains from the selected areas. Information concerning the performance of cowpea genotypes under *Alectra* strain infestation would be valuable to cowpea breeders in planning future cowpea selection and development programmes aimed at increasing cowpea yield in the country. The purpose of this chapter therefore was to determine the response of cowpea genotypes for resistance to *Alectra* strain infestation.

4.2 Materials and Methods

4.2.1 Study area

A screen house pot experiment to determine the response of improved cowpea varieties was conducted at Tanzania Agricultural Research Institute (TARI)- Ilonga Centre, in Morogoro, Tanzania, (06° 42’S, 37°02’ E, Altitude 506 meters above sea level).

4.2.2 Materials

Table 4.1: The strains and cowpea genotypes used in pot experiment

Strain	***Alectra* populations**	**Name of locations *Alectra* populations were collected**			
1	7	Lyadebwe	Ikwega	Welela	Mbalawala
		Kondoa	Iyambi	Ilunda	
2	4	Nyamahana	Mangalali	Kising'a	Ngamu
3	2	Gawaye	Mbande		
4	2	Nkungi	Mhenda		
Genotypes	**Reaction to *A. vogelii***	**Source**			
B301	Resistant	IITA			
Mkanakaufiti	Resistant	Malawi			
Vuli AR 1	Resistant	TARI Ilonga			
Vuli AR 2	Resistant	TARI Ilonga			
Vuli-1	Susceptible	TARI Ilonga			

4.2 3 Method

Petri dish method was used during germination test. This method allows more detailed studies on induction of germination. Preconditioning of *Alectra* seeds was performed under sterile conditions in a Laminar Air flow cabinet before the seeds become responsive to germination stimulants. The active seed germination stimulant of the parasitic weeds of Orabanchaceae called GR24 was used to test for germination of *Alectra* seeds. This stimulant acts as the strigolactones (plant hormones) that induce germination of parasitic weed seeds. In *A. vogelii*, it acts as elicitor of ethylene biosynthesis, leading to subsequent *Alectra* seed germination. Preconditioning of *Alectra* seeds was performed to leach out chemical inhibitors from the *Alectra* seed, thus increasing the permeability of the aleurone layer on *Alectra* seeds.

Alectra seeds (5 g) were surface sterilized in 5 % (v/v) sodium hypochlorite solution for 30 minutes in a test tube, with gentle agitation. The seeds were then rinsed thoroughly with 100 ml of sterile distilled water, then spread on a glass fiber filter paper (Whatman GFA), put into sterile petridishes and wet with 2.5 ml of sterile distilled water. The petridishes were then sealed with parafilm and wrapped with aluminium foil to prevent

water losses and exclude light. There after the Petridish were placed in an incubator for 14 days at 30 ^{0}C for conditioning. Period of conditioning allows the seed to germinate because seeds imbibe water before exposure to germination stimulant. After conditioning, the *Alectra* seeds were treated with a sterile germination stimulant (GR 24) to induce germination. Equal volume of 2.5 ml of the GR24 stimulant was added to each petridish having the pre-conditioned seeds. When the radicle protruded through the seed coat, the seeds were considered to have good germination.

4.2.4 Experimental design

The experiment employed the pot experiment method used by Botanga and Timko (2005) with slight modification. About 500 *Alectra* seeds of each strain were thoroughly mixed separately with 250 ml of sterilized sieved sand to form the inoculum stock. The inoculum stock was used to inoculate the top 5 cm of each experimental pot which contained a mixture of soil and sand (3:1 v/v). After inoculation, the *Alectra* seeds were pre-conditioned for seven days before sowing the cowpea seeds to enhance *Alectra* seed germination. After soil inoculation with *Alectra* seeds, the pots were watered daily to field capacity for seven days consecutively to precondition the seeds to break their dormancy and ensure optimum germination. After pre-conditioning of *Alectra* seeds, 3 seeds of cowpea were sown per pot in 3 replications at uniform depth in holes with 5 cm deep. The seedlings were thinned out and two were maintained per pot at 2 weeks after germination. The soil was kept moist by watering regularly every two days or when necessary. Plants that favoured attachment and emergence of many *A. vogelii* were classified as susceptible like Vuli-1 and those that appeared with few infections, were categorized as resistant/tolerant genotypes.

The experiment was designed as a Spilt Plot Experiment laid in a Randomized Complete Block Design (RCBD) with three replications. *Alectra* strains were used as main plot (factor A) and the cowpea genotypes were used as sub plot (factor B) (Table 4.1). Five cowpea genotypes were used as treatments in this experiment, namely; Vuli AR1, Vuli ARI 2, Mkanakaufiti, and with local checks B 301 (resistant) and Vuli- 1 (susceptible) in 2016/2017 farming season. The genotype Vuli-1 is the locally grown cowpea cultivars.

4.2.5 Data collection and analysis

Data were collected and analysed on various characters. These characters are height of the plant, number of leaves per plant, branches per plant, number of nodes per plant, leaf area index (LAI), number of days to 50% flowering of cowpea plants, days to 95% physiological maturity, days to *Alectr*a emergence, and number of *Alectra* per plant. The plants were harvested at physiological maturity, when more than 95% of the pods were dry and brown.

Yield in a crop is governed by yield components (Oladejo *et al*., 2011).Yield variables measured include the number of pods per plant, pod length, number of seeds per pod, 100 seed weight, and seed yield. The number of pods per plant was obtained by randomly selecting five plants within the sub-plot and counting the pods on them. The average number of pods per plant was then determined. Also, ten pods from each sub plot per replication were selected and their lengths measured with a meter rule. For the average number of seeds per pod, twenty pods from each sub plot per replication were shelled and the seeds counted and the average was found. Four lots of 100 seeds from the shelled pods of each sub- plot were counted and weighed. The average was then taken as the weight of 100 seeds. After harvesting pods from sub- plot, they were shelled, the seeds were weighed and the average was calculated to determine the final yield expressed in

grams per plant (g plant^{-1}). These data were analysed using GenStat Discovery 16th Edition.

4.3 Results

There were significant differences (P = 0.001), among cowpea genotypes in response to *Alectra* strains (Table 4.2). Results showed that, four resistant genotypes (B301, Mkanakaufiti, Vuli AR1 and Vuli AR2 were also infested by *A. vogelii.* Cowpea genotypes supported *Alectra* emergence whereas Vuli-1 supported earliest emergence of the weed, followed by Vuli AR1, and latest *A. vogelii* emergence was in B301. The effect of cowpea genotypes on *Alectra* emergence was first observed in strain 4 of *A. vogelii*, followed by strain 2 and latest in strain 1.

There were significant differences (P = 0.001), on the effect of genotypes on number of *Alectra* shoots at 35 DAP, 49 DAP and 63 DAP. At 35 DAP, Vuli-1 had higher number of *Alectra* shoots per plant, followed by Mkanakaufiti and lowest was recorded in B301 (Table 4.2). At 49 DAP, Vuli-1 had higher number of *Alectra* shoots per plant, followed by Vuli AR1 and Vuli AR2 which had the same number of *Alectra* shoots per plant and lowest in B301. At 63 DAP, Vuli-1 had higher number of *Alectra* shoots per plant, followed by Mkanakaufiti and lowest in B301. The genotype Vuli-1 was highly infested by emerged *Alectra* shoots, however less *Alectra* shoots were observed on the cowpea genotype B 301.

Table 4.2: Effect of cowpea genotypes on the emergence and number of *Alectra* shoots per plant

Strains	Days to *Alectra* emergence	Number of 35 DAP	Shoots per 49 DAP	Plant at 63 DAP
1	39.33 a	1.47a	6.47 a	9.00 a
2	36.93 a	1.73 a	5.67 a	7.00 a
3	37.73 a	3.49 a	6.94 a	7.33 a
4	35.93 a	4.13a	6.75 a	7.93 a
GM	37.48	2.71	6.46	7.82
CV%	5.6	52.80	19.40	17.30
SE±	2.09	1.43	1.25	1.35
P- value	0.33	0.16	0.64	0.37
Genotypes				
B 301	42.83 c	0.67 a	1.33 a	1.25 a
Mkanakaufiti	37.25 b	2.62 a	4.08 b	6.83 b
Vuli AR 1	36.75 b	2.42 a	5.33 b	5.0 b
Vvuli AR 2	37.42 b	2.58a	5.33 b	6.25 b
Vuli-1	33.17 a	5.25 b	16.17 c	19.75 c
GM	37.48	2.71	6.46	7.82
CV%	7.90	85.50	45.10	37.6
SE±	2.97	2.32	2.91	2.94
P- value	0.001	0.001	0.001	0.001

Means in the same column followed by the same letter(s) are not statistically different (P< 0.05) by Duncan's New Multiple Range Test.

Interaction effect on cowpea genotypes and *A. vogelii* showed that, days to *Alectra* emergence were different among cowpea genotypes and *Alectra* strains (Fig. 4.1). However, *Alectra* emerged earlier in Vuli-1 with strains 2, 3 and 4, followed by the same genotype Vuli-1 with strain 1 and Vuli AR1 with strain 4, all with the same number of days to *Alectra* emergence, whereas *Alectra* emerged latest in B301.

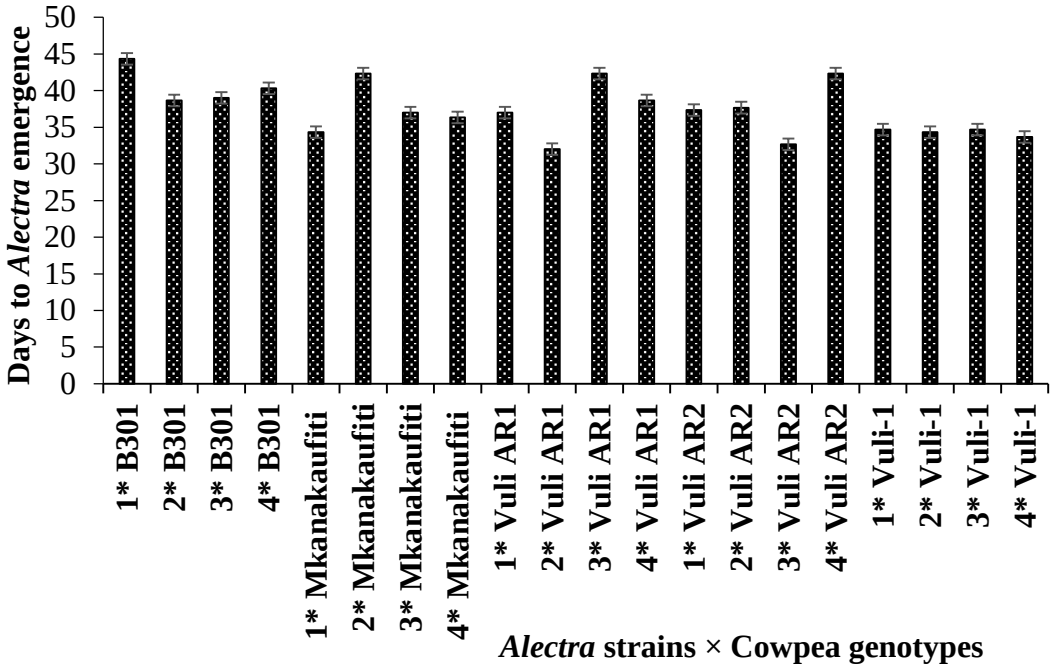

Figure 4.1: Interaction effect of cowpea genotypes and strains of *Alectra vogelii* on days to *Alectra* emergence

The genotypes Vuli AR1 with strain 1 and B301 with train 3 had lowest number of *Alectra* shoots (Fig. 4.2). At 35 DAP, more *Alectra* shoots per plant were observed in Vuli-1 with strain 4, followed by Vuli AR1 with strain 4 and lowest in three different genotypes which are B 301 with strain 1 and 3, Vuli AR1 with strain 1 and 3 and Vuli AR2 with strain 2. At 49 DAP, Vuli-1 with strain 1, 2, 3 and 4, recorded more *Alectra* shoots per plant followed by Mkanakaufiti with strain 3. At 63 DAP, Vuli-1 had the highest number of *Alectra* shoots per plant in all strains, followed by Mkanakaufiti and lowest was in B 301.

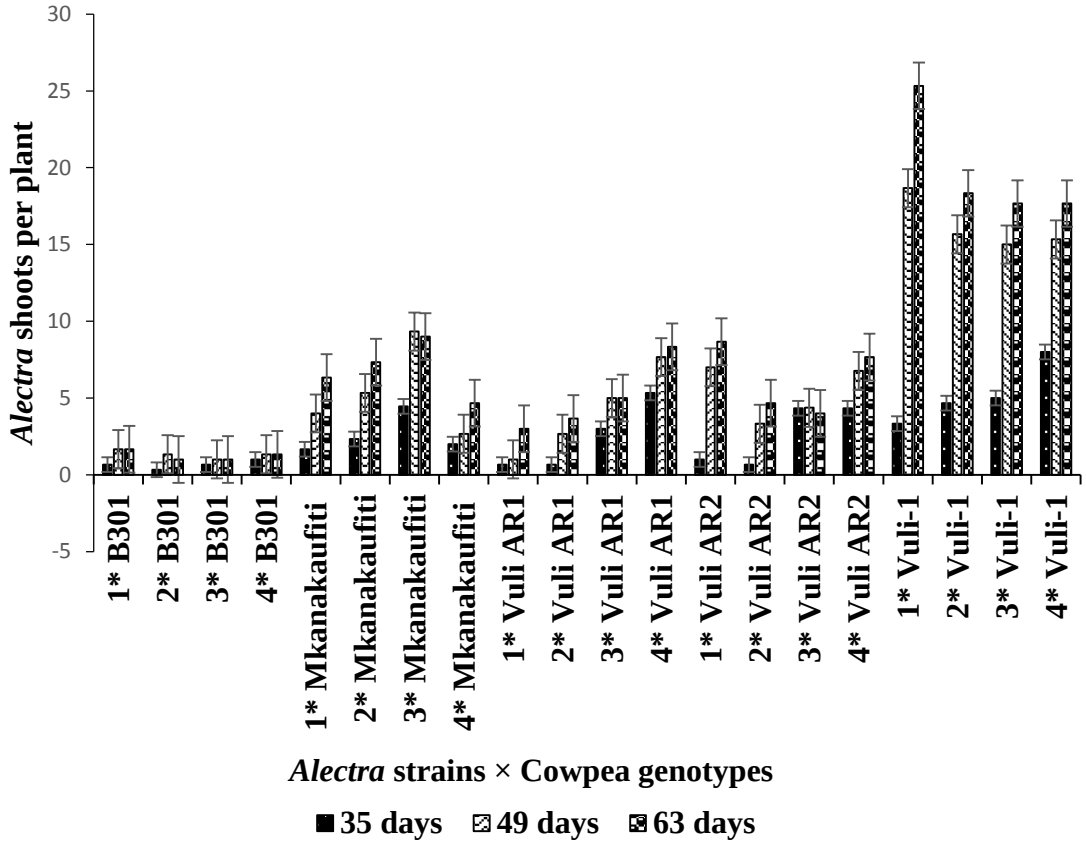

Figure 4.2: Interaction effect of cowpea genotypes and strains of *Alectra vogelii* on number of *Alectra* shoots per cowpea plant

The height of cowpea was significantly affected by different strains (Table 4.3). The tallest plant was observed in strain 3, followed by strain 4 and shortest plant was observed in strain 1. With respect to genotypic effects, tallest plants were observed in Vuli AR1, followed by Mkanakaufiti and the shortest plants were in Vuli 1.

Cowpea genotypes showed variation in number of leaves per plant, though there were no significant differences. The plants in strain 3 showed higher number of leaves, followed by strain 1, and lowest in strain 4. More leaves were in Vuli AR1 followed by Vuli AR2 and lowest in Vuli-1.

The plants in strain 4 had more branches, followed by strain 3 and lowest in plants sown in strain 2. The effect of these strains showed that, the genotype vuli AR2 had higher number branches, followed by Vuli AR1, and lowest with B301.

The plants in strain 3 had the highest number of nodes, followed by plants in strain 1 and lowest from the plants that were in strain 2. The effect of these strains on number of nodes indicated that, the genotype Vuli AR2 produced more number of nodes per plant followed by Vuli AR 1 and the lowest number of nodes was in B301.

There were some variations with no significant differences for leaf area index (LAI) within the strains. However, plants in strain 1 recorded larger values of LAI, followed by plants in strain 2 and the lowest value was for plants in strain 4. Cowpea genotype Vuli ARI was with highest LAI, followed by Mkanakaufiti and lowest LAI was in B 301 and differences were significant.

The early flower on set plant was recorded in strain 1 and the latest in strain 3 and 4. There were significant differences among the cowpea genotypes on days to flower on set. The effect of strains on cowpea genotypes showed that, early flowering plants were recorded in genotype Vuli AR2 followed by two genotypes Vuli-1 and B 301 with the same number of days to flower on set while the latest were observed in Mkanakaufiti.

The results indicated that there were variations, however there were no significant differences on number of days to 50% flowering, among the strains of *A. vogelii*. In the strains, early days to 50% flowering was recorded in plants which were planted in strain 3 and 4, followed by strain 2 and latest in strain 1. The plants in strain 1 were the latest to attain days to 50% flowering. Generally, there were significant differences ($P<0.05$) for

days to 50% flowering among the genotypes whereas early days to 50% flowering plants were recorded in genotype B 301, followed by Vuli AR1 while the latest were observed in Mkanakaufiti.

Variations with no significant differences were noted among strains on the number of days to 95% physiological maturity. Cowpea genotypes in strain 2 and 4 were the earliest to reach 95% physiological maturity, followed by strain 1 and latest was on genotypes in strain 3. There were significant differences ($P<0.05$) for number of days to physiological maturity. The earliest 95% physiological maturity was observed in genotype vuli -1, followed by vuli AR2, while the latest was Mkanakaufiti.

Table 4.3: Effect of strains of *Alectra vogelii* on some growth characteristics of cowpea genotypes

Strains	Plant height (cm)	Leaves plant^{-1}	Branches plant^{-1}	Nodes plant^{-1}	Leaf Area Index	Flower on set	50% flowering	95% maturity
1	49.300 a	20.890 a	7.218 a	6.600 a	4.380 b	43.400 a	50.000 a	69.070 a
2	60.680 ab	20.800 a	7.013 a	6.244 a	4.349 ab	43.600 a	49.670 a	68.800 a
3	68.180 b	22.470 a	8.284 b	7.071 a	4.342 ab	43.870 a	49.470 a	69.600 a
4	63.160 ab	20.640 a	8.340 b	6.367 a	4.231 a	43.870 a	49.470 a	68.800 a
GM	60.330	21.200	7.710	6.570	4.330	43.680	49.650	69.070
CV%	12.000	5.300	4.300	6.800	1.400	1.300	1.100	1.200
SE±	7.240	1.115	0.328	0.447	0.059	0.561	0.563	0.802
P- value	0.080	0.255	0.004	0.215	0.085	0.701	0.638	0.604
Genotypes								
B 301	52.270 ab	20.870 a	7.019 a	6.322 a	4.217 c	44.330 b	46.580 a	70.000 c
Mkanakaufiti	65.360 c	20.970 a	7.267 a	6.456 a	4.436 d	46.330 c	53.750 d	76.330 d
Vuli AR 1	71.020 c	21.380 a	8.222 b	6.681 a	5.517 e	42.000 a	48.580 b	67.830 b
Vuli AR 2	62.250 bc	22.140 a	8.414 b	6.919 a	3.994 b	41.420 a	50.000 c	66.330 ab
Vuli-1	50.760 a	20.650 a	7.647 ab	6.475 a	3.464 a	44.330 b	49.330 bc	64.830 a
GM	60.330	21.200	7.710	6.570	4.330	43.680	49.650	69.070
CV%	21.100	13.600	13.900	14.200	3.100	3.600	2.500	2.800
SE±	12.755	2.893	1.068	0.932	0.134	1.560	1.240	1.902
P- value	0.002	0.739	0.013	0.563	0.001	0.001	0.001	0.001

Means in the same column followed by the same letter(s) are not statistically different (P< 0.05) by Duncan's New Multiple Range Test.

Interaction of strains of *A. vogelii* and cowpea genotypes on some growth characteristics (Appendix 1), showed that for height, number of leaves, number of branches, nodes, days to flower on set, days to 50% flowering, and days to 95% physiological maturity among cowpea genotypes were not statistically significant (P >0.05). Significant interactions between strains and genotype were observed on number of *Alectra* shoots at 63 DAP and on leaf area index.

The tallest combination genotype was Mkanakaufiti with strain 3, followed by Vuli AR1 with strain 4 and the shortest was Vuli-1 with strain 1 (Fig. 4.3).

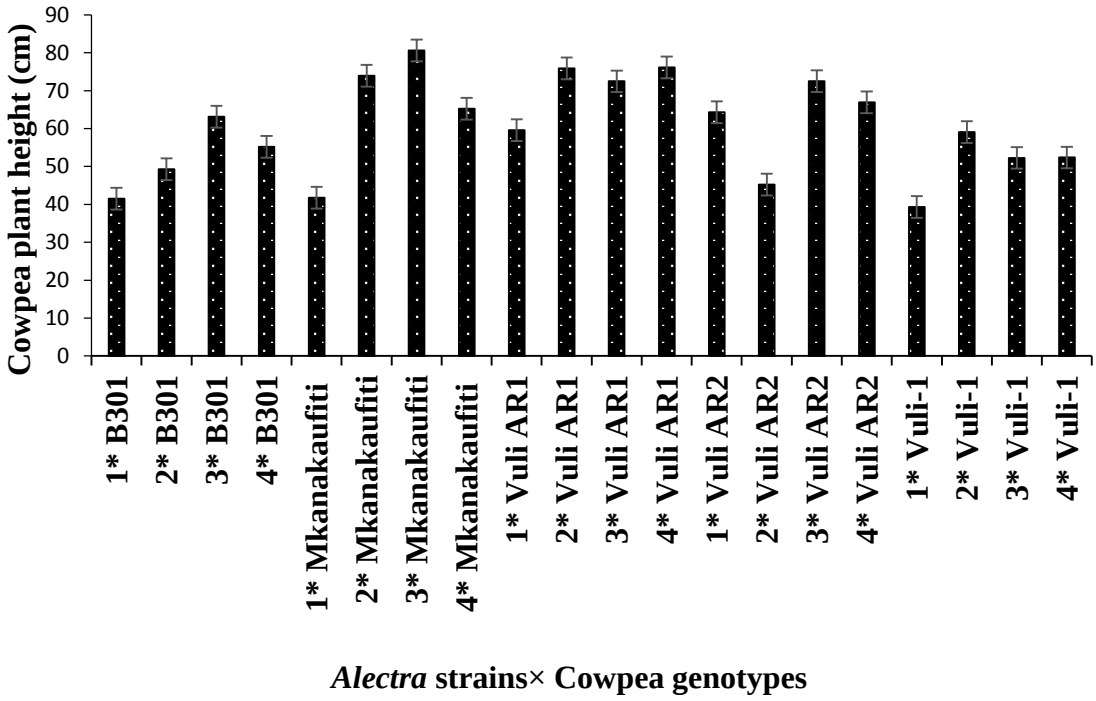

Figure 4.3: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on cowpea plant height

More leaves were observed in Vuli AR 1 with strain 3 and Vuli AR2 with strain 2 and less number of leaves was in Vuli AR1 with strain 2 (Fig. 4.4).

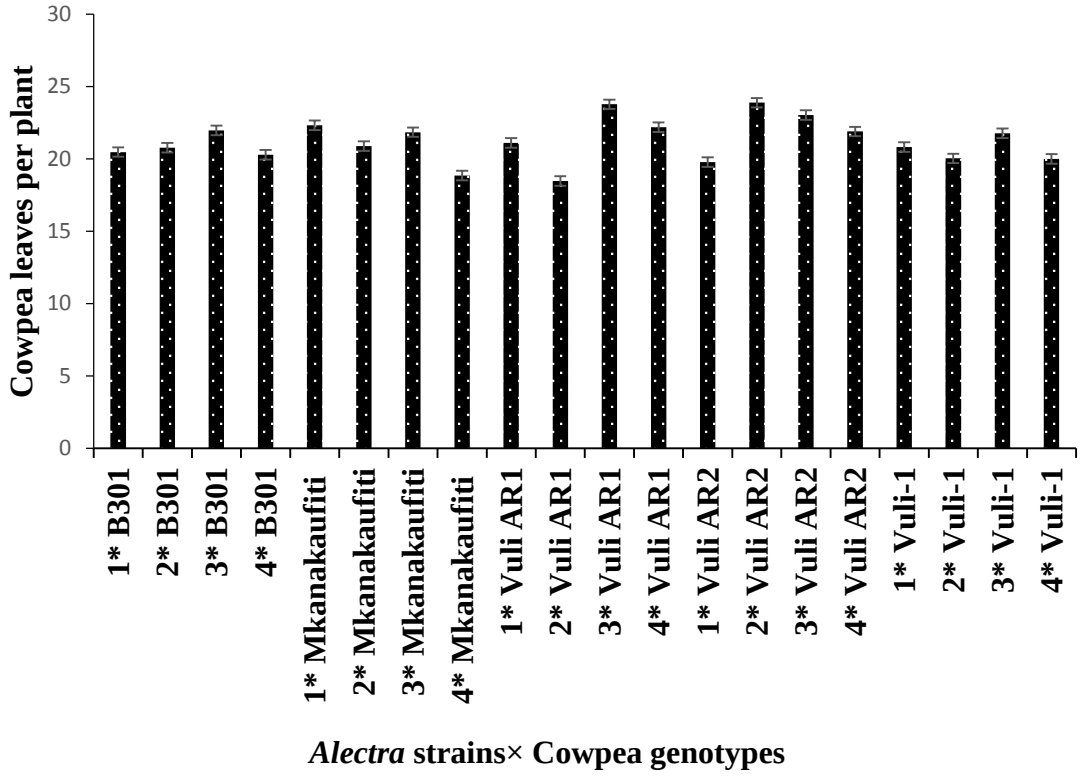

Figure 4.4: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on cowpea leaves per plant

The genotype vuli AR2 with strains 3 and 4, had highest number of branches, followed by vuli AR1 with strain 4 whereas B 301 with strain 1 had the lowest number (Fig. 4.5).

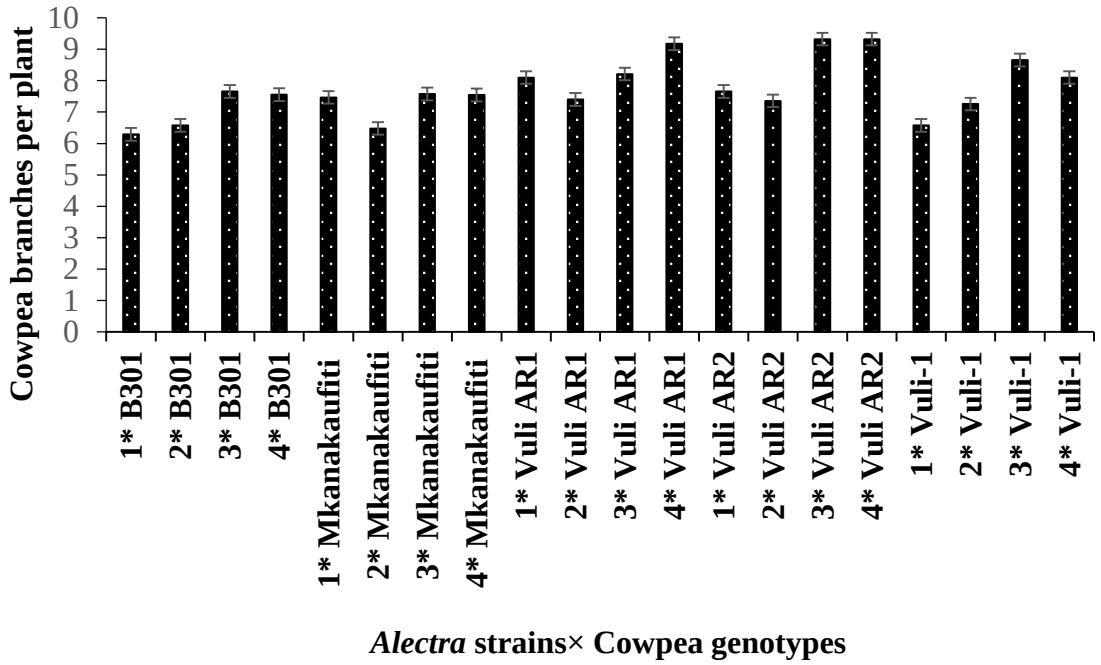

Figure 4.5: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on cowpea branches per plant

The genotypes Vuli AR2 and Vuli-1 with strain 3 gave high number of nodes, followed by Vuli-1 with strain 3 and the lowest in B301 with strain 2 (Fig. 4.6).

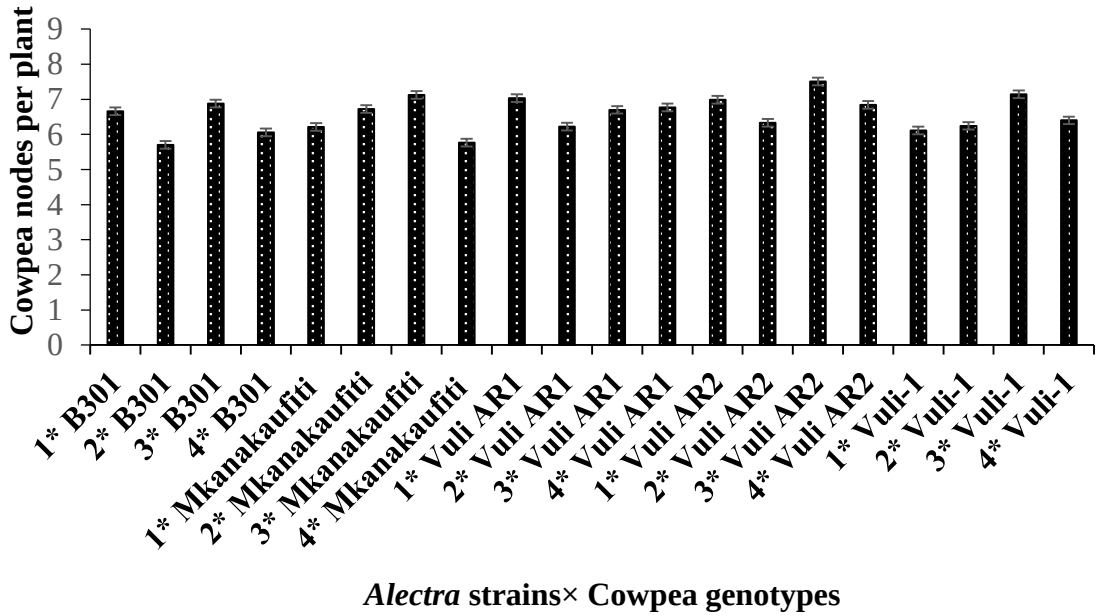

Figure 4.6: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on cowpea nodes per plant

There were significant effects of interaction among genotypes and *Alectra* strains in Leaf Area Index (LAI). The highest LAI was in genotype Vuli AR1 with strain 3, and the lowest LAI was recorded in the Vuli-1 with strain 3 (Fig. 4.7).

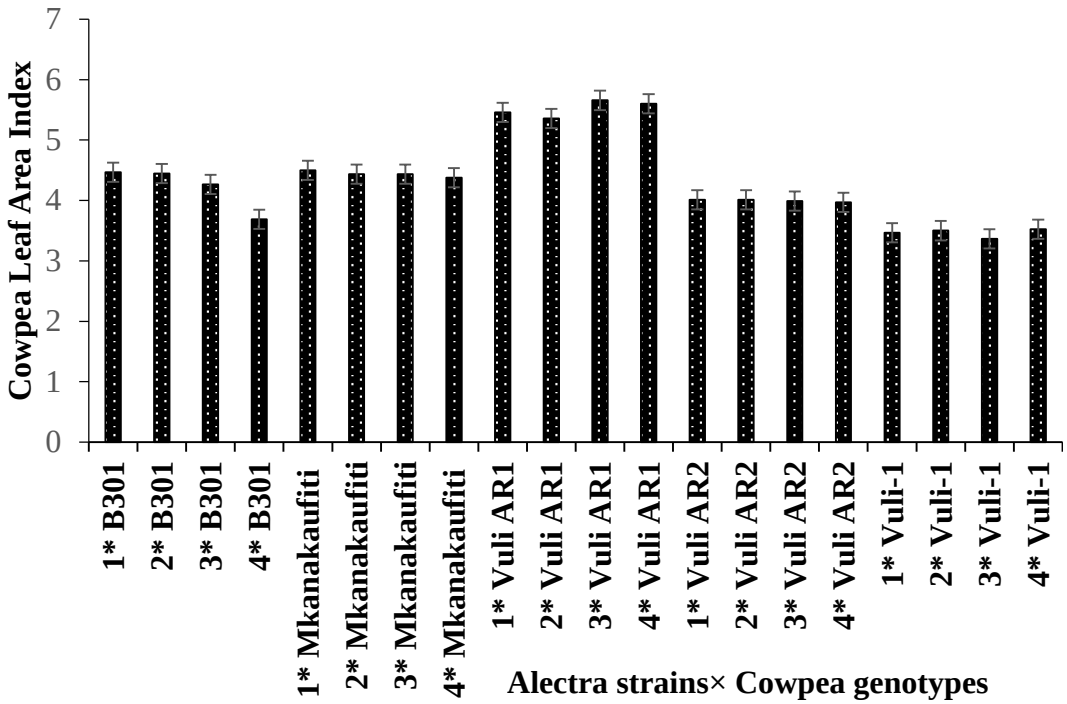

Figure 4.7: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on Leaf Area Index of cowpea

The results indicated Vuli AR2 as an early flowering genotype followed closely by Vuli AR 1 and Mkanakaufiti was the latest flowering genotype (Table 4.3). Thus, early flowering plants were recorded in Vuli AR 2 with strains 1 and 3, followed by Vuli AR1. The latest flowering combination genotype was in genotype Mkanakaufi with strain 4 (Fig. 4.8).

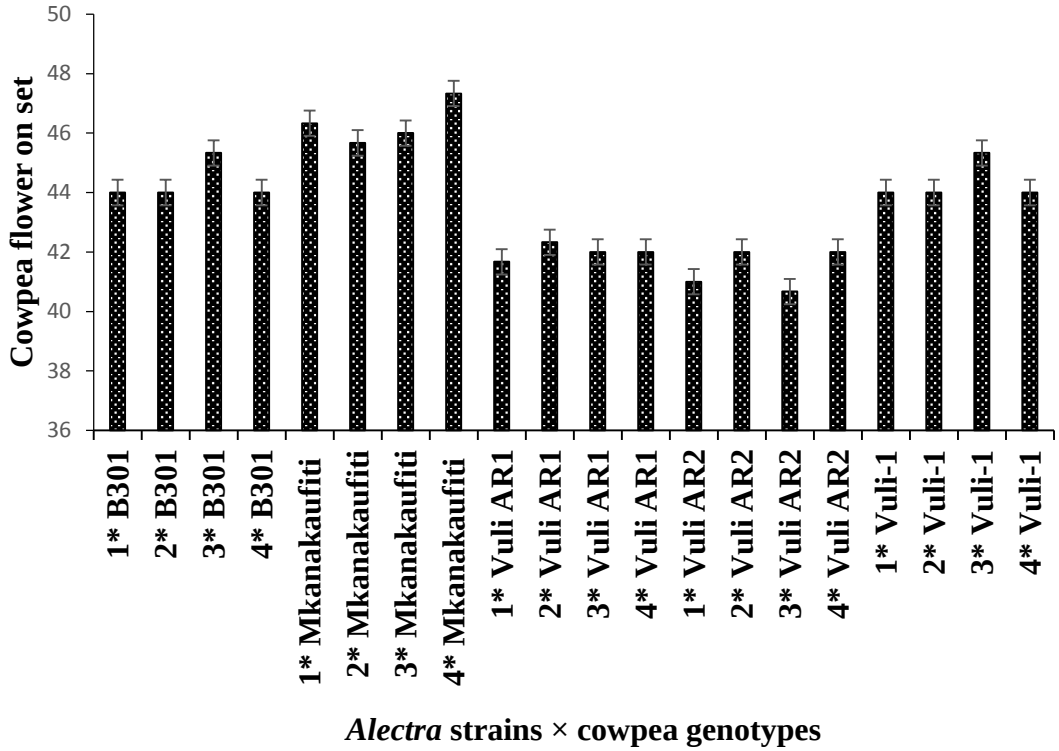

Figure 4.8: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on days to cowpea first flower on set

The genotpe B301 recorded early days to 50 % flowering whereas Mkanakaufiti was the lastet to attain 50 % flowering (Table 4.3). The 50 % flowering were earliest in B301 with strains 1 and 4, but Mkanakaufiti with strains 1 and 2 were the latest (4.9).

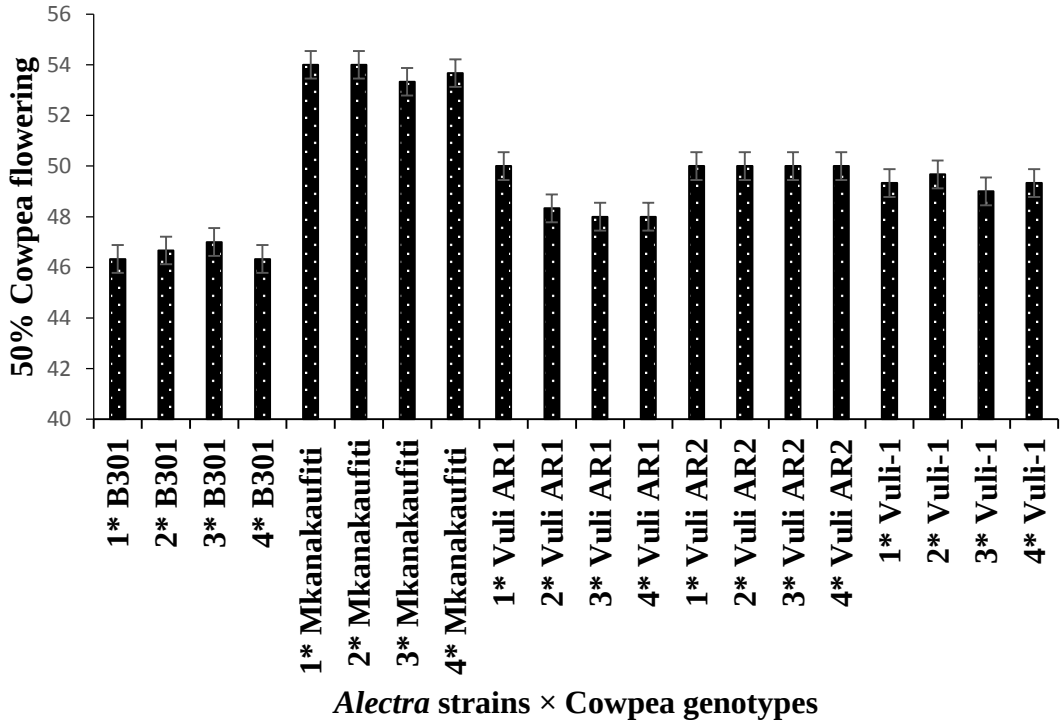

Figure 4.9: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on days to 50% cowpea flowering

The variations were noted among strains and genotype combinations on the number of days to 95% physiological maturity (Fig. 4.10). Cowpea genotype Vuli-1 with strain 2 was the earliest to reach 95% physiological maturity and the latest was Mkanakaufiti with all strains.

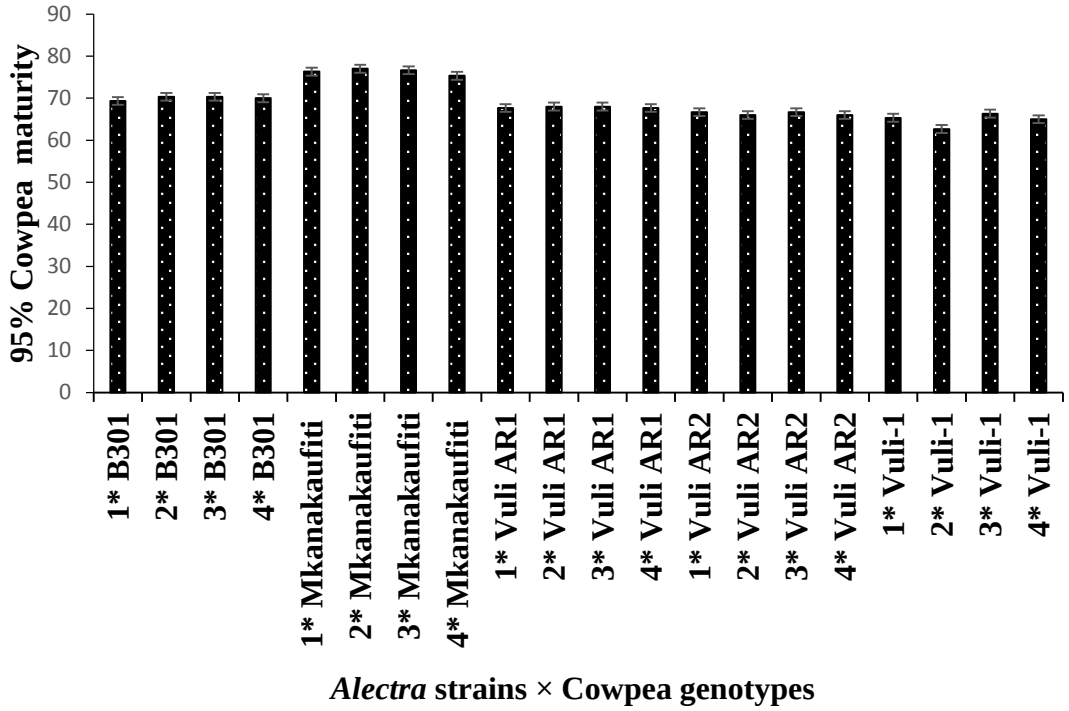

Figure 4.10: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on days to 95% cowpea pod physiological maturity

All four strains showed no significant differences (P >0.05), on seed yield and yield components (Table 4.4), however, significant differences between genotypes were for 100 seed weight, seed per pod, pod length and pod weight.

The effect of genotypes showed that, the genotype Vuli ARI gave longest pods per plant, followed by B301 and shortest was Vuli AR2.

There were significant differences among genotypes on number of seeds per pod and in 100-seed weight. The genotype B 301 produced highest number of seeds per pod, followed by Vuli 1 and few numbers of seeds were produced by Mkanakaufiti (Table 4.4).

The effect of genotypes showed that, the highest mean values for 100 seed weight were produced by Vuli AR 2, followed by Vuli AR 1, and the lowest was B 301. There was no significant difference in seed yield per plant between genotypes.

Table 4.4: Effect of strains of *Alectra vogelii* on cowpea seed and yield components

Strain	number of pods plant^{-1}	weight of pods plant^{-1}(g)	length of pod plant^{-1} cm)	number of seeds pod^{-1}	100 seed weight (g)	Yield plant^{-1} (g)
1	6.356 a	12.080 a	17.020 a	13.830 a	14.210 a	8.077 a
2	6.356 a	12.860 a	15.790 a	12.000 a	14.380 a	8.499 a
3	6.356 a	13.310 a	16.580 a	13.530 a	14.120 a	8.883 a
4	6.356 a	12.340 a	16.380 a	13.010 a	13.950 a	8.370 a
GM	6.550	12.650	16.440	13.090	14.160	8.460
CV%	15.500	20.600	3.700	8.900	3.200	18.200
SE±	1.015	0.606	0.606	1.166	0.453	1.540
P- value	0.956	0.937	0.197	0.325	0.708	0.932
Genotype						
B 301	7.078 a	15.410 b	17.230 bc	15.620 b	13.120 ab	9.253 a
Mkanakaufiti	6.933 a	11.700 a	15.620 ab	10.570 a	13.990 b	8.342 a
vuli AR 1	5.792 a	10.660 a	17.680 c	12.380 a	15.670 c	7.706 a
vuli AR 2	6.203 a	12.450 a	14.560 a	12.350 a	15.950 c	8.300 a
vuli-1	6.758 a	13.040 a	17.110 bc	14.540 b	12.100 a	8.687 a
GM	6.550	12.650	16.440	13.090	14.160	8.460
CV%	23.700	21.500	12.900	19.000	10.900	20.200
SE±	1.555	2.719	2.117	2.492	1.547	1.709
P- value	0.242	0.003	0.005	0.001	0.001	0.282

Means in the same column followed by the same letter(s) are not statistically different (P< 0.05) by Duncan's New Multiple Range Test.

There was no significant interaction of strains and genotypes on pods per plant (Fig. 4.11). The highest number of pods per plant was recorded by B301 with strain 4 followed by Vuli-1 with strain 3. The lowest number of pods was recorded in Vuli AR1 with strain 2.

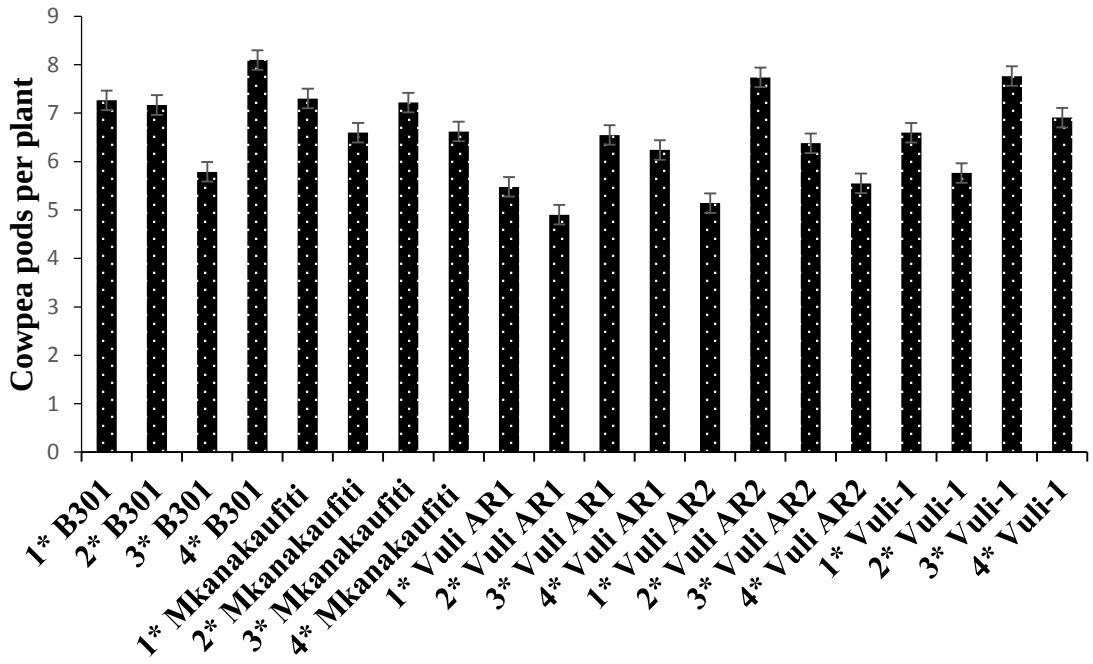

Figure 4.11: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on cowpea pods per plant

The genotype, B301 with strain 2 gave highest weight of pods followed by the same genotype B301 with strain 4 while the lowest weight of pods was from vuli AR1 with strain 2 (Fig. 4.12). However, such differences were not statistically different.

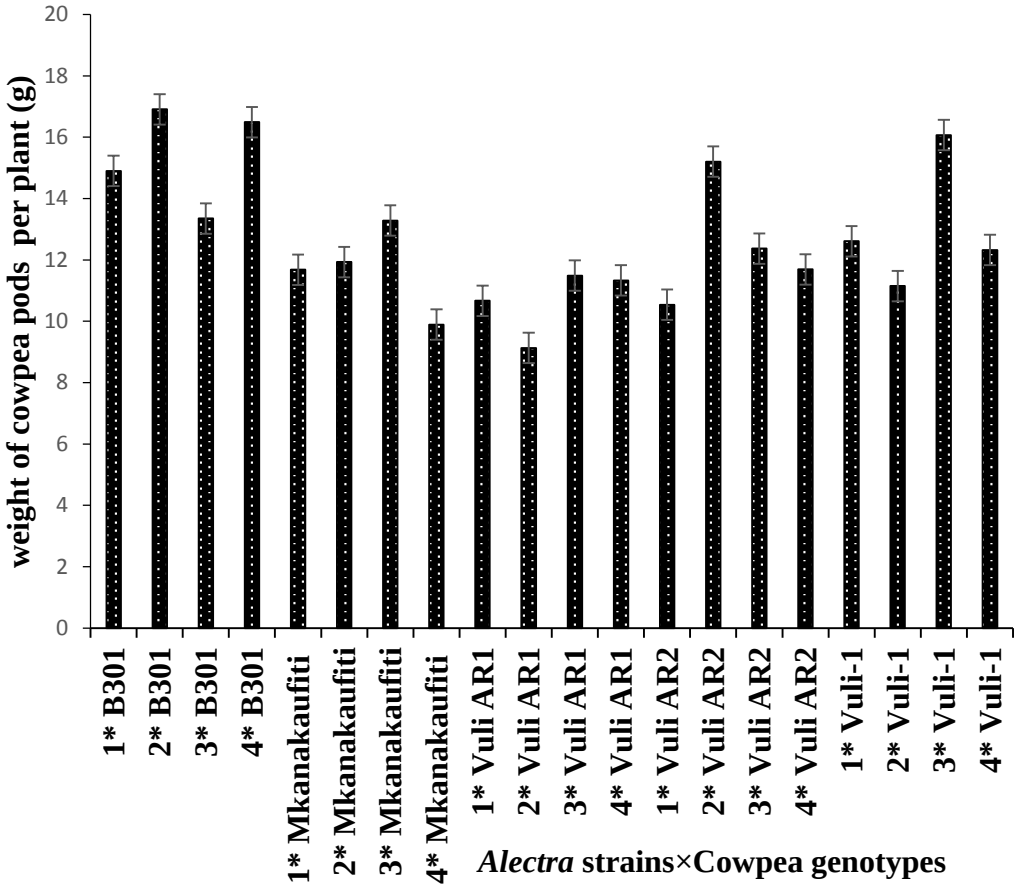

Figure 4.12: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on weight of cowpea pods per plant

The length of pods per plant differed significantly among genotypes (Table 4.4). In genotype and strain combination, longest pod was observed in Vuli AR 1 with strain 1 followed vy genotype Vuli AR 1 with strain 3 (Fig. 4.13). However, the combinations of genotype and strain were not statistically different.

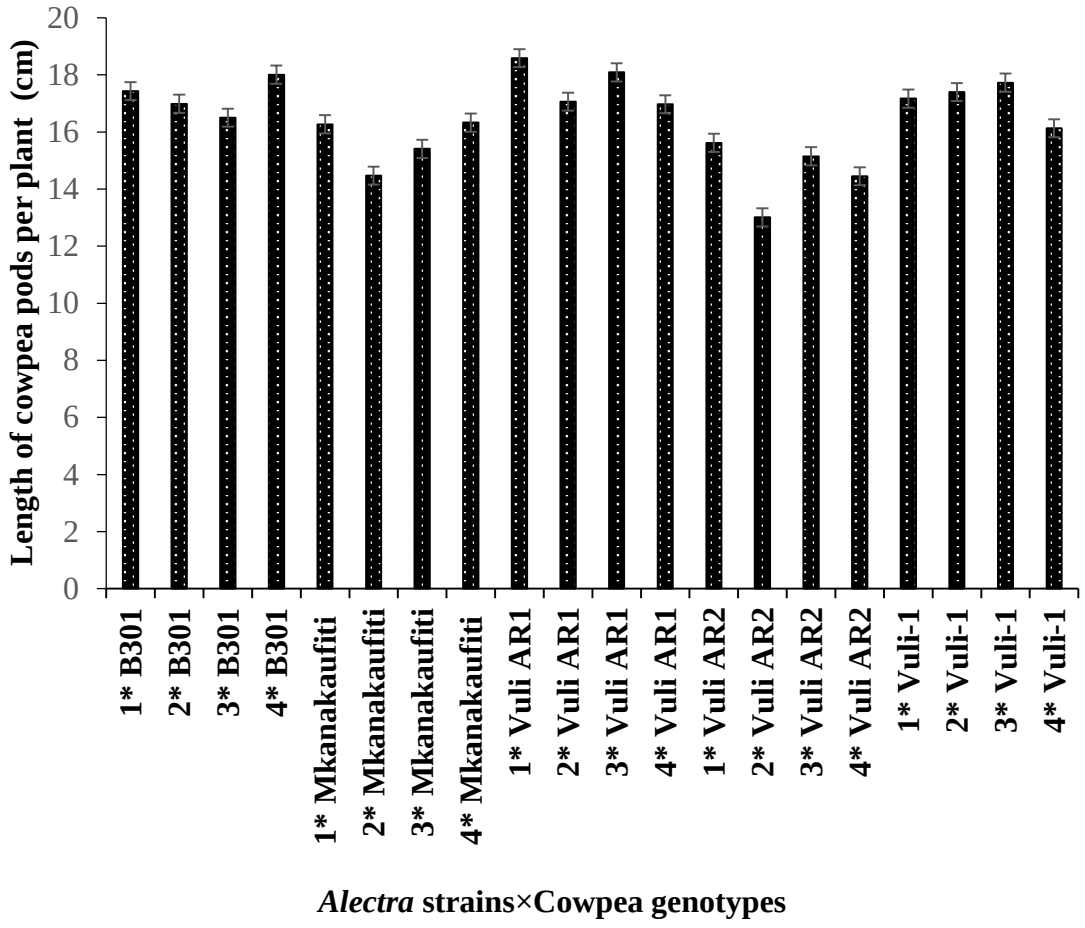

Figure 4.13: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on length of cowpea pods per plant

There were some variations though not significant among cowpea genotypes in response to *Alectra* infestation in number of seeds per pod. The highest number of seeds was observed in B301 with strain 1 followed by the same genotype B 301 with strain 2 and lowest in Mkanaufiti with strain 3 (Fig. 4.14).

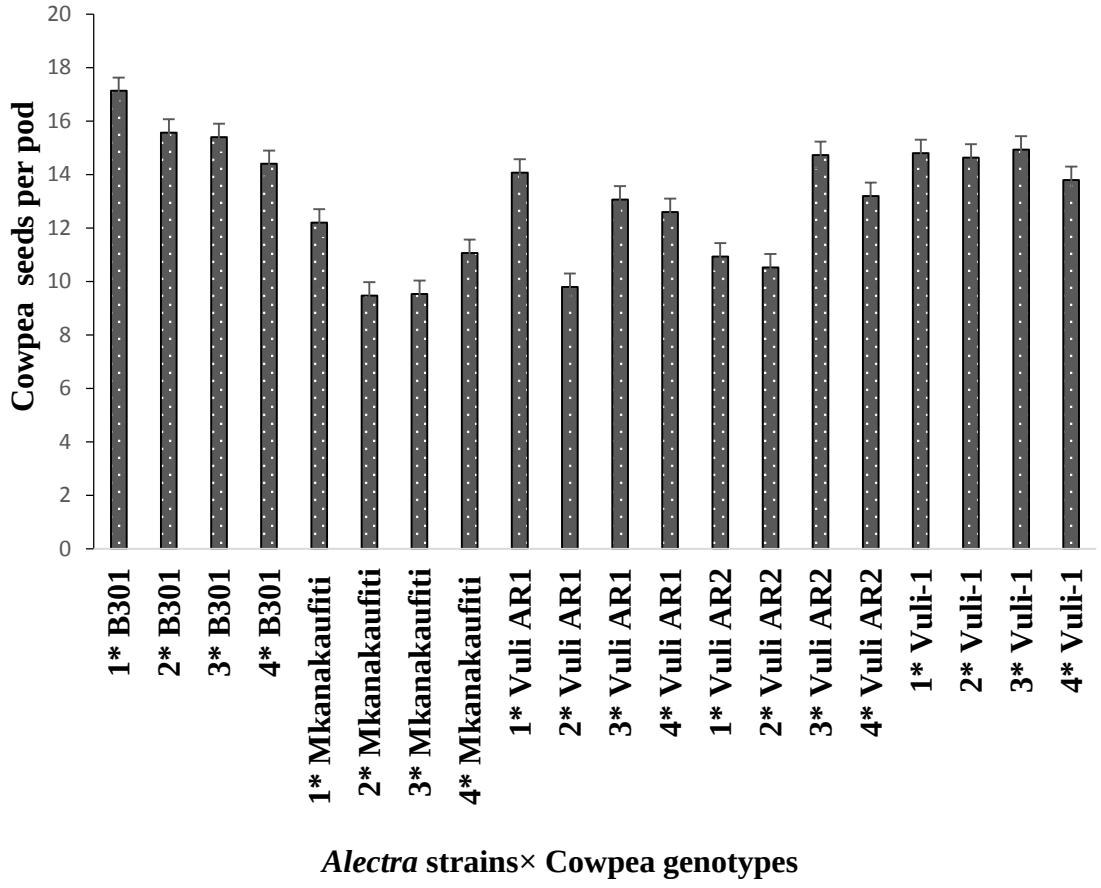

Figure 4.14: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on cowpea seeds per pod

The seed combinations of Vuli AR 1 with strain 2, 3 and 4 and Mkanakaufiti with strains 2, 3 and 4 recorded highest weight, while the lowest was from Vuli-1 with strain 4 (Fig. 4.15). However, the differences were not statistically significant.

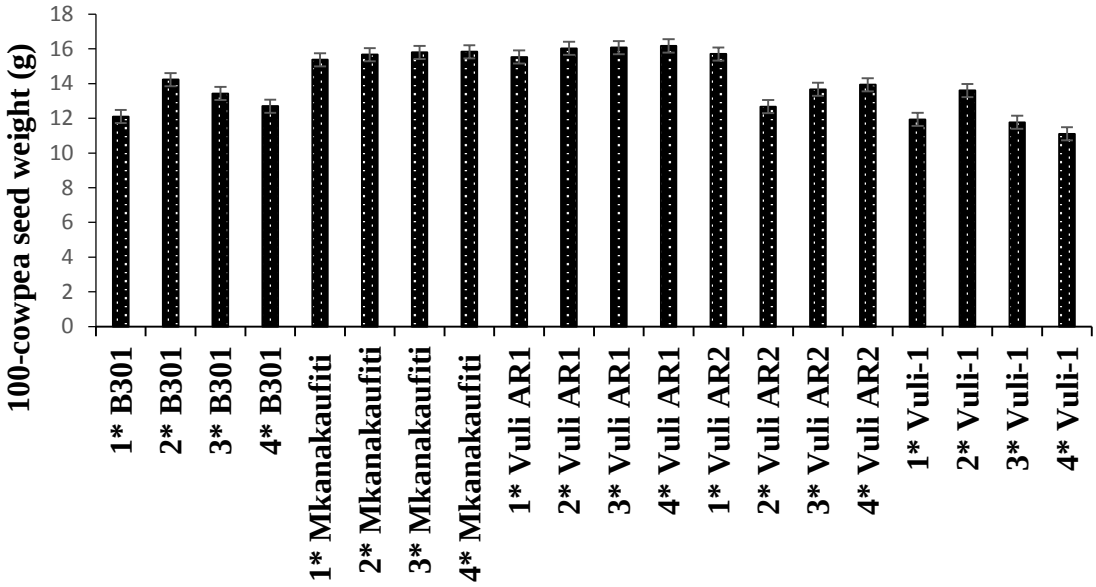

Figure 4.15: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on 100- cowpea seed weight

Vuli-1 with strain 3 recorded highest yield; followed by B 301 with strain 4. The lowest yield was produced by Vuli AR 1 with strain 2 (Fig. 4.16). However, the differences were not statistically different.

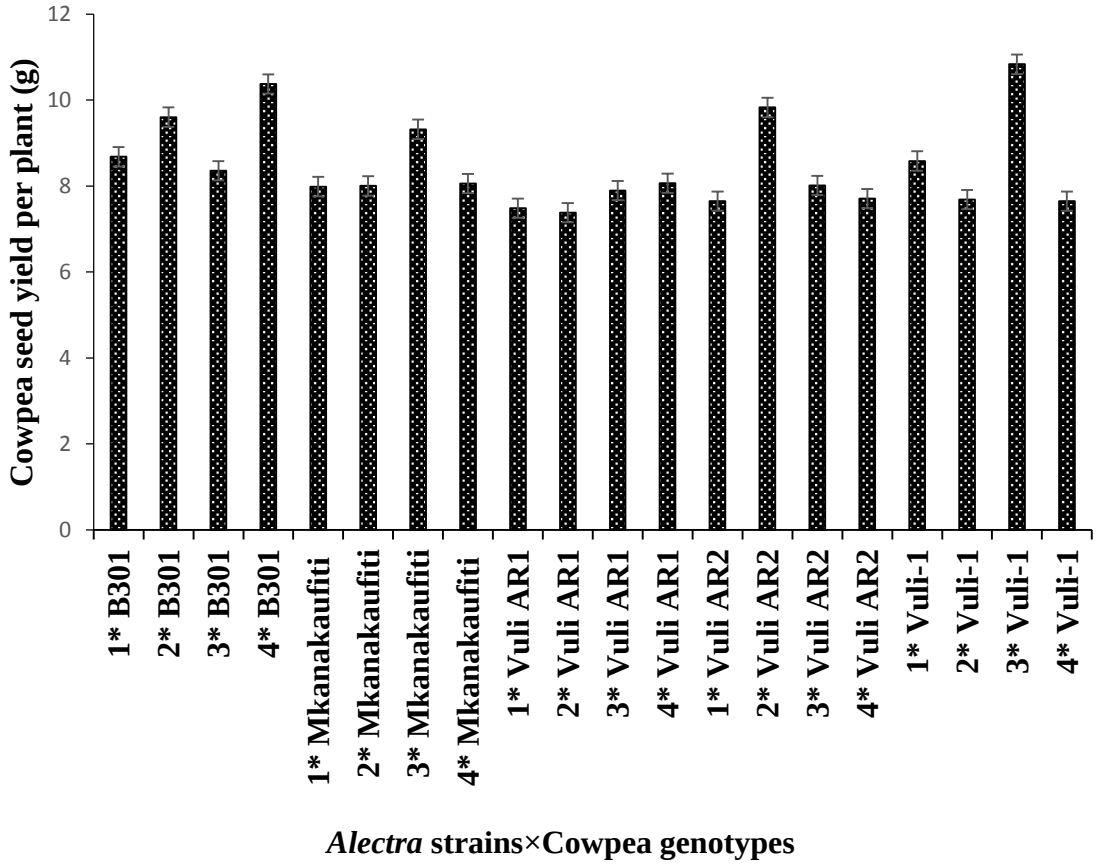

Figure 4.16: Interaction effect of strains of *Alectra vogelii* and cowpea genotypes on cowpea seed yield per plant

Results of linear correlation (r) analysis conducted to study the degree of relationship among various growth and yield components as they were affected by the strains of *Alectra vogelii* are presented in Table 4.5. The findings showed that the height of plant was highly significant and positively correlated with number of nodes per plant, leaf area index and 100- seed weight. Also, the number of leaves per plant was highly significant and positively correlated with number of branches per plant, and number of nodes per plant. Number of branches per plant was highly significant and positively correlated with number of nodes per plant.

The data showed that the leaf area index was highly significant and positively correlated with 100 seed weight, was highly significant and negatively correlated with number of

Alectra shoots at 49 DAP and was highly significant and negatively correlated with number of *Alectra* shoots at 63 DAP. Number of days to flower on set was highly significant and positively correlated with 95% to physiological maturity. The days to 50% flowering were highly significant and positively correlated with number of days to physiological maturity, while were significant and positively correlated with 100- seed weight. The number of days to 95% physiological maturity was highly significant and positively correlated with 100-seed weight, was highly significant and negatively correlated with number of *Alectra* shoots at 49 DAP and was highly significant and negatively correlated with number of *Alectra* shoots at 63 DAP.

The number of pods per plant was highly significant and positively correlated with weight of pods per plant and seed yield per plant and was significant and positively correlated with days to *Alectra* emergence. The length of pods per plant was highly significant and positively correlated with number of number of seeds per pod. The weight of pods per plant was significant and positively correlated with number of seeds per pod, highly significant and positively correlated with seed yield per plant, highly significant and positively correlated with number of days to *Alectra* emergence. The correlation showed that, 100- seed weight was significant and negatively correlated with number of *Alectra* shoots at 35 DAP, and highly significant and negatively correlated with number of *Alectra* shoots at 49 DAP. Number of days to *Alectra* emergence were highly significant and negatively correlated with 35 DAP, 49 DAP and, 63 DAP. Number of *Alectra* shoots per plant was highly significant and negatively correlated with 35 DAP and 49 DAP.

4.4 Correlation analysis

Table 4.5: Linear correlation among growth variables and yield components

S/N PH	PH	LP	BP	NP	LAI	DFS	50% F	95% M	NPP	LPP	WPP	NSP	100-SW	SYP	DAE	35 D	49 D
LP	0.097																
BP	0.386	0.343**															
NP	0.416**	0.408**	0.588**														
LAI	0.383**	0.092	0.072	0.051													
DFS	-0.168	-0.065	-0.247	-0.215	-0.219												
50% F	0.202	-0.037	0.005	0.078	-0.043	0.292											
95% M	0.177	-0.073	-0.208	-0.105	0.250	0.532**	0.507**										
NPP	-0.011	-0.099	0.214	0.105	-0.162	0.201	0.013	0.133									
LPP	-0.061	-0.155	-0.126	-0.018	0.143	-0.005	-0.164	-0.100	-0.208								
WPP	-0.145	-0.115	0.184	0.121	-0.254	0.061	-0.212	-0.045	0.830**	-0.052							
NSP	-0.282	0.021	-0.065	0.050	-0.209	-0.086	-0.417	-0.368	0.040	0.565**	0.292*						
100-SW	0.430**	0.151	0.171	0.099	0.619**	-0.021	0.309*	0.386**	-0.211	0.101	-0.208	-0.238					
SYP	-0.039	-0.136	0.165	0.104	-0.199	0.077	-0.083	-0.003	.791**	-0.175	0.839**	0.104	-0.244				
DAE	0.138	0.051	0.057	0.150	0.182	-0.060	-0.236	0.169	.287*	0.030	.290*	0.118	0.179	0.213			
35 D	0.143	-0.092	0.162	0.062	-0.228	-0.003	0.114	-0.153	0.076	-0.059	-0.047	0.032	-.256*	-0.036	-.519**		
49 D	-0.087	-0.088	0.031	-0.002	-.481**	0.079	0.132	-.380**	0.07	-0.041	0.018	-0.003	-.357**	0.085	-.458**	0.505**	
63 D	-0.161	-0.03	0.08	0.004	-.507**	0.144	0.185	-.358**	0.015	0.072	-0.047	0.011	-0.246	0.005	-.403**	0.430**	0.866**

ns = not significant

***Significant at 0.05**

****Significant at 0.01**

PH = Plant height (cm), LP= number of leaves per plant, BP= number of branches per plant,
NP = Number of nodes per plant, LAI=Leaf area index, DFS= Days to flower on set, 50% F = number of days to 50% flowering,
95% M= Days to 95% pod physiological maturity, NPP= number of pods per plant, LPP= length of pods per plant (cm), WPP=weight of pods per plant, NSP =number of seeds per pod, 100-SW = 100 see weight, SYP= seed yield per plant, DAE= days to *Alectra* emergence, 35 D= *Alectra* shoots at 35 days after planting, 49 D= *Alectra* shoots at 49 days after planting and 63 D = *Alectra* shoots at 63 days after planting.

4.5 Discussion

The significant mean squares observed for the *A. vogelii* strains and cowpea genotypes, indicated differences which exist among improved cowpea genotypes in their response to strains of *A. vogelii* (Appendix 1). Such differences were also reported by Alonge *et al.* (2001), that cowpea varieties have genotypic differences in their response to *A. vogelii*. These observed responses of cowpea genotypes to *A. vogelii* strains indicate that the genes controlling these parasites are non-allelic and independent of one another (Omoigui *et al.*, 2012).

The difference in days to emergence was observed among the strains of *A. vogelii* and among cowpea genotypes. Cowpea genotypes differ in days to emergence due to the differences in the thickness of the seed coat and tissue layers among the genotypes (Onyishi *et al.,* 2013). Also, differences in days to *A. vogelii* emergence is the result of cowpea genotypes to stimulate *Alectra* seed germination and allowing emergence of shoots (Gelete, 2010). Among cowpea genotypes, Vuli-1 supported earliest emergence whereas B 301 was the latest in supporting the emergence of *A. vogelii.* *Alectra* emergence occurred latest in genotype B 301 than the other resistant genotypes which are Mkanakaufiti, Vuli AR1 and Vuli AR2. The days to *Alectr*a emergence in B 301 coincided with its days to flower on set (Table 4.2 and 4.3). B301 is having the attributes of low production of stimulants for the germination of *Alectra* seeds as well as attachment and prevention of haustorial formation and subsequent development of the seedling of the parasite. *A. vogelii* was first noticed at 37.5 days after planting. The studies on cowpea, soybean, and groundnuts reported the emergence of *A. vogelii* at 55 DAP, 75 DAP and 109 DAP respectively (Kabambe *et al.,* 2008; Rugare *et al.,* 2013) which were contrary with 37.5 DAP found in this study. This would suggest that, the *A. vogelii* strains used in

this study were very aggressive, or cowpea genotypes in this study were able to allow the emergence of *A. vogelii* strains due to their ability to produce high levels of stimulants.

All genotypes allowed for *Alectra* shoot counts. The Vuli-1 had highest number of emerged *Alectra* shoots than the remaining genotypes. The local genotype B301, which is identified to be resistant to *A. vogelii* has been observed to exhibit its resistance to all the four strains, however it supported very few *Alectra* shoots (Fig. 4.2). The *Alectra* shoots in B301 was due to genes of resistance contained in the resistance. The resistance in genotype B 301 has been reported to be controlled by a single major gene, which may not be durable (Gnanamanickam *et al.,* 1999) because resistance conferred by a single major gene (vertical resistance), frequently fails to provide long term control to parasitic weeds. If such varieties are grown over a broad area they potentially lead to serious breakdown of resistance. The genotype Vuli AR1 is the derivative of B301 but Fig 4.2 indicates that, Vuli AR 1 reaction differes with strains. It supports lesser shoots per plant with strain 1 and 2 of the parasitic weed. Thus, presence of other genes might mask to varying degrees of the genes from B 301. Therefore, breeding for vertical gene resistance requires pyramiding of more than one gene from diverse resistance sources into a single genotype as vertical resistance is associated with a common phenomenon of the resistance breakdown (Gnanamanickam *et al.,* 1999). This would provide a better option so as to delay breakdown, broaden the resistance genetic base and provide much needed durable resistance.

The genotype B 301 attained 50% flowering earlier and the latest was Mkanakaufiti (Table 4.3). The mean value to 50% flowering in B 301 was 46.58 DAP. Days to 50% flowering reported by Ishayaku and Singh (2003), on two cultivars of cowpea were 31 and 38 days. Thus, different genes might result to differential maturity periods. The

attribute of flowering is controlled by a single dominant gene in cowpea (Ishayaku and Singh, 2003). The difference in the genotypes on time to flowering varies depending on the environmental factors like temperature, altitude, soil conditions, and photoperiod during the period for growth and development.

Time to 50% flowering determines the maturing period of genotypes (Table 4.3). Thus the 50% to flowering provides an opportunity for selection of earliness on different cowpea genotypes. Earliness is an important trait as it facilitates mechanism of *Alectra* resistance through escape from *Alectra* and may enable selection for planting in *Alectra* infested areas. The earlier the genotype flowers, the earlier the physiological maturity is reached (Shegro *et al.,* 2010). Latsest genotype B 301 resulted to latest days to *A. vogelii* emergence and earliest genotype Vuli-1 resulted to earliest days to *Alectra* emergence (Table 4.1, 4.2 and 4.3). Thus, choice of late genotypes will assist in the control of parasitic weeds. But, the earliness character (days to flowering, pod filling and days to physiological maturity enables B 301 to flower, pod fill and mature early and therefore escape the effect of *A. vogelii*. The genotype B301 gave higher seed yield and this was attributed by its resistance to *A. vogelii*. Seed yield is the major universal breeding objective of the cow crop (Oladejo *et al.,* 2011), being representing the final product from physiological and developmental process which occur from time of sowing to maturity.

Generally, the performance of a genotype B 301 was estimated from the analysis of its growth and yield variables. Superiority in the variables such as earliness for days to 50% flowering, number of pods per plant and seed yield per plant, implied that the genotype B301 was more vigorous in growth and subsequently led to higher seed yield. Seed yield is an important trait in plants because it is the final aggregate product of many interwoven physiological, biochemical and development traits controlled by different arrays of genes.

In order to achieve high seed yield, understanding traits of yield components is paramount (Oladejo *et al.,* 2011). The genotype B 301 proved to be the best genotype for resistance of *A. vogelii.* It produced the highest mean seed yields across strains. Thus, should be used as donor parent to provide the desirable traits to a recipient.

The genotype Mkanakaufiti was recorded as a late flowering on set genotype. The delayed onset of flowering in the genotypes, reduced number of flowers, number of pods, weight of pods and seeds in cowpea due to *Alectra* infestation (Alonge *et al.,* 2001). The number of seeds per pod was affected by of *A. vogelii*, and Mkanakaufiti genotype produced the least number of seeds per pod.

Genotypes did not differ significantly on leaves per plant (Table 4.4), however among all, Vuli AR 1 had highest number of leaves. This characteristic is important for the genotype if its leaves are used as vegetables and also can be used as livestock feed during the dry season of the semi-arid areas when fodders are scarce. The genotype Vuli AR1 also recorded the highest mean leaf area index (LAI) and the lowest leaf area index was recorded in genotype Vuli- 1 (Fig. 4.7). Varietal differences among the cowpea genotypes or differences in anatomical, morphological and physiological features affect the leaf area resulting to differences on leaf area index of the genotypes (Onyishi *et al.,* 2013).

The genotype Vuli AR1 gave the longest pods per plant (Table 4.4). The pod length is a genotypic characteristic. This implies that if the genotype has longer pod length, the seeds within the pods become widely spaced, compared to the genotypes with short pods. The character for long pod length is important in crop improvement because the longer pods more space is provided for seeds (Onyishi *et al.,* 2013). The genotype Vuli AR2 was early genotype to flower on set trait, while the late flower on set genotype recorded in this

study was Mkanakaufuti. The genotype Vuli AR2 recorded the highest 100 seed weight thus high seed size. The genotype B301 has very small seeds (Hela *et al.,* 2013), that is the reason for its lowest 100- seed weight. For longest pods and 100 seed weight, Vuli AR1 and Vuli AR2 respectively are best.

The genotype Vuli-1 was was able to produce high seed yield per plant under *A. vogelii* infestation which could suggest that, this genotype have some degree of tolerance to this parasitic weed. Nevertheless, since it had very low yield reduction despite high infestation, it can be considered as being tolerant to *Alectra.* Among the types of resistance, tolerance is considered as a type of horizontal resistance which is polygenic in contrast to vertical resistance which is monogenic (Kwaga *et al.,* 2010). Normally, the horizontal resistance has co-existence between the host and the parasite and it is more sustainable than vertical resistance which breaks down faster with time (Kwaga *et al.,* 2010). Despite high parasitim of *A. vogelii*, the tolerant genotypes produce high yield, which implies they are efficient in the production of assimilates to give high yields and in turn support the parasites (Kwaga *et al*., 2010). For number of seeds per pod, days to 95% pod maturity, and yield per plant, Vuli-1 ranked the best. The genotype combines number of seeds per pod, early maturity and its yield being higher than the improved genotypes, Mkanakaufiti, Vuli AR1 and Vuli AR2.

Regarding linear correlation analysis among growth variables and yield components, there was significant and positive correlations with weight of pods per plant, seed yield per plant with number of seeds per pod, seed yield per plant, days to *Alectra* emergence with length of pods per plant. This means that, the characters on pods per plant and weight of pods per plant when are selected during breeding programme, the seed yield per plant is well also selected.

Linear correlation on growth and yield variables indicated that, the leaf area index and 95% physiological maturity were highly significant and positively correlated with 100-seed weight, thus the effect of *Alectra* on LAI and 95% physiological maturity affected directly 100-seed weight, and indirectly affected the yield per plant of the genotypes. The LAI was affected by number of *Alectra* shoots per plant because there was highly significant and negative correlation with *Alectra* shoots at 49 DAP and 63 DAP. Number of shoots per plant caused a reduction in the rate of leaf expansion and photosynthesis per unit leaf area. The physiological maturity at 95% was highly significant and negatively correlated with number of *Alectra* shoots per plant, at 49 DAP and 63 DAP while 100-seed weight was significant and negatively correlated with number of *Alectra* shoots per plant, at 35 DAP, and was highly significant and negatively correlated with number of *Alectra* shoots per plant at 49 DAP.The negative relationship recorded between days to 95% maturity and *Alectra* shoots, indicated that 100-seed weight increased with earliness in maturity and became less as *Alectra* infestation increased.

The correlation analysis was performed principally in order to know the extent of association between growth variables, yield traits and *A. vogelii* which can bring genetic changes during improvement for resistance. Increasing major components of seed yield such as pods per plant, pod lenth, weight of pods, seeds per pod and 100 seed weight, allows improving cowpea yield potential (Makanur *et al*., 2013). Similarly, Alonge *et al.* (2001) found that *Alectra* reduced number of pods per plant, pod weight, number of seeds per pod and seed yield in cowpea. This was attributed to reduced leaf area and photosynthetic activity of parasitized cowpea plants. The high significant negative correlations recorded between 100 seed weight and *Alectra* infestation indicated that cowpea yield was reduced as *Alectra* infestation increased. This means that, reduced seed yield per plant is associated with increase in number of *Alectra* per plant. The correlation

between yield per plant and number of *Alectra* per plant indicates that higher number of yields per plant was obtained in the genotype B 301 which is resistant to *A. vogelii.*

The highly significant interaction effect between *Alectra* and genotype on seed yield variables obtained in this study showed how seed yield was indirectly affected by *A. vogelii*. The result therefore suggested that, the genotype B 301 and Vuli-1 respectively requires attention priority in improvement programs because they are potential good yielders and B 301 has resistance against *A. vogelii*. Indications were that, for areas infested with *Alectra,* the elite progenies desired from genotype B301 crosses could be planted aiming at higher seed yields and resistance to *Alectra.* Under higher *Alectra* infestation, resistance varieties could be adopted but including the crosses with other genotypes which are B301 and Vuli-1 to obtain high yields. In this study therefore, cowpea genotypes responded differently to four strains because the ability of host plants to tolerate these weeds involves a number of different mechanisms. Development of improved genotypes with resistance to a single strain is often straight- forward if a good source of genes for resistance is available. Efficient, easily controlled and practical screening procedures should be in place to provide good selection pressure. Unfortunately, this is seldom the case with many strains of parasitic weeds like *A. vogelii*. Breeding based on only a few dominant genes are at serious risk of breakdown of the resistance. This requires the breeders to continuously search for new sources of resistance.

The presence of *Alectra* infestation, yield and yield components of improved cowpea genotypes are highly associated with the level of resistance and performance of other growth parameters of the crop. Thus, in order to have higher yields use of resistant varieties and controlling the weed with other management strategies should be practiced.

The direct effect of *A. vogelii* is to reduce leaf area and photosynthetic activity which inturn reduces number of pods, number of seeds per pod, pod weight and seed yield in cowpea (Alonge *et al.,* 2001; Zitta *et al.,* 2014).

4.6 Conclusion and Recommendation

Strains of *A. vogelii* did not differ significantly on all the studied variables. Significant genotypic effects were evident for all the studied variables except for pods per plant and yield per plant. Significant interaction between genotpyes and strains was evident for leaf area index and number of shoots at 63 days after planting. *A. vogelii* had significant effect on pod length, number of seeds per pod, 100 seed weight however there was no significant effect on total yield per plant. There was varied number of days to emergence of *A. vogelii* among cowpea genotypes. Genotype B 301 a resistant genotype showed that, strain 3 reduces its number of pods per plant. The study showed that, each strain responds differently to each cowpea genotype. Apart from the effects of *A. vogelii*, the differences in performance are even due to cowpea inherent genetic differences. Venture requires to develop a genotype and extensively test it across a wider geographic area using many populations of *Alectra*. This will ensure stability and durability of the variety without easy breakdown once it is moved to another area with more virulent strains. This study has confirmed that it is possible to exploit host plant resistance as part of control options in the management of *Alectra* in cowpea. The combination of different resistance mechanisms into a single cultivar will provide durable outcome of the resistance in the field. This can be achieved by pyramiding resistant genes in cowpea using existing molecular markers.

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CHAPTER FIVE

5.0 GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, 8 primer pairs out of 23 pairs of primers used in the present study allowed enough distinction among the *A. vogelii* populations. These 8 markers can be used in subsequent experiments to detect molecular markers for polymorphic genes with economic importance. The analysis of descriptive population genetic statistics and molecular analysis of variance confirmed the presence of genetic variability among and within *A. vogelii*. AMOVA and cluster analysis revealed substantial variation in *A. vogelii,* implying that gene flow occurred that resulted in several strains. Another reason could be adaptation of the *A. vogelii* in the different agro-ecologies that resulted in wider diversity. The *Alectra* populations are highly variable. The ultimate source of variability was due to gene flow. Exchange of cowpea seeds by the farmers across the regions could also be responsible for the introduction and subsequent maintainance of variable. Cluster analysis also revealed that the majority of populations of a given geographical location tend to group together. The *Alectra* was significantly and negatively correlated with some growth and yield variables of the cowpea genotypes. The significant differences found among the genotypes in reaction to strains of *A. vogelii* suggest the combined sustainable control options of *A. vogelii*. Breeding strategy for resistance to *A. vogelii* seems not a straight forward venture. It is possible to exploit host plant resistance as part of control options.

5.2 Recommendations

The following are recommended for further studies:

i) A study should be done on identification and characterization of useful genes of germpalsms with additional source of resistance to help bringing much diversity to broaden the genetic base of cowpea cultivars.

ii) The valuable appropriate breeding efforts should employ the hypersensitive reaction mechanisms when developing high yielding *Alectra* resistant genotypes.

iii) The main focus should be on intogression of resistant genes into adapted cultivars with pyramiding the resistance, routine screening against the parasite and future matching of resistant cowpea genotypes with *A. vogelii* strains.

iv) To increase production and productivity to the farmers, the pivot concern in cowpea breeding programme for resistance should be to improve yielding potential, quality of the grains and nutritional values.

APPENDICES

Appendix 1: ANOVA for the studied variables (mean squares)

Source of variation	d.f.	DAE	35 DAP	49 DAP	63 DAP	PH	LP	BP	NP	LAI	DF	50% F	95% M	PP	WP	LP	SP	100-SD	SYP
Replications	2	312.07	28.533	5.176	12.817	879.3	1.842	49.526	3.5866	0.02319	5.017	0.650	2.817	16.235	52.749	7.441	17.267	5.701	19.308
Strains	3	30.950	25.696	4.729	11.572	957.8	10.94	7.272	1.9967	0.06353	0.772	0.950	2.133	0.522	4.508	3.913	9.666	0.491	1.676
Residual Error	6	21.867	10.226	7.868	9.106	2.620	6.220	0.539	0.9971	0.01768	1.572	1.583	3.217	5.147	34.062	1.833	6.793	1.024	11.857
Genotypes	4	143.560	32.213	385.846	590.642	899.4	4.146	4.305	0.6535	6.88479	47.517	82.725	241.767	3.495	38.055	20.479	47.843	32.504	3.867
Strain*Genotypes	12	3.436	4.557	15.974	18.197	241.2	6.093	0.613	0.4042	0.10271	1.272	0.658	1.967	2.573	9.496	1.71	5.467	2.653	3.080
Residual Error	32	8.792	5.361	8.48	8.658	162.7	8.367	1.142	0.8678	0.01804	2.433	1.537	3.617	2.419	7.393	4.481	6.21	2.392	2.921
Total	**59**																		
P- value (Strains)		0.327	0.155	0.638	0.366	0.083	0.255	0.004	0.215	0.085	0.701	0.638	0.604	0.956	0.937	0.197	0.325	0.708	0.932
P- value (Genotypes)		0.001	0.001	0.001	0.001	0.002	0.739	0.013	0.563	0.001	0.001	0.001	0.001	0.242	0.003	0.005	0.001	0.001	0.282
P- value (Strain*Genotypes)		0.957	0.602	0.076	0.047	0.182	0.714	0.874	0.920	0.001	0.884	0.940	0.869	0.420	0.420	0.961	0.574	0.387	0.428

DAE = days to *Alectra* emergence, 35 DAP= *Alectra* shoots per at 35 days after planting (DAP), 49 DAP= *Alectra* shoots per at 49 days after planting (DAP), 63 DAP= *Alectra* shoots per at 63 days after planting (DAP), PH= plant height (cm), LP= leaves per plant, BP= branches per plant, NP= Nodes per plant, LAI= leaf area index (LAI), DF= days to flower on set, 50% F= days to 50% flowering, 95% M= days to 95% days to pod physiological maturity, PP = pods per plant, WP= weight of pods per plant (g), LP= length of pods per plant (cm), SP= seeds per pod, 100- SD = 100-seed weight (g) and SYP= seed yield per plant (g)