

**DIVERSITY OF *Striga gesnerioides* (L.) Walp ON COWPEA IN GHANA**

**FREDERICK JUSTICE AWUKU**

**(10277011)**

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## DECLARATION

I hereby declare that except for references to works of other researchers, which have duly been cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

.....

Frederick Justice Awuku  
(Student)



.....

Prof. Pangirayi Tongoona  
(Supervisor)

.....

Dr Agyemang Danquah  
(Supervisor)

.....

Dr Francis Kusi  
(Supervisor)

## ABSTRACT

*Striga gesnerioides* is a devastating parasitic weed of cowpea, especially in Ghana and the rest of West Africa. It is the major cause of cowpea yield reduction in Ghana. Despite seven different races being known across West Africa, the races of the parasite present in Ghana and their diversity have not been studied. This study investigated the distribution and diversity of *Striga gesnerioides* on cowpea in Ghana. The study revealed widespread distribution of the parasite within the Northern, Upper East and Upper West regions of the northern sector of the country. Morphological variation within the parasite showed two flower colour types: purple and white, with a ratio of purple to white being 3:1. The parasite was also found parasitizing two weeds: *Tephrosia pedicellata*, a leguminous weed, and *Ipomoea tribola* a non-leguminous weed. The reaction of 29 cowpea genotypes to eight *Striga* populations from endemic zones revealed that some cowpea genotypes were resistant to all *Striga* populations, some susceptible to all *Striga* populations and others showed differential response. The genotypes which showed differential responses probably indicated the presence of more than one race of *Striga gesnerioides* in Ghana. Molecular studies on 35 *Striga gesnerioides* collections using 73 markers designed using *Striga gesnerioides* sequences retrieved from the National Centre of Biotechnology Information (NCBI) database revealed two main clusters. This shows the probable existence of multiple strain of *Striga gesnerioides* in Ghana. The combined results of response of differential cultivars and the molecular study revealed the probable races of *Striga gesnerioides* in Ghana from this study are SG3, SG2 and SG5. More studies however are needed to positively confirm the presence of these races in the country.

## DEDICATION

I dedicate this work to my entire family. My dear wife, Sandra Awuku and lovely daughter Sigrid Anyamah Awuku, My dad Simon Tonnah, my mum, Dora Awuku and all my siblings, Derrick, Cecilia, Eunice and Joyce.



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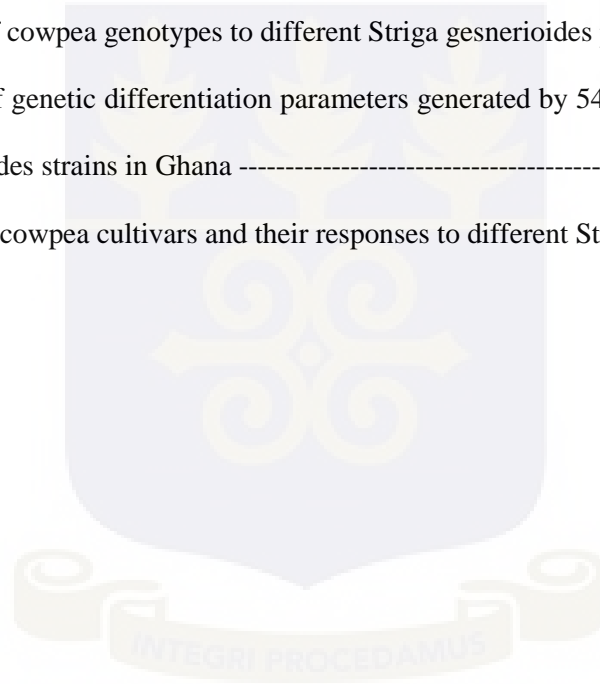
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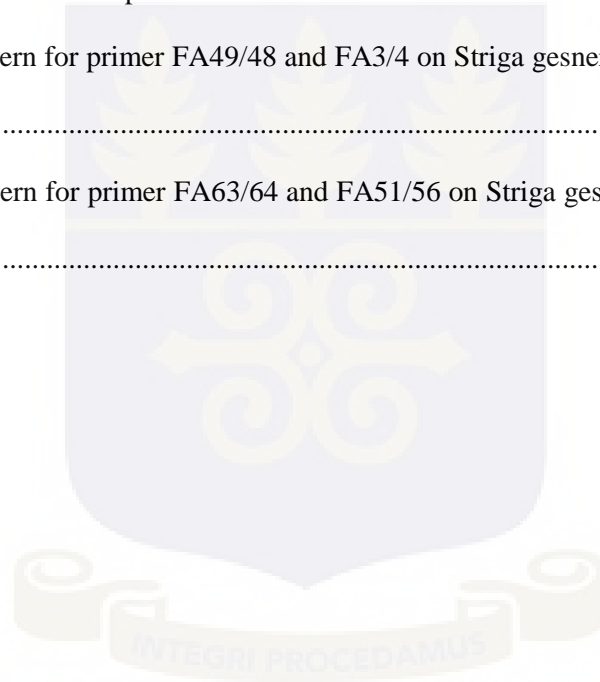
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## LIST OF ABBREVIATION

$\mu$ l	
Micro liter.....	38
2.4-D	
2,4-Dichlorophenoxyacetic acid.....	13
AFLP	
Amplified Fragment Length Polymorphism.....	20
AP-PCR	
Arbitrarily Primed Polymerase Chain Reaction.....	20
ASLP	
Amplified Sequence Length Polymorphism.....	19
CAPS	
Cleaved Amplified Polymorphic Sequence.....	19
cm	
Centimetre.....	31
CSIR	
Council for scientific and industrial research.....	29

CTAB

Cetyl trimethylammonium bromide..... 33

DAF

DNA Amplification Fingerprinting.....20

DAMD

Directed Amplification of Minisatellites DNA..... 20

DGGE

Denaturing Gradient Gel Electrophoresis..... 19

DHPLC

Denaturing High Performance Liquid Chromatograph..... 19

DNA

Deoxyribonucleic acid..... 19

DTSE

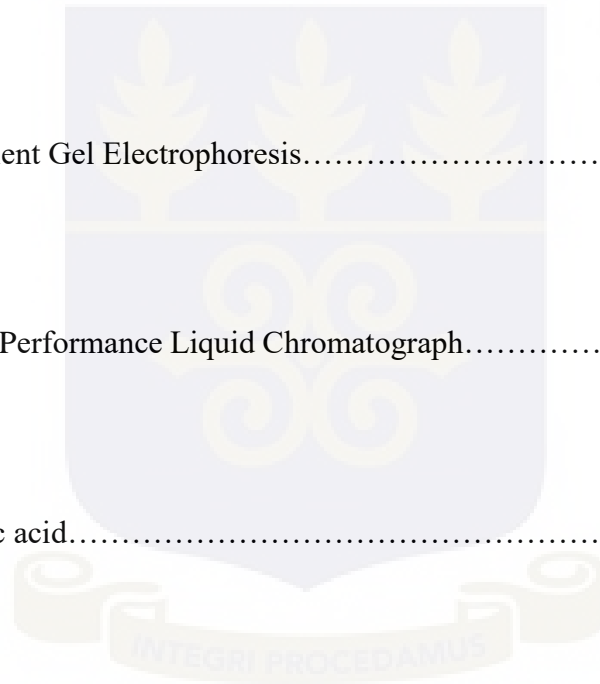
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EDTA

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GC%

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GPS	
Global Positioning System.....	40
HDA	
Heteroduplex Analysis.....	19
IITA	
International Institute of Tropical Agriculture.....	26
ISSR	
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ITS	
Internal Transcribed Spacer.....	19
LG	
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mM	
Milli Molar.....	38
NCBI	
National Centre on Bioinformatics Information.....	34
PCR	
Polymerase chain reaction.....	39

PVP	
polyvinyl pyrrolidone.....	37
RAPD	
Random Amplified Polymorphic DNA.....	20
RFLP	
Restriction Fragment Length Polymorphisms.....	19
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SARI	
Savannah Agricultural Research Institute.....	26
SAS	
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SCAR	
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SES	
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SG	
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SPAR

Single Primer Amplification Reaction.....20

SSA

Sub Saharan Africa.....2

SSCP

Single-Strand Conformation Polymorphism.....19

SSLP

Simple Sequence Length Polymorphism.....19

SSR

Simple Sequence Repeats.....3

STMS

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STR

Short Tandem Repeat.....19

TAE

Tris acetate EDTA.....39

TGGE

Thermal Gradient Gel Electrophoresis.....19

Tm

Melting Temperature.....36

UAM

University of Agriculture, Makurdi.....26

UPGMA

Unweighted Pair Group Method with Arithmetic Mean.....40

USA

United States of America.....9

UV

Ultraviolet.....39

VNTR

Minisatellites or Variable Number of Tandem Repeats.....19



## CHAPTER ONE

### 1.1 INTRODUCTION

Cowpea is an important grain legume in Ghana and West Africa (Anele *et al.*, 2011). Its importance is well documented as a protein rich source, soil fertility improvement crop and fodder for animals (Boukar *et al.*, 2011). Cowpea though very important, is faced with several biotic and abiotic production constraints. An important biotic constraint is *Striga gesnerioides* infestation (Asare *et al.*, 2013).

*Striga gesnerioides* is amongst the world's worst obligate parasitic weeds, reducing the yield and quality of legumes especially in semi-arid regions of the world (Botanga *et al.*, 2002; Ramesh *et al.*, 2006). *S. gesnerioides* is the only species of the genus *Striga* that is virulent on dicots (Mohamed *et al.*, 2007; Ramesh *et al.*, 2006; Welsh & Mohamed, 2011). A major host of *S. gesnerioides* is cowpea, a widely cultivated legume in Ghana and West Africa. *S. gesnerioides* also parasitizes other legumes such as Bambara groundnut, common bean, mung bean, and many legume fodder crops, including Hyacinth bean, Siratro and Velvet bean (Langyintuo *et al.*, 2003; Rubiales *et al.*, 2006; Timko *et al.*, 2007).

Yield losses ranging from 36.5 – 44.2 % have been reported on farmers' fields due to infestation by *S. gesnerioides* (Berner *et al.*, 1994). Hearne (2009) reported that 40 % of losses in crop production in Africa is due to *Striga spp.* This represents an annual value loss of agricultural revenue of about 7 billion dollars (Hearne, 2009). Due to severe loss from *S. gesnerioides* infestation, supply of cowpea does not meet demand in Ghana. This has contributed partly to the high import of 3,380 metric tonnes of cowpea grains to supplement the country's production of 219,300 tonnes as at 2010 (Egbadzor *et al.*, 2013).

One major cause of food insecurity in the world is *Striga* as it affects food crops that constitute about 70% of diet in semi-arid sub-Saharan Africa (SSA) with cowpea forming about half the total protein present in the same diet (Lane & Bailey, 1992). Several control measures have been suggested, which include; cultural practices, chemical control, biological control and host plant resistance (Boukar *et al.*, 2004; Raj 1949). No single method has completely and effectively controlled the infestation (Dubé & Olivier, 2001; Parker, 2012). The difficulty in controlling this parasite and development of stable resistant cultivars is partly due to its differential virulence (Botanga & Timko, 2006).

*Striga gesnerioides* exhibits differential virulence across the West Africa sub-region with some countries having more than one race (Lane & Bailey, 1992). At least seven distinct races of *S. gesnerioides* have been identified throughout West Africa, designated SG1 through to SG7. There are two different races reported in Benin designated SG4 and SG4z which are the most virulent so far. Some of these races are related, such as SG1 and SG5; SG4 and SG6; whilst SG4 and SG3 are divergent (Botanga & Timko, 2006; Li *et al.*, 2009; Omoigui *et al.*, 2012; Toure *et al.*, 1998). Studies have shown that the genetics of cowpea *Striga*-resistance varies according to the biotype of the parasite (Karanja *et al.*, 2013; Toure *et al.*, 1998). Dubé & Olivier (2001) pointed out that Cowpea *S. gesnerioides* resistance is conferred by oligogenic independent dominant genes. Other studies pointed out that resistance is conferred by monogenic recessive genes and others reported monogenic dominant genes (Huang *et al.*, 2012; Moore *et al.*, 1995; Singh *et al.*, 1993; Toure *et al.*, 1998).

Crop production in the savannah ecological zone of Ghana is characterized by a prolonged dry season, an erratic rainfall period as well as low soil fertility with low levels of external

inputs. These climatic and soil conditions support the growth of *S. gesnerioides* resulting in high yield losses in cowpea production by farmers who have very little idea on its management. The growth of *S. gesnerioides* is stimulated by strigolactone, which is in the root exudates of the cowpea (Fernandez-Aparicio *et al.*, 2011). Studies have shown that under stress conditions such as poor soil fertility and water deficit the cowpea plant strives to support itself by producing strigolactone, resulting in high levels of *Striga* germination (Lane *et al.*, 1996).

A major concern with this weed is its differential virulence. Knowing the different races of *S. gesnerioides* within the country will bring to bear important information on the race of the parasite present in the country and form the basis for the cowpea breeding programme for further breeding activities. However, there is currently no published information or report on the diversity nor the race(s) of *S. gesnerioides* in Ghana.

Few molecular diversity studies have been conducted on *Striga gesnerioides*. The few studies reported have used Amplified Fragment Length Polymorphic (AFLP) markers. Botanga & Timko, (2006) and Dube & Belzile (2010) examined genetic diversity in *Striga gesnerioides* using AFLPs. Dube & Belzile (2010) concluded that, there was a low number of polymorphic bands of 6% from a total of 1200 bands. Simple Sequence Repeats (SSR) markers are highly polymorphic as compared to AFLPs and give a better degree of variability within populations as has been observed from several crop diversity studies (Teka, 2014). There are no developed SSR markers for *Striga gesnerioides* diversity studies. It will thus be very good to develop SSRs to examine the diversity of this parasite due to their advantages over AFLPs.

The objective of this study was to determine the diversity of *S. gesnerioides* collections from different endemic regions in northern Ghana.

The specific objectives were to:

- determine existence of genetic diversity among *S. gesnerioides* populations from cowpea fields in Northern Ghana, and
- evaluate the response of differential cowpea cultivars to *S. gesnerioides* and determine the races of *Striga* in Ghana



## CHAPTER TWO

### 2.1 LITERATURE REVIEW

#### 2.1.1 Parasitic plants

A parasitic plant is a plant that derives some or all of its nutritional requirements from another living plant. They make up about 1% of angiosperms and are in almost every biome in the world (Westwood et al., 2012). All parasitic plants have modified roots, named haustoria (singular haustorium), which penetrate the host's vascular system. The haustoria provide them with the ability to extract water and nutrients from the host (Estep et al., 2012).

About 4000 flowering plant species have adapted to parasitize other plants. Unfortunately for farmers, a small number of these species have become weeds, posing severe constraints to major crops including grain and forage legumes (Rubiales & Mónica, 2012). Most of these parasitic plants are able to detect their host by chemical exudates or certain signalling processes from the host which facilitate germination and attachment to the host and derive benefits for their growth and development (Musselman, 1980).

Species within the Orobanchaceae are some of the most economically destructive of which *Striga* is a part. Legumes are major food crops in West Africa under severe parasitic weed infestation. Major parasitic weeds that affect legume production are broomstraps, *Alectra vogelii*, Dodder, *Striga gesnerioides* (Parker, 2012). *Striga gesnerioides* is a major problem to farmers in West Africa (Ghana, Mali, Burkina Faso, Niger, Nigeria, Senegal, Togo and Benin) and tobacco production in Zimbabwe (Timko et al., 2007). Grain yield

of cowpea is reduced drastically following infestation by *Striga gesnerioides* (Aggarwal *et al.*, 1988).

### 2.1.2 *Striga gesnerioides*

*S. gesnerioides* is one of about 30 species of the genus *Striga* and the only spp. of the genus parasitizing dicots (Spallek *et al.*, 2013). Seeds of *S. gesnerioides* are found in capsules of approximate diameter 2.40 mm and length 5.96 mm with each capsule containing approximately 800 seeds of approximate weight 2.0 mg/capsule (Ayensu *et al.*, 1984)

(Plate 2:1).



Plate 2:1 *Striga gesnerioides* seed capsules (A), microscopic view of *Striga* seeds stained with 1% Iodine potassium iodide at x400 (photo by Frederick J. Awuku)

### 2.1.3 Origin and Taxonomy of *S. gesnerioides*

The origin of *Striga gesnerioides* is not different from that of the genus *Striga*. Its origin has not been pin pointed to a location as has been for most domesticated crops. According to Atera & Itoh (2011) the origin of *Striga* is believed to be the same as that of domesticated sorghum to be between the mountains of Ethiopia and the Nubian hills of

Sudan. It is also believed that monocot parasitizing *Striga* evolved from those *Striga* on Sorghum (Westwood *et al.*, 2012). *Striga gesnerioides* as it is currently known scientifically belongs to the following taxonomic tree:

**Domain:** Eukaryota

**Kingdom:** Plantae

**Phylum:** Spermatophyta

**Subphylum:** Angiospermae

**Class:** Dicotyledonae

**Order:** Scrophulariales

**Family:** Scrophulariaceae

**Genus:** *Striga*

**Species:** *Striga gesnerioides*

*Striga gesnerioides* was originally described as *Buchnera gesnerioides* in 1801 by Willdenow (Oxelman *et al.*, 2005), *Buchnera orobanchoides* by R. Brown in 1814, *Striga orobanchoides* in 1836 by Bentham. In Ghana, several local names are given to *Striga gesnerioides* due to the devastating effect it has on cowpea. Among the people of the Upper East region it is called 'Wang' meaning rival, among the people of the Northern region 'Wubulim' meaning destroyer and 'Dochaa' meaning cowpea cannot survive and among the people of the Upper West region 'Saka' meaning locust and 'Wonbile' meaning destroyer (Personal communication, 2016), these names translate into the devastating effect of the parasite on its host and nature of fields under infestation.

### 2.1.4 Life cycle of *S. gesnerioides*

The life cycle of *S. gesnerioides* is not completely different from the general life cycle of the genus *Striga* (Figure 2:1). *S. gesnerioides* life cycle takes an alternating wet and dry season in the tropics. The seeds of *Striga* remain viable in the soil for almost 20 years (Ayensu *et al.*, 1984). Germination of *Striga* seeds requires pre-treatment and a stimulant ‘strigol’ or strigolactone produced by the host root exudate (Botanga & Timko, 2006). Pre-treatment is provided by rains at the beginning of the rainy season before planting and establishment of host root system. *Striga* seeds within 2 mm of the host root zone will germinate in response to the stimulant produced by its host (Hearne, 2009).

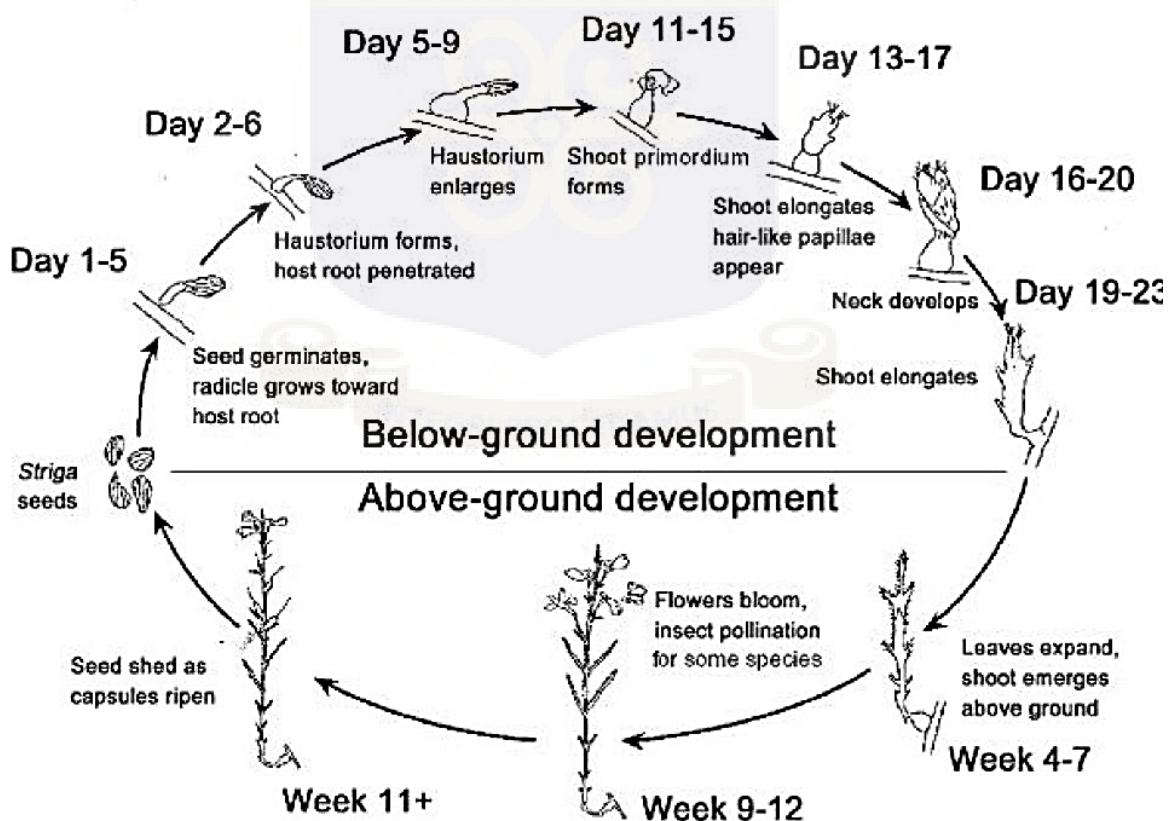


Figure 2:1 General life cycle of *Striga* source: (Aggarwal *et al.*, 1988)

The growing radicle of the *Striga* seed forms a haustorium that attaches and links to the vascular system of its host. The parasite develops rapidly at this stage but remains underground for some weeks till it is well established and has colonized the root zone of its host. On emerging above the ground, it flowers and releases hundreds of seeds in each seed pod. Though the aerial part possesses normal chlorophyll, the parasite largely depends on its host. The seeds are light and tiny and mostly dispersed by wind. Dispersed seed remains dormant in the soil until it receives a stimulant from an appropriate host when it germinates (Cook et al., 1966).

#### **2.1.5 Distribution of *Striga gesnerioides***

*Striga* is wide spread all over Africa, India and tropical Asia (Figure 2:2) (Jansen & Cardon, 2005). Botanga & Timko (2006), mentioned its presence in Florida in the United States of America (USA). Its introduction in the USA might probably be through exchange of contaminated seed from infested regions around the world. It is also recorded in Australia (Barkley et al, 1980). *Striga gesnerioides* is widely spread throughout West Africa probably due to it being a major centre of cowpea production (Lane et al., 1996). Although *Striga gesnerioides* infestation is of serious concern to cowpea production in Ghana, its distribution has not been documented.



### 2.1.7 Host range of *Striga*

The genus *Striga* is known for its devastating effect on its host. This genus has quite a wide range of hosts from wild to domesticated crops. Most hosts of the genus are cereals, with sorghum being a major host. Millet and rice are other cereals that are hosts to *Striga*. The species that attack cereals are *S hermonthica*, *S. asiatica* and *S. forbesii*. Legumes are another group of crops that are hosts to *Striga*, with the majority within this group being cowpea. *Striga* is a major constraint to production of the above-mentioned cereals and legumes on the Africa continent. **Table 2:1** summarises the host range of some selected species of the genus that are of economic importance.

**Table 2:1** Host range of some selected *Striga* species of economic importance

<i>Striga</i> species	Host range
<i>S. hermonthica</i> (Del.) Beth	Sorghum, pearl millet, maize, rice, wild grasses
<i>S. asiatica</i> (L.) Kuntze	Sorghum, pearl millet, finger millet, maize, upland rice, sugar cane, wild grasses
<i>S. gesnerioides</i> (Willd.) Vatke	Cowpea, tobacco, sweet potato, convolvulaceae and fabaceae
<i>S. aspera</i> (Willd) Benth	Rice, Wild grasses, Maize, Sorghum, Sugar cane
<i>S. euphrasioides</i> Benth	sorghum, maize, sugarcane, upland rice, wild grasses
<i>S. forbesii</i> Benth	sorghum, maize, rice
<i>S. bilabiata</i> (Thunb.) kuntza	wild grasses
<i>S. elegans</i> Benth	wild grasses
<i>S. macrantha</i> Benth	wild grasses
<i>S. aequinoctialis</i> Chev. Ex hutch, and Dalz	wild grasses
<i>S. klingii</i> (Skann)	sorghum, millet, wild grasses

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<i>S. elegans</i> Benth	cereals, wild grasses
<i>S. brachychalyx</i> Skan	cereals, wild grasses
<i>S. latericea</i> Vatke	sugarcane, cereals

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Sources: (Atera & Itoh, 2011; Musselman, 1980; Sibhatu, 2016; Spallek *et al.*, 2013)

### 2.1.8 *Striga* control measures

There have been several efforts by researchers and farmers to eradicate the problem of *Striga* infestations on legume and cereal crops with a major focus on *Striga gesnerioides* and *hermonthica*, but these have yielded very little impact. The various methods used for *Striga* control can be grouped into; cultural practices, biological control, herbicides and resistance breeding. Most of these have been used in the control of *Striga hermonthica* on cereals.

#### 2.1.8.1 Cultural control of *Striga*

Cultural control of *Striga* is one of the oldest control measures. Cultural control includes but not limited to the use of expensive farm inputs, labour intensive approaches such as hand weeding and changes in cropping systems (Berner *et al.*, 1997; Berner *et al.*, 1994).

Hand weeding is often resorted to by the rural poor farmers, whom mostly are in developing countries. The approach is not helpful in preventing the damage caused by the attachment of the parasite to its host nor prevents it from attaching. Weeding is usually done after emergence of the *Striga* and by which time the damage has been done while the *Striga* was beneath the soil surface. This method though not helpful immediately, helps in

the long run by reducing the *Striga* population and a build-up of *Striga* seed in the soil (Obilana & Ramaiah, 1992).

*Striga* is known to thrive well in very poor soils, the use of farm inputs such as fertilizers; organic or inorganic fertilizers to increase the soil fertility in *Striga* endemic zones reduces *Striga* infestation. These methods have not been helpful as they are expensive to practise by the rural poor farmers and also result in an accumulation of residues and usually acidifies the soil because of the poor rainfall in the regions (Teka, 2014).

Changes in cropping systems has been reported to help in the control of *Striga* on crops. Rotating between crops such as host and non-host crops helps fallow the farm land for a while before returning to planting host plants which helps reduce the seed bank of the parasite. This practise could be combined with mixed cropping of the host plant with trap crops that favour the suicidal germination of *Striga* seeds (Obilana & Ramaiah, 1992; Teka, 2014).

#### **2.1.8.2 Chemical control of *Striga***

Chemical control though expensive has proved useful in the control of *Striga*. Pre- and post-emergence *Striga* herbicides have been successfully used to control *Striga* on cereals. This includes Goal as pre-emergence herbicide and 2,4-D, dicamba, dicamba in combination with urea or chlorsulfuron, Glufosinate and Oxyfluorfen as post emergence herbicides (Dugje *et al.*, 2006; Obilana & Ramaiah, 1992; Spallek *et al.*, 2013; Teka, 2014). These herbicides do not prevent the damage of *Striga* to the crop prior to emergence, thus still not reliable in the short term. Germination stimulants such as

Ethylene, ethephon, strigol, strigolactone in dicots results in the suicidal germination of *Striga* seeds in the absence of its host, hence reduces the seed build up drastically (Cook *et al.*, 1966; Pescott, 2013; Steinkellner *et al.*, 2007; Teka, 2014).

### **2.1.8.3 Host resistance control of *Striga***

Host resistance control to *Striga* seems to be the most lasting solution to the *Striga* infestation problem and other parasitic weeds. Host resistance is the introduction of host plants that will prevent the attachment and subsequent emergence of the parasite or induce a hypersensitive reaction to kill attached parasite. Host plant resistance is the most affordable and durable way of curbing the *Striga* problem. Several maize varieties have been developed with resistance to *Striga hermonthica* (Pescott, 2013) across west Africa. Other crops (host) have been developed to have resistance to parasitic weed infesting them. Toure *et al.* (1998), identified cowpea with resistance to *Striga gesnerioides* infestation. Omoigui *et al.* (2015) also identified sources of resistance in cowpea to *Striga gesnerioides* infestation. The genotype IT97K-499-35 (Songotra) and B301 have been used for over a decade now as resistant sources to *Striga gesnerioides* over the west African sub region (Singh *et al.*, 1993).

### **2.1.9 Diversity of parasitic weeds**

Parasitic weeds are a severe constraint to cowpea production in sub Saharan Africa, therefore an understanding of their diversity will lead to the identification of races or biotypes and hence improve the chance of breeding success. *Striga hermonthica*, *Striga asiatica*, *Striga gesnerioides*, *Striga aspera* and *Striga forbesii* are the most harmful of the *Striga* genus. Presence of biotypes of *Striga* has long been thought to be responsible for

the breakdown of resistance in crops (Joel, 2014) . Gethi *et al.* (2005) and Dawud (2017) made similar observation in Tanzania and West Africa respectively on sorghum. There has also been reports of the breakdown of resistance of cowpea variety B301 to *Striga gesnerioides* race 4z in Benin. The wide geographical distribution set conditions for genetically structured populations. The genetic variations among the weed populations allow for quick breakdown of resistance in crops hence making control of the weed difficult (Gethi *et al.*, 2005). Information on the diversity within the parasite is needed to effectively control them.

Diversity studies in *Striga* have been performed using several approaches including; morphological, physiological and genetic traits analysis. The advent of techniques to estimate genetic diversity has enabled scientists to focus their work on describing hybrids, local races and general genetic diversity inherent in plant parasites. Much work has been done on genetic diversity of *Striga* spp. such as *S. asiatica*, *S. aspera* and *S. hermonthica* (Estep *et al.*, 2011) but very little on *Striga gesnerioides*.

Navajas & Fenton (2001), combined allozyme electrophoresis and Random Amplified Polymorphic DNA (RAPD) markers to investigate genetic diversity in *S. hermonthica* populations from Nigeria and Kenya. Using cluster analyses, both methods showed high levels of genetic diversity existing between and within the *Striga* populations (Joel, 2014). Gethi *et al.* (2005) studied the genetic diversity of 24 *S. hermonthica* populations from Kenya using AFLPs. He reported very low genetic diversity (at 90 % similarity) between and within the populations studied. This was attributed to the homogeneity of the Kenyan populations of *S. hermonthica* due to substantial gene flow between the populations and noted that seed dispersal basically could have been through contaminated seeds. This

agreed with studies on *S. asiatica* and *S. gesnerioides* colonization events in the United States where both showed genetic uniformity in introduced populations suggesting a single successful colonization event (Welsh & Mohamed, 2011).

Musimwa (2005), worked on the genetic variability, host specificity and resistance in *Striga asiatica*-host plant interactions and concluded that there were physiological strains of the parasitic weed *S. asiatica*, which were adapted to specific hosts within and between species. Another observation he made was that, molecular techniques such as RAPDs and isozymes can be effectively used to differentiate strains of parasitic weed; *S. asiatica* however there is a need to determine the relationship between molecular differences and differential virulence (Musimwa, 2005).

Diversity study among 12 *Striga hermonthica* population from four regions Mali using 12 microsatellite markers revealed, extensive diversity among them with most being heterozygous for most markers. Large amount of gene flow was observed with broad distribution of allelic diversity across the populations but the genetic differentiation observed was very (Estep *et al.*, 2011).

*Striga gesnerioides* being a species of *Striga* has a history of low genetic variability (Musselman, 1980). A study made on the genetic variability within and between 43 populations of five of the previously recognized races of the parasite present in West Africa using Amplified Fragment Length Polymorphism (AFLP) markers showed almost no genetic variability within and between the populations. This study showed no race differentiation for the populations used but observed geographical groupings of the populations. AFLP markers previously identified to discriminate between races of *Striga*

failed for their collection of *Striga* populations (Dubé & Olivier, 2001). SSR markers will probably be more useful in discriminating between *Striga gesnerioides* populations than AFLPs due to their numerous advantages over AFLPs.

There is very little information on the diversity of parasitic weeds on domesticated crops. Studies by some researchers show that there exists some diversity among these parasitic weeds which is mostly attributed to the geographical location of the parasite, origin and host specificity (Welsh & Mohamed, 2011). Diversity studies on *Striga* are limited to the species *hermonthica*, *asiatica* and *gesnerioides* (Dubé & Olivier, 2001). Most work on *Striga hermonthica* diversity studies reveals great diversity of the species between geographical locations than host specificity (Estep et al., 2011; Joel, 2014; Welsh & Mohamed, 2011). This is probably due to the wide adaptation of the parasite to different ecologies.

There has been an increase in different strains of *Striga gesnerioides* in the West Africa sub region (Timko, 2016). This is due to the parasite overcoming resistances in resistant cultivars hence seven (7) races of the parasite are known in the sub region (**Figure 2:3**). These races are different with respect to their degree of virulence. It will be good to be able to know the differences between the races of *Striga gesnerioides* using molecular markers such that a race is identifiable by one or two markers. This will help in the linking of molecular markers to the virulence differentiation of races of *Striga gesnerioides*.

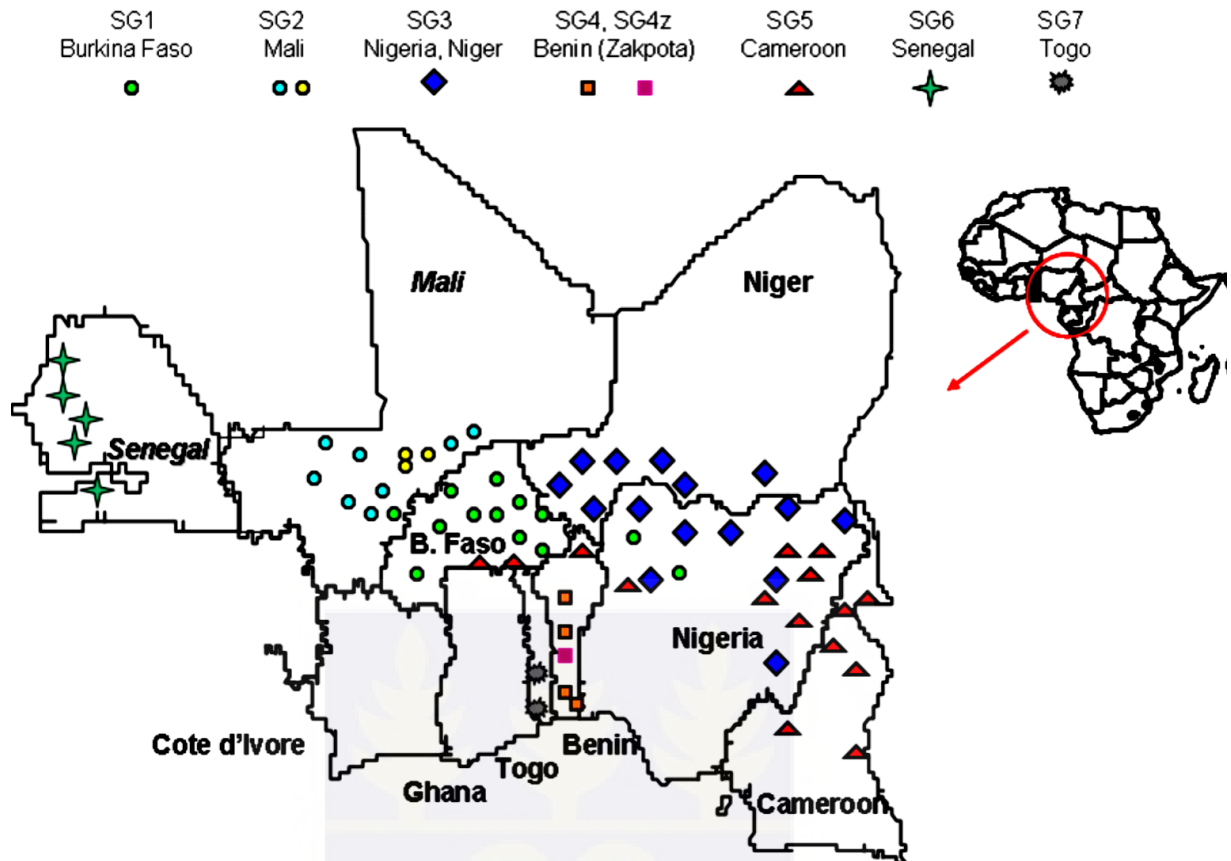


Figure 2:3 Races of *Striga* across West Africa source: (Timko, 2016)

Genetic diversity studies on *Striga gesnerioides* by Botanga & Timko (2005), revealed genetic differences based on host specificity in populations of *S. gesnerioides* parasitizing *Indigofera hirsuta* in central Florida and populations parasitizing *I. hirsuta* and cowpea from West Africa. The Florida strain and the West African strain parasitizing *I. hirsuta* were more closely related compared to the Florida strain and the West African strain parasitic on cowpea. This they did using AFLPs markers. There have been very few studies on the diversity of *Striga gesnerioides* within countries under heavy infestation and on the continent of Africa.

This study will design SSR markers to assess the diversity in *Striga gesnerioides* on cowpea in Ghana.

### **2.1.10 Molecular techniques in diversity studies**

Molecular techniques are the modern tools used in diversity studies, usually involving the use of molecular markers. Molecular markers are short DNA sequences with a known physical location on a large sequence, chromosome or genome. Markers used in diversity studies are usually grouped into Morphological markers, Biochemical markers and Molecular markers (Duminil & Michele, 2009).

It is of importance that diversity study should not be based solely on morphological markers but should be combined with molecular markers (Navajas & Fenton, 2001). Molecular markers are more readily available and reliable than morphological markers (Kumar *et al.*, 2009). The types, advantages and disadvantages of different molecular markers is summarised in **Table 2:2** and **Table 2:3**. The molecular markers that have been extensively used in diversity studies of parasitic weed are AFLPs (Cortese *et al.*, 2010; Estep *et al.*, 2011; Pineda-Martos *et al.*, 2014; Welsh & Mohamed, 2011). SSRs are better in diversity studies than AFLPs in that they have high genomic abundance, highly reproducible and cover very well the entire genome and produce high polymorphism with multiple alleles (Kumar *et al.*, 2009). The technique involving the use of SSRs is also easy to automate, hence is preferred to AFLPs (Kumar *et al.*, 2009).

**Table 2:2 Summary of techniques used in diversity study and their associated molecular markers**

<b>Name of Technique</b>	<b>Markers used</b>
<b>Biochemical markers</b>	Allozymes
<b>Molecular markers</b>	
<b>Non-PCR based techniques</b>	Restriction Fragment Length Polymorphisms (RFLP) Minisatellites or Variable Number of Tandem Repeats (VNTR)
<b>PCR-based techniques</b>	
<b>DNA sequencing</b>	Multi-copy DNA, Internal Transcribed Spacer regions of nuclear ribosomal genes (ITS) Single-copy DNA, including both introns and exons
<b>Sequence-Tagged Sites (STS)</b>	Microsatellites, Simple Sequence Repeat (SSR), Short Tandem Repeat (STR), Sequence Tagged Microsatellite (STMS) or Simple Sequence Length Polymorphism (SSLP) Amplified Sequence Length Polymorphism (ASLP) Sequence Characterized Amplified Region (SCAR) Cleaved Amplified Polymorphic Sequence (CAPS) Single-Strand Conformation Polymorphism (SSCP) Denaturing Gradient Gel Electrophoresis (DGGE) Thermal Gradient Gel Electrophoresis (TGGE) Heteroduplex Analysis (HDA) Denaturing High Performance Liquid Chromatography (DHPLC)
<b>Multiple Arbitrary Amplicon Profiling (MAAP)</b>	Random Amplified Polymorphic DNA (RAPD) DNA Amplification Fingerprinting (DAF) Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) Inter-Simple Sequence Repeat (ISSR) Single Primer Amplification Reaction (SPAR) Directed Amplification of Minisatellites DNA (DAMD) Amplified Fragment Length Polymorphism (AFLP) Amplified Fragment Length Polymorphism (AFLP)

Source: (Cortese *et al.*, 2010; Kumar *et al.*, 2009; Navajas & Fenton, 2001)

**Table 2:3 Advantages and disadvantages of common markers used in diversity studies**

<b>Type of Marker</b>	<b>Advantages</b>	<b>Disadvantages</b>
<b>Restriction Fragment Length Polymorphism (RFLP)</b>	High genomic abundance Co-dominant markers Highly reproducible Can use filters many times Good genome coverage Can be used across species No sequence information Can be used in plants reliably (well-tested) Needed for map based cloning	Need large amount of good quality DNA Laborious (compared to RAPD) Difficult to automate Need radioactive labelling Cloning and characterization of probe are required
<b>Randomly Amplified Polymorphic DNA (RAPD)</b>	High genomic abundance Good genome coverage No sequence information Ideal for automation Less amount of DNA (poor DNA acceptable) No radioactive labelling Relatively faster	No probe or primer information Dominant markers Not reproducible Cannot be used across species Not very well-tested
<b>Simple Sequence Repeat (SSR)</b>	High genomic abundance Highly reproducible Fairly good genome coverage High polymorphism No radioactive labelling Easy to automate Multiple alleles	Cannot be used across species Need sequence information Not well-tested

<b>Amplified Fragment Length Polymorphism (AFLP)</b>	High genomic abundance High polymorphism No need for sequence information Can be used across species Work with smaller RFLP fragments Useful in preparing contig maps	Very tricky due to changes in patterns with respect to materials used Cannot get consistent map (not reproducible) Need to have very good primers
<b>Sequence-Tagged Site (STS)</b>	Useful in preparing contig maps No radioactive labelling Fairly good genome coverage Highly reproducible Can use filters many times	Laborious Cannot detect mutations out of the target sites Need sequence information Cloning and characterization of probe are required
<b>ISOZYMES</b>	Useful for evolutionary studies Isolation lot easier than that of DNA Can be used across species No radioactive labelling No need for sequence information	Laborious Limited in polymorphism Expensive (each system is unique) Have to know the location of the tissue Not easily automated

Source: (Appiah *et al.*, 2011; Kumar *et al.*, 2009; Navajas & Fenton, 2001)

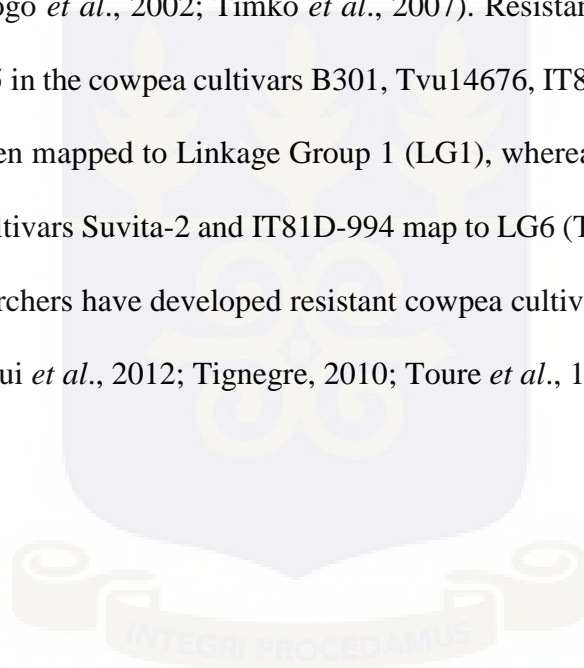
### **2.1.11 Differential cultivars in race identification of parasitic weeds**

Differential cultivars are different varieties of a crop that respond differently to the same crop parasitic host (Rispaill *et al.*, 2007). This is usually due the presence of different set(s) of resistance genes present in the host plant. These differences in genes usually arise as a result of emergence of different strains of the parasites (Goldwasser *et al.*, 2000). Emergence of different strains of these parasite brings about differential virulence in the parasite (Thorogood *et al.*, 2009). Hence, some cultivars may have resistance to certain strains and not to others due presence or absence of specific resistance genes. This results in the parasite being virulent on other cultivars and non-virulent on others. These phenomena of absence and presence of resistance genes within a crop host to a particular strain of parasite has been used to classify parasites of crops based on their virulence on the crop and subsequently identification of different strains or race(s) of the parasite (Lane & Bailey, 1992; Li *et al.*, 2009). This concept of differential virulence is not different from that of the gene for gene action in that, for each virulent gene in the parasite there needs to be an avirulent in the host to neutralise its effect. This means that for each cultivar that lack this avirulent gene, will be susceptible to parasite. Hence within an accession of cultivars, a particular parasite would exhibit differential virulence by being virulent on cultivars that lacks such counter genes and not virulent on other which do not. In this same vein, if the parasite evolves with a virulent gene which the cultivar does not have a counter gene to then the resistance in that cultivar will be broken. Hence depending on the situation prevailing at the geographical location of a parasite, it can have several virulent genes. Therefore, the geographical location of a parasite plays a major role in its virulence. Different strains of the same parasite will exhibit differential virulence if the host cultivars do not all have all set of counter genes to neutralise the effect of the several virulent genes within the parasite.

In cultivated rice, differential cultivars have been identified with different response to *S. hermonthica*, *S. asiatica*, and *S. aspera*, with very limited number of resistant and tolerant cultivars identified (Timko *et al.*, 2012). Over the past two or three decades of concerted efforts by researchers/scientist to solve the problem of *Striga gesnerioides* infestation on cowpea, several cowpea cultivars have been identified to possess some resistance to different races. The first of *S. gesnerioides* resistance in cowpea was made in Burkina Faso, which were cultivars 58-57 and Suvita2. These cultivars were only resistant in Burkina Faso but not in other West African countries. Hence the existence of different strains of *Striga gesnerioides* was conceived. Suvita2 was resistant to *Striga* strains from Mali but susceptible to strains from Niger (Toure *et al.*, 1998). The search for resistant cultivars for different strains of *Striga gesnerioides* resulted in the identification of cultivars B301 and IT82D-849 which were resistant across Mali, Niger and Nigeria (Aggarwal *et al.*, 1988). B301 was later found to be susceptible to a strain of *Striga* localised to the region of Zakpota in Benin (Lane *et al.*, 1996). As a result of these differences in virulence of the *Striga* strains from different locations and the differential response of cowpea cultivars to these strains, a study was conducted with the aim of evaluating the differential host resistance responses using differential cowpea cultivars. From that study, the response of the cultivars was used to group the *Striga* strains into five proposed distinct races in West and central Africa (Lane *et al.*, 1996). Botanga and Timko (2006), using differential cultivar response and AFLPs markers in a genetic study of collections of *Striga* isolates re-classified the races of *Striga gesnerioides* in West Africa to be at least seven (7), making two additions to that of Lane *et al.* (1996). They designated these races SG1 to SG6; SG1 (Burkina Faso), SG2 from Mali, SG3 from Niger and Nigeria, SG4 from Benin, SG4z from a localised region Zakpota in Benin, SG5 from Cameroon and SG6 from Senegal. They also identified molecular relatedness between

these races, SG1 was closely related to SG5, SG4 and SG5 being distantly related but SG4 and SG4z shared almost the same molecular profile as revealed by the AFLPs used. Hence differences in virulence separated the two Strains SG4 and SG4z into two races. There has been reports of an additional race SG7 in Togo (Timko, 2016).

Since the identification of different races of *Striga gesnerioides* study has been conducted using segregating F2 populations to map various genes controlling resistance to some of the races in certain cowpea cultivars using molecular techniques such as AFLPs, RAPDs and SSRs (Ouédraogo *et al.*, 2002; Timko *et al.*, 2007). Resistance to *Striga* races SG1, SG2, SG3, and SG5 in the cowpea cultivars B301, Tvu14676, IT82D-849, and Tvu14676, respectively has been mapped to Linkage Group 1 (LG1), whereas resistance to SG1 and SG4z in cowpea cultivars Suvita-2 and IT81D-994 map to LG6 (Timko *et al.*, 2007). Ever since, several researchers have developed resistant cowpea cultivars to different races (Li *et al.*, 2009; Omoigui *et al.*, 2012; Tignegre, 2010; Toure *et al.*, 1998).



## CHAPTER THREE

### 3.1 MATERIALS AND METHODS

#### 3.1.1 Experimental location

Two separate experiments were carried out in this study, a screen house pot experiment and a laboratory experiment. The screen house experiment was conducted at Manga Station (11°00.977 N, 000°15.912 W) of the Savannah Agricultural Research Institute (SARI) in the Upper East Region of Ghana. This region falls within the Sudan Savannah agro-ecological zone of Ghana. The laboratory work was conducted at the Kirkhouse Trust funded molecular and biotechnology lab at SARI Nyankpala.

#### 3.1.2 Screen house experiment

The screen house experiment consisted of pot screening of Twenty-nine (29) differential cowpea cultivars of known responses to known races of *S. gesnerioides* with eight populations of *Striga* from the endemic zones in Ghana.

The cowpea genotypes were obtained from International Institute of Tropical Agriculture (IITA), Nigeria; University of Agriculture, Makurdi (UAM), Nigeria; and SARI, Ghana (Table 3.1). These were used for the screening experiment.

**Table 3.1 *Striga gesnerioides* race specific resistant cowpea genotypes**

Sample ID	Genotype	Race Responds
1	IT84S-2049	susceptible check
2	B524	3
3	IT97K-461-4	susceptible check
4	IT98K-409-4	3
5	IT93K-693-2	1
6	IT98K-205-10	3
7	IT82D-849	1, 2, 3, 5
8	IT99K-494-6	3
9	IT98K-1092-1	3
10	IT00K-1263	3
11	IT97K-819-118	3
12	IT99K-7-21-2-2	3
13	IT98K-615-6-1	3
14	IT99K-573-2-1	3
15	SUVITA2	1, 2, 4, 5, 6
16	UAM09-1051-1	3
17	IT81D-994	1, 2, 5
18	IT97K-1042-3	3
19	IT93K-452-1	3
20	UAM09-1046-6-1	3
21	IT97K-499-35	Resistant check
22	IT00K-1217	3
23	UAM09-1046-6-2	3
24	IT98K-216-44	3
25	IT03K-338-1	3
26	UAM09-1055-6	3
27	IT99K-573-1-1	3
28	IT90K-205-8	3
29	TVX 3236	Susceptible check

*Striga* populations were collected from eight *Striga* endemic areas in the northern, upper east and upper west regions of Ghana. The specific location and the regions within which the *Striga* was collected are presented in **Table 3:2**. Matured *Striga* plants (plants with weathered flowers and not completely dried) were harvested from farmers' fields at endemic zones to *Striga gesnerioides* into polythene/ rubber lined sacks and air dried. The capsules of dried *Striga* plants were then threshed and sieved through pore sizes of 250  $\mu\text{m}$ , 200  $\mu\text{m}$ , 180  $\mu\text{m}$  and finally 150  $\mu\text{m}$  to obtain pure *Striga* seeds. The pure *Striga* seeds were labelled per endemic zones and stored at room temperature. In all, eight populations of *Striga* were used in this study.

**Table 3.2** *Striga gesnerioides* endemic zones in Ghana from which samples were collected

<b>Region</b>	<b>Endemic Zone</b>
<b>Upper East</b>	Manga
	Googo
	Garu
<b>Upper West</b>	Lawra
	Babilla
<b>Northern</b>	Gushiegu
	Walewale
	Gambaga

Soil used in this experiment was taken from the Manga Station of the CSIR-SARI. A mixture in the ratio of 70% loam to 30% sand, based on weight was used. The soil was steam sterilized to get rid of contamination from native *Striga* seeds within the soil. The drum method of sterilization was used (**Plate 3:1**). This consists of a metallic drum with an internal division at the one third length from the bottom. The division was made by welding a flat bar across the diameter of the drum at the position and a wire mesh/net was also welded on top of the bar. This marked the threshold level of water for sterilization.

Two jute sacks were then placed on the mesh or net to prevent the soil from entering the water. The drum was then filled with the soil mixture. The setup was covered again with two jute sacks and a metallic lid placed on it and secured. Fire was set under the drum with the soil and water. The steam from the boiling water, sterilized the soil as it moved from the bottom to the top of the drum through the soil. Once the steam was seen at the top, it was left for an hour for complete sterilization. After sterilization, the soil was scooped out and cooled.



**Plate 3.1** Photograph of the drum approach, Drum filled with water to the mesh level (A), Drum with jute sack sealed/place on the mesh (B), Drum filled with soil mixture for initiation of sterilization (C)

### **3.1.2.1 Inoculation, preconditioning**

Pots of diameter 35 cm and height 35 cm were filled with sterilized soil and inoculated with 20 mg of pure *S. gesnerioides* seeds (approximately 10,000 seeds) from each endemic zone. With this, about half the soil in each pot was poured into a wider pot. A unit of measure (spatula) was tested by measuring the seed weight it could contain. This was ascertained to be 20 mg of pure *Striga* seeds. A spatula full of pure *Striga* seed was then transferred into the wider pot with the soil mixture. The contents of this pot was then mixed thoroughly and transferred into the experimental pot. This was done for all pots constituting a strip. In-between strips the spatula was washed and rinsed off any residual *Striga* seed from the previous strip. For every strip, a different wider pot was used for mixing of soil mixture and pure *Striga* seed.

The pots were preconditioned by watering for three days before planting. Three cowpea seeds were planted in each pot and thinned to 2, two weeks after emergence.

### **3.1.2.2 Experimental setup**

A split plot arrangement with three replications was used in this study. The varieties were assigned to the main plots and the *Striga* populations the sub plots. Each plot consisted of nine splits with each containing 29 pots for the differential cowpea genotypes used. Of the nine splits, eight were inoculated with the different *Striga* population. The ninth split was not inoculated to serve as the control and to check the success of the sterilization process.

### 3.1.2.3 Morphological Data Collection

Morphological data were taken on both the cowpea and its parasite, *S. gesnerioides*. The only data taken on the cowpea was its reaction to the *Striga* populations used. That is either susceptible or resistant.

The reaction of each cowpea genotype to each of the *Striga* populations used was taken. A Cowpea genotype that showed susceptibility to a *Striga* population was scored as '1', a susceptible genotype is one that shows *Striga* emergence or attachment. A Cowpea genotype that showed resistance to any *Striga* population was scored as '0', a resistant genotype is one that shows no emergence nor attachment of *S. gesnerioides*.

Data taken on *Striga gesnerioides* plants were days to emergence (DTSE), *Striga* emergence score (SES) and *Striga* attachment score (SAS). Days to the emergence of *Striga* was taken by counting the number of days from the time of planting of the cowpea to the time *Striga* plants emerged to the surface of the soil. *Striga* emergence scores were taken by scoring pots/cowpea genotype with emerged *Striga* plants as "1" and those with no emergence as "0". This data was recorded throughout the development of the cowpea plants up to physiological maturity. The root zone of each plant in a pot was washed in a bigger basin and the root of each plant examined for attachment of *Striga* plants. Cowpea genotypes showing attachment were scored as '1' and those without attachment as '0'.

#### **3.1.2.4 Data Analysis**

GPS coordinates was taken at all locations where *Striga* samples were taken using the Garmin etrex 30 GPS. The coordinates were plotted onto the Ghana map using the software ArcGIS (<https://www.arcgis.com>).

The reaction of the 29 cowpea varieties to the eight population of *Striga gesnerioides* collected from endemic zones were scored as '1' for susceptible varieties and '0' for resistant varieties. The virulence of the *Striga populations* on the cowpea varieties were also scored as '1' for virulence and '0' for non-virulence. The data was subjected to cluster analysis using the Jaccard similarity test and an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method for both cowpea genotypes and the *Striga gesnerioides* populations (Abe et al., 2003). The software used for this was GenStat 12<sup>th</sup> edition and DARwin version 6 (Egbadzor et al., 2014).

#### **3.1.3 Laboratory Experiment**

The laboratory analysis was carried out at the biotechnology laboratory of the Savannah Agricultural Research Institute (SARI), Nyankpala. It consisted of molecular characterization of the *Striga* strain from different locations within endemic zones using simple sequence repeats (SSR) markers. DNA extraction was done using CTAB protocol as described in section 3.1.3.4 and gel electrophoresis and PCR were carried out as described in section 3.1.3.6

**3.1.3.1 *Striga* samples for laboratory experiment**

Thirty-Five *Striga* samples were used in this laboratory work (Table 3:3). These samples were collected from different locations within endemic areas for *S. gesnerioides* on cowpea in Ghana.

**Table 3.3 Districts and regions of *Striga gesnerioides* sample collection for laboratory experiment**

<b>ID</b>	<b>Name of location</b>	<b>Number of samples</b>	<b>District</b>	<b>Region</b>
1	Buguya-kura	2	West Mamprusi	Northern
2	Norilogubi	2	East Mamprusi	Northern
3	Yankazia	3	East Mamprusi	Northern
4	Barinya	1	East Mamprusi	Northern
5	Misiga	2	Bawku Municipal	Upper East
6	Yaro yire	1	East Mamprusi	Northern
7	Jagbani	2	West Mamprusi	Northern
8	Tabier	2	Laura	Upper West
9	Babile	2	Laura	Upper West
10	Nahadague	1	Sisala East	Upper West
11	Manga	4	Binduri	Upper East
12	Googo	3	Bawku West	Upper East
13	Boko	3	Binduri	Upper East
14	Zuabuliga	2	Pusiga	Upper East
15	Nankpawei	2	Sisala East	Upper West
16	Dimajan	1	Sisala East	Upper West
17	Nyankpala-UDS	1	Tolon kunbumgu	Northern
18	Manga	1	Binduri	Upper East

### 3.1.3.2 SSR primers for PCR amplification

Seventy-three SSR primers (Table 3:4) were used in this work. The primers were designed using primer3 based on sixteen *S. gesnerioides* gene sequences retrieved from the National Centre on Bioinformatics Information (NCBI) data base.

**Table 3.4 SSR primers used for diversity studies and their expected product size and annealing temperatures**

Primer Name	Forward	Reverse	Product size (bp)	Annealing Temperature
FA1/2	GCCGGAACAAAGACTCGATC	TCACCAGCCTCCAAGTTGAT	244	57
FA3/4	TAGGTTGGAAGTGGGACCAC	TCTGTACAGGAGGAGGTCGA	218	58
FA5/6	TGTCACCAGAAGAGCCGAAT	TCACCAGCCTCCAAGTTGAT	193	56
FA7/8	GACCTCCCAAACAACACCAC	GTGGTCCCAGTTCCAACCTA	231	58
FA9/10	TAGGTTGGAAGTGGGACCAC	CTAACACCGAGAGCAGCAAC	157	58
FA11/12	GCTCTACGCCTGGAAGATCT	CAACGCATAAATGGCTGGGA	248	57
FA13/14	TAAAACCTTCCAAGGCCCGC	CAACGCATAAATGGCTGGGA	207	56
FA15/16	CTTCGCGGTGGACTTGATTT	ATTTCTTCGCATGTACCCGC	173	56
FA17/18	GCTCTACGCCTGGAAGATCT	AAATCAAGTCCACCGCGAAG	203	57
FA19/20	TAAAACCTTCCAAGGCCCGC	AAATCAAGTCCACCGCGAAG	162	56
FA21/22	CTGGTTGCGTCATTGGCTAA	TCCGAGCCAAAGTTCTAGCA	217	56
FA23/24	CTGGTTGCGTCATTGGCTAA	CCGAGCCAAAGTTCTAGCAC	216	57
FA25/26	TTCCTCTGGTTGCGTCATTG	TCCGAGCCAAAGTTCTAGCA	222	56
FA27/28	GCATCCCATTAGTAAGCCGG	TCCGAGCCAAAGTTCTAGCA	173	57
FA29/30	GGCATCCCATTAGTAAGCCG	TCCGAGCCAAAGTTCTAGCA	174	57
FA31/32	CCTAGTCATGTCCGTCGTCA	CGACGTGAATGGAGAACACC	194	58
FA31/34	CCTAGTCATGTCCGTCGTCA	CCGACGTGAATGGAGAACAC	195	58
FA35/36	GAGTGTGCCATGGAGAGACT	CCACCCCAACAGCCAGTATA	242	58
FA37/38	GCGCACTACAGTCGTACAAG	CTCCCCATGGTCGTCTTCAT	167	58
FA39/40	TTAGGGGATGCCTTCTGTGG	CGCCTTACCACCTCTAGAA	189	58
FA47/38	ACTTCGAGCCTGTTAAGCCT	CTCCCCATGGTCGTCTTCAT	212	57
FA49/38	TTCGAGCCTGTTAAGCCTCA	CTCCCCATGGTCGTCTTCAT	210	57
FA51/52	CATGCGCCAAGGAAAACAGA	GATGGTTCACGGGATTCTGC	184	57
FA53/54	GCAGAATCCCGTGAACCATC	GGGTGCCTCGTGAATTCATT	194	57
FA51/56	CATGCGCCAAGGAAAACAGA	ACTCGATGGTTCACGGGATT	188	56
FA57/54	AATCCCGTGAACCATCGAGT	GGGTGCCTCGTGAATTCATT	190	56
FA59/52	ATGCGCCAAGGAAAACAGAT	GATGGTTCACGGGATTCTGC	183	56
FA61/62	CGGCTGTGGTGAAAAGACAA	AATCTGCAAGGTCAGGGTCA	192	56
FA63/64	CGGCGGGATGTTAACGAATT	CCGCCAGATATGTTTGCAA	159	56
FA65/62	ACAAAGGGCGGGAAGACTAA	AATCTGCAAGGTCAGGGTCA	233	56
FA67/62	AACAAAGGGCGGGAAGACTA	AATCTGCAAGGTCAGGGTCA	234	56

<b>FA69/70</b>	CGGCGGGATGTTAACGAATT	CGCAGCGCCGTATATTCTTT	231	56
<b>FA71/52</b>	CGCCAAGGAAAACGGATAGG	GATGGTTCACGGGATTCTGC	180	58
<b>FA71/74</b>	CGCCAAGGAAAACGGATAGG	ACTCGATGGTTCACGGGATT	184	57
<b>FA71/76</b>	CGCCAAGGAAAACGGATAGG	TCGATGGTTCACGGGATTCT	182	57
<b>FA77/78</b>	AGGAAAACGGATAGGAGCGT	GATGGTTCACGGGATTCTGC	175	57
<b>FA79/74</b>	AGGAAAACGGATAGGAGCGT	ACTCGATGGTTCACGGGATT	179	56
<b>FA81/82</b>	GTGGTGGGAACGATGAAACC	CGGGCGAATATTTACTCTTGCA	171	58
<b>FA83/82</b>	GGTGGGAACGATGAAACCTG	CGGGCGAATATTTACTCTTGCA	169	58
<b>FA85/82</b>	TGGTGGGAACGATGAAACCT	CGGGCGAATATTTACTCTTGCA	170	57
<b>FA81/76</b>	GTGGTGGGAACGATGAAACC	TGCAATCTCTATTTACGCCGT	153	57
<b>FA83/76</b>	GGTGGGAACGATGAAACCTG	TGCAATCTCTATTTACGCCGT	151	57
<b>FA91/92</b>	TGGATTCACACCCCTTACACT	ACATACCATTTTCCCACGCA	216	56
<b>FA93/94</b>	CCTTGCGTGGGAAAATGGTA	GGATTACTATCGCGAAAAGGGG	163	58
<b>FA97/92</b>	TGTTTTGGATTCACACCCCT	ACATACCATTTTCCCACGCA	221	54
<b>FA101/102</b>	TGATTTCCAGATCTCCCGG	GTGCCTGCAGTTTTTCATTGC	172	57
<b>FA101/104</b>	TGATTTCCAGATCTCCCGG	CGTGCCTGCAGTTTTTCATTG	173	57
<b>FA105/106</b>	GCATCCCATTAGTAAGCCGG	TCCGAGCCAAAGTTCTAGCA	173	57
<b>FA107/106</b>	GGCATCCCATTAGTAAGCCG	TCCGAGCCAAAGTTCTAGCA	174	57
<b>FA109/102</b>	GATTTCCAGATCTCCCGGA	GTGCCTGCAGTTTTTCATTGC	171	57
<b>FA105/116</b>	GCATCCCATTAGTAAGCCGG	CCGAGCCAAAGTTCTAGCAC	172	58
<b>FA107/116</b>	GGCATCCCATTAGTAAGCCG	CCGAGCCAAAGTTCTAGCAC	173	58
<b>FA119/120</b>	CCCATTAGTAAGCCGGTTCG	TCCGAGCCAAAGTTCTAGCA	169	57
<b>FA121/122</b>	CCGAACCTGGTGTCTTAGT	AACGTGGAAAAGCACCTGTC	241	57
<b>FA123/122</b>	ACCGAACCTGGTGTCTTAG	AACGTGGAAAAGCACCTGTC	242	57
<b>FA125/126</b>	AGCAACTCCGCAACTTGAAG	TCCACACCTTCTTAGCAGCA	216	56
<b>FA127/128</b>	AGAAGCAACTCCGCAACTTG	TCCACACCTTCTTAGCAGCA	219	56
<b>FA129/128</b>	CAAGAAGCAACTCCGCAACT	TCCACACCTTCTTAGCAGCA	221	56
<b>FA131/132</b>	CGCCAAGGAAAACGGATAGG	GATGGTTCACGGGATTCTGC	174	58
<b>FA133/134</b>	CTGCGGAAGGATCATTGTCG	CCTATCCGTTTTCTTGCGG	158	58
<b>FA131/136</b>	CGCCAAGGAAAACGGATAGG	ACTCGATGGTTCACGGGATT	178	57
<b>FA131/138</b>	CGCCAAGGAAAACGGATAGG	TCGATGGTTCACGGGATTCT	176	57
<b>FA139/140</b>	AGGAAAACGGATAGGAGCGT	GATGGTTCACGGGATTCTGC	169	57
<b>FA141/142</b>	GCAGAATCCCGTGAACCATC	CCCAACACGACAGGAAGAGA	224	58
<b>FA143/144</b>	GCAGAATCCCGTGAACCATC	TCCCAACACGACAGGAAGAG	225	58
<b>FA143/146</b>	GCAGAATCCCGTGAACCATC	CTCCCAACACGACAGGAAGA	226	58
<b>FA143/148</b>	GCAGAATCCCGTGAACCATC	TGTTGGGTCTAAGGGTACGC	250	58
<b>FA149/142</b>	AATCCCGTGAACCATCGAGT	CCCAACACGACAGGAAGAGA	220	57
<b>FA151/152</b>	GCAGGAGTTCATTTGGGCA	GTTTTCGCCTACTTGCTGCA	163	56
<b>FA151/154</b>	GCAGGAGTTCATTTGGGCA	TGTTTTCGCCTACTTGCTGC	164	56
<b>FA155/156</b>	ATCCGAAGCCTGCAATTTGG	CTTTTCGGAAGGCGGCTTAG	246	57
<b>FA157/158</b>	GCAATAAAGGCTCGGTGTCA	TTGTCTTTTACCACAGCCG	171	56
<b>FA159/158</b>	TGCAATAAAGGCTCGGTGTC	TTGTCTTTTACCACAGCCG	172	56

### 3.1.3.3 Preparation of samples for DNA extraction

*S. gesnerioides* samples collected from locations within endemic zones were brought to the laboratory in Ziploc bags on ice (Plate 3:2). The samples were then air dried overnight and placed in newly labelled Ziploc bags with 5 mini pack silica gels to dry the samples. The dried samples were ground using a mortar and pestle to fine powder with 0.02 g of polyvinyl pyrrolidone (PVP 40, 000) added. The ground samples were then put into labelled 2 ml Eppendorf tubes and preserved at room temperature for laboratory analysis.

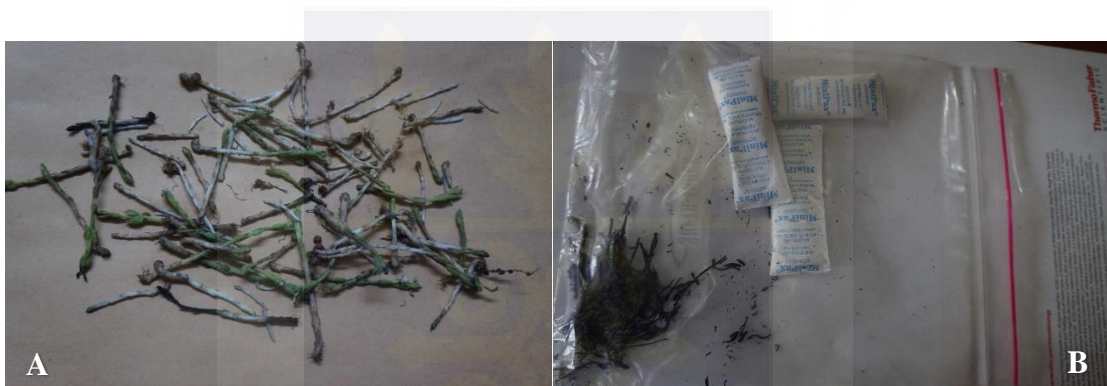


Plate 3:2 Air dried *Striga* samples (A), *Striga* sample in Ziploc bag with mini pack silica gel (B)

### 3.1.3.4 DNA Extraction

Total genomic DNA was extracted using the CTAB protocol by Doyle and Doyle (1990). About 200 mg of ground dried samples were put into labelled separate 2 ml Eppendorf tubes containing 1 ml of Pre-warmed CTAB buffer 2% CTAB, 20 mM EDTA, 100 mM Tris-HCl and 1.4M NaCl with 0.2% mercapto-ethanol (2  $\mu$ l/1 ml of CTAB buffer) added to each Eppendorf tube and placed in a water bath at 60 °C for 1 hour. During this time the content of the tubes were mixed gently by inverting for 6 -7 times. Two hundred micro litres (200  $\mu$ l) of potassium acetate was added and put on ice for 20 minutes, 700  $\mu$ l of chloroform: Isoamyl alcohol (24: 1) mixture was added to each tube and mixed gently by

inverting, this was left undisturbed for 5 minutes. The tubes were then centrifuged at 10000 rpm for 15 min and the middle aqueous layer transferred into labelled new 2 ml tube. The *Striga* exudes some dye which masks the clear separation of aqueous and organic layers, hence care was taken to avoid contamination. The same volume of chloroform: Isoamyl alcohol in new tube was added, mixed gently and left undisturbed for 5 min. This was centrifuge at 10 000 rpm for 10 min and the supernatant transferred into new labelled 1.5 ml tubes. Five hundred micro litres (500  $\mu$ l) of ice cold isopropanol was added to get a white precipitate, for maximum precipitation of the DNA, the tubes were left over night at a temperature of - 20 °C. The tubes were centrifuged the next day for 5 min at 10 000 revolutions per minute (rpm) and the supernatant removed carefully leaving pellets of DNA. The pellets were washed twice with 300 ml of 75% ice cold ethanol depending on pellet size, each followed by centrifugation at 15000 rpm for 5 min and allowed to air dry. About 300 ml of x1 TE buffer was added to the dried pellet and kept at 4 °C for dissolution of the pellet. Two micro litres (2  $\mu$ l) of RNase A was added to each tube and incubated at 37 °C for an hour to get rid of RNA in the DNA.

#### **3.1.3.5 DNA quality test**

A 2% agarose gel (2 g agarose, 100 ml of x1 TAE buffer and 5  $\mu$ l of ethidium bromide) was used in checking the quality of DNA obtained. Eight micro litres (8  $\mu$ l) each of the purified DNA after RNase treatment was pipetted into separate 0.2  $\mu$ l PCR tubes and 2  $\mu$ l of 10 % loading dye (0.025 g xylene, 0.025 g cyanol and 30 % glycerol) was added to each tube. Nine micro litres (9  $\mu$ l) of each sample was loaded into the wells with 8  $\mu$ l of 1 kb ladder loader into the first and last well. Electrophoresis was carried out in an x1 TAE buffer for 2 hours at 120 V and visualized on a UV trans-illuminator and photographed.

Extraction was repeated for samples with poor quality or no DNA bands. Good quality DNA samples were stored at - 20 °C until needed for amplification.

### **3.1.3.6 PCR amplification and gel electrophoresis**

Polymerase chain reaction (PCR) of the extracted DNAs were carried out using the following condition: denaturation at 94 °C for 30 seconds, annealing at X °C (depending on primer, (Table 3.4)) for 30 seconds and extension at 72 °C for 30 seconds for a cycle length of 35, in a reaction volume of 10µl (3 µl of ddH<sub>2</sub>O, 1 µl of DNA, 1 µl of primer and 5 µl of premix). The premix used was the puReTaq Ready-To-Go PCR Beads. The beads were dissolved with 20 µl of nuclease free water and divided into two then 1 µl of DNA and 1 µl of primer (0.5 µl each of forward and reverse) was added to constitute a PCR reaction.

The PCR products were resolved on a 6% horizontal polyacrylamide gel at a voltage of 120, running time of two hours. The gel was stained with ethidium bromide and visualized under a trans-illuminator.

### **3.1.3.7 Molecular Data Collection**

Product bands after gel electrophoresis were scored based on clear bands and using a standard 50 bp ladder run alongside the samples. Two clear bands for each sample were scored based on their weights as compared with the ladder as x/y, where x is the band size for the heavier weight and y for the lighter weight.

### 3.1.3.8 Data Analysis

Scoring for clear bands for each marker was done as '1' for presence of allele and '0' for absence of allele. Two clear bands were scored for each marker. The score for presence and absence of alleles were used in a cluster analysis based on the markers using the software GenStat 12<sup>th</sup> edition and DARwin version 6. This was also subjected to principal component analysis using the same software. The bands scored were analysed for summary genetic variation for gene diversity, allele number, heterozygosity, major allele frequency and polymorphic information content, using the software PowerMarker version 3.2.5 (Liu et al., 2003).

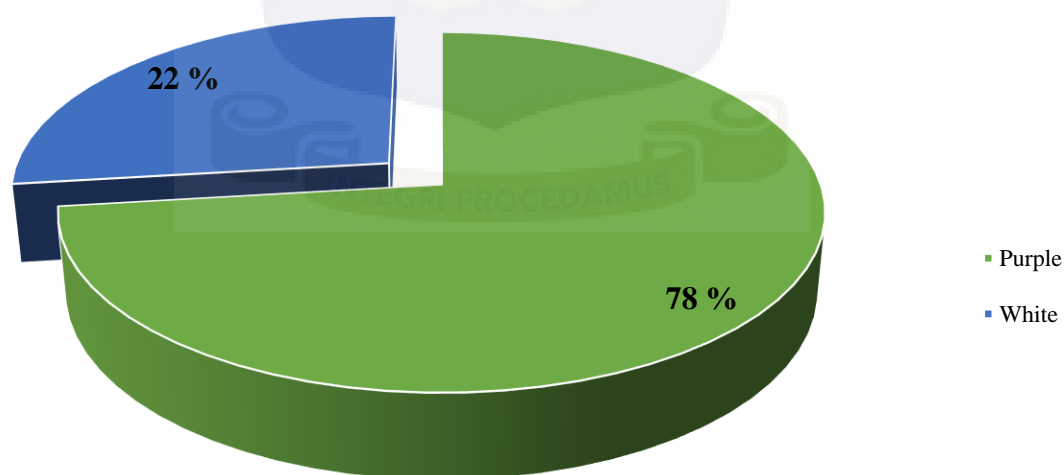


## CHAPTER FOUR

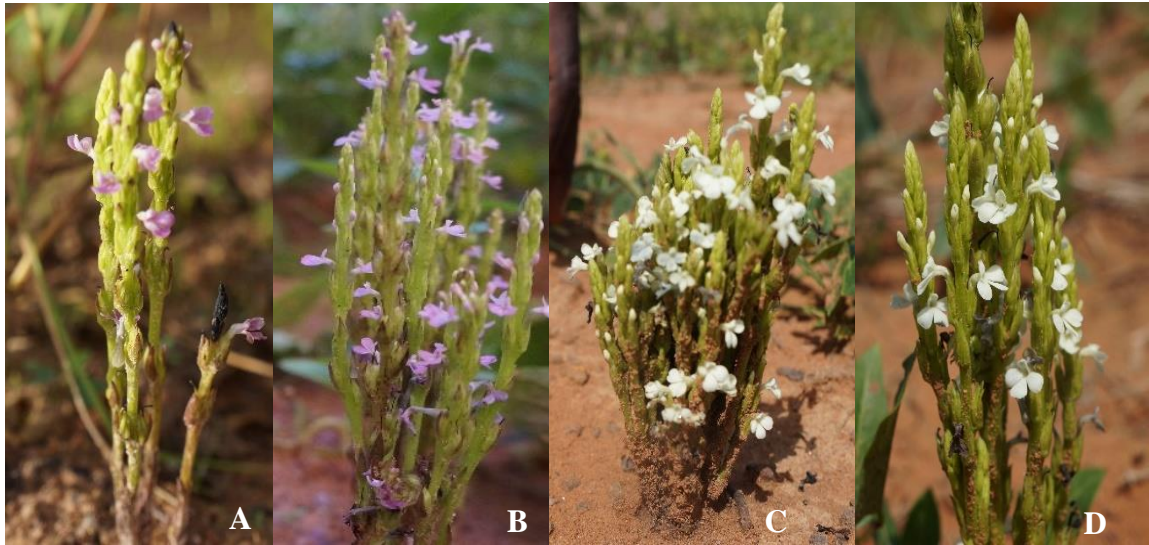
### 4.1 RESULTS

#### 4.1.1 Morphological variation in *Striga gesnerioides*

Morphological variations were observed in *Striga* populations during collection. The cowpea growers in these areas also confirmed the existence of morphological variations in the striga on their cowpea plants. Morphological variation observed was in the colour of the flower at maturity (**Plate 4:1**). A total of 35 *Striga* samples were collected. Seventy-eight (70) percent of the *Striga* samples collected had purple coloured flowers, with variations within the purple showing different expression levels ranging from dark to vary faint purple. The remaining 22 percent were white in colour (**Figure 4:1**). At some locations, the two flower colours were found on the same field, that is, patches of the field had white while most of the fields had *Striga* with purple flowers. A ratio of 3:1 (78:22) for purple to white flower was observed.



**Figure 4:1** Percentage representation of purple and white flowered *Striga gesnerioides* among samples collected



**Plate 4:1** Variations in flower colour of *Striga gesnerioides*, A and B shows purple flowers, C and D shows white flowers. (Photo credit: Frederick J. Awuku)

#### 4.1.2 *Striga gesnerioides* on other weeds

In this study, *Striga gesnerioides* was found on a leguminous weed, *Tephrosia pedicellata* and a non-leguminous weed *Ipomoea tribola* (Plate 4:2 and Plate 4:3). The parasitic plant on the weed *Tephrosia pedicellata* was in two colours: purple and green colour. The green coloured parasitic plant appeared the same as that on the cowpea plant. The parasitic plant on the weed *Ipomoea tribola* was green in colour as that on the cowpea plant.



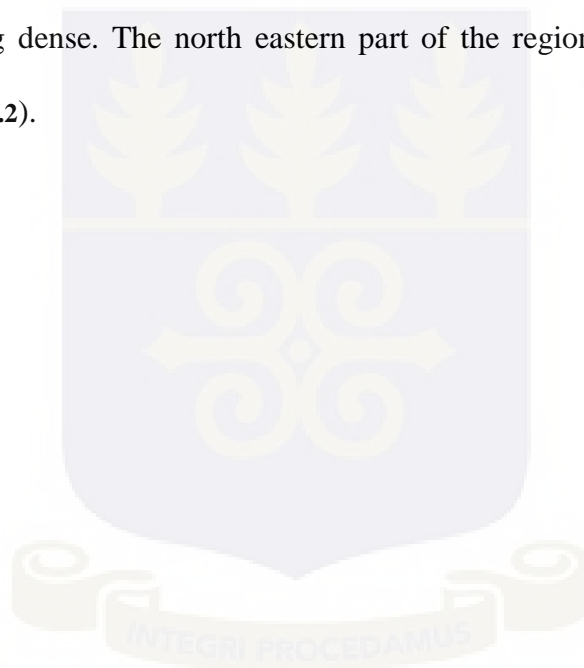
Plate 4:2 *Striga gesnerioides* on the weed *Tephrosia pedicellata*. Arrow points to *Striga gesnerioides*

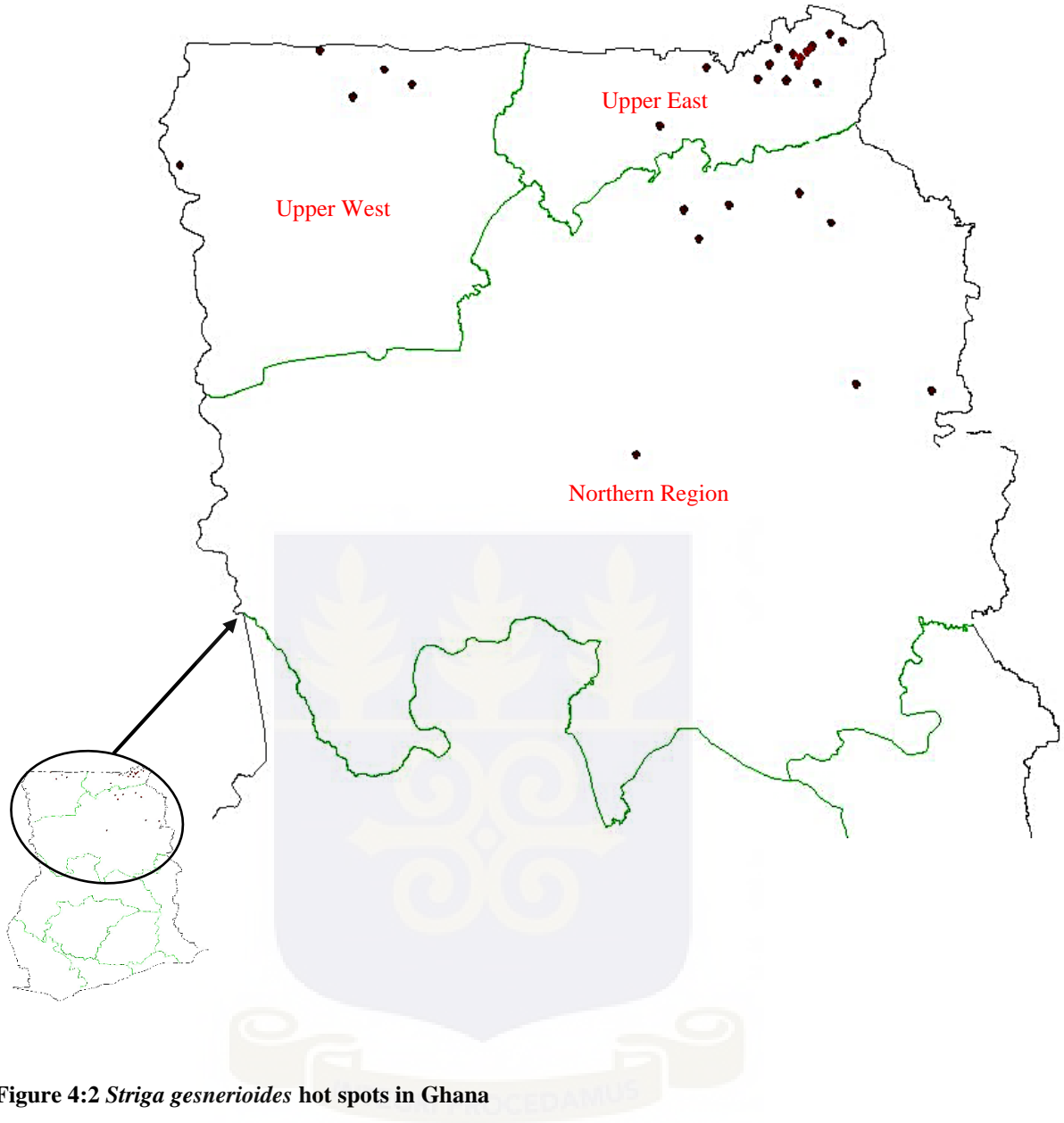


Plate 4:3 *Striga gesnerioides* on *Ipomoea tribola*, circles represents attachment of *Striga gesnerioides* to the roots of *Ipomoea tribola*, red circles shows attachment of *Striga gesnerioides* to *Ipomoea tribola*

#### 4.1.3 *Striga gesnerioides* hot spots in Ghana

*Striga gesnerioides* is a major parasitic cowpea weed that is widely distributed in cowpea producing regions of Ghana. From the figure above, it is distributed in three regions: Upper East, Upper West and the Northern. The Upper East region registered the highest incidence of *Striga*, Upper West region equally has *Striga gesnerioides* widely distributed across, with most cowpea producing areas abandoned due to heavy infestation. Also, most areas under cultivation still suffer attacks from this parasitic weeds. For the northern region, the weed is sparsely distributed, with the portions sharing boundaries with the Upper East and West regions being dense. The north eastern part of the region shows the most weed infestation (Figure 4.2).





**Figure 4:2** *Striga gesnerioides* hot spots in Ghana

#### 4.1.4 Virulence of different *Striga gesnerioides* population on selected cowpea cultivars

*Striga gesnerioides* exhibit differential virulence on cowpea varieties. In this study nine different *Striga gesnerioides* treatments (designated N1 – N9) were used as inoculum on twenty-nine cowpea varieties. Of the nine inoculum, N1 to N8 were pure *Striga gesnerioides* seeds from different locations, while N9 was a control (no inoculum). The inoculum grouped the cowpea varieties into three; the groups were based on inoculum virulence with respect to the cowpea varieties (Table 4:1)

The control treatment (N9) showed no virulence on any of the varieties. Varieties IT98K-205-10 and IT98K-618-6-1 had problems with germination and as such, no results were obtained. All eight inoculums were virulent on the following varieties: IT84S-2049, B524, IT97K-461-4, IT98K-409-4, IT93K-693-2, IT97K-1042-3, IT93K-452-1, IT98K-216-44 and TVX 3236. Of these, IT84S-2049, IT97K-461-4 and TVX 3236 were susceptible checks (Table 3:1) and hence, the expectation was that, all inoculum should be virulent on them. None of the inoculums were virulent on the following cowpea varieties: IT82D-849, IT98K-1092-1, IT99K-573-2-1, UAM09-1051-1, IT97K-499-35, IT00K-1217, UAM09-1046-6-2, IT99K-573-1-1, UAM09-1055-6 and IT90K-205-8. The variety IT97K-499-35 was the local resistant check. The eight inoculums showed differential virulence to the following cowpea genotypes: IT99K-494-6, IT00K-1263, IT97K-819-118, IT99K-7-21-2-2, Suvita2, IT81D-994, UAM09-1046-6-1 and IT03K-338-1. N4 and N8 were virulent on IT99K-494-6. Except for N4, all other inoculums were virulent on IT00K-1263. Inoculum N6 showed virulence on only genotype IT97K-819-118. N2, N4 and N5 were also virulent on genotype IT99K-7-21-2-2. Inoculum N2 and N3 were virulent on only Suvita2. Except for N3 and N6 all other inoculums were virulent on IT81D-994. Only

inoculum N5 showed virulence on UAM09-1046-6-1 and inoculum N1 showed virulence on IT03K-338-1.

**Table 4:1 Response of cowpea genotypes to different *Striga gesnerioides* populations**

ENTRY	Strains of <i>Striga gesnerioides</i>								
	N1	N2	N3	N4	N5	N6	N7	N8	N9
IT84S-2049	1	1	1	1	1	1	1	1	0
B524	1	1	1	1	1	1	1	1	0
IT97K-461-4	1	1	1	1	1	1	1	1	0
IT98K-409-4	1	1	1	1	1	1	1	1	0
IT93K-693-2	1	1	1	1	1	1	1	1	0
IT82D-849	0	0	0	0	0	0	0	0	0
IT99K-494-6	0	0	0	1	0	0	0	1	0
IT98K-1092-1	0	0	0	0	0	0	0	0	0
IT00K-1263	1	1	1	0	1	1	1	1	0
IT97K-819-118	0	0	0	0	0	1	0	0	0
IT99K-7-21-2-2	0	1	0	1	1	0	0	0	0
IT99K-573-2-1	0	0	0	0	0	0	0	0	0
SUVITA2	0	1	1	0	0	0	0	0	0
UAM09-1051-1	0	0	0	0	0	0	0	0	0
IT81D-994	1	1	0	1	1	0	1	1	0
IT97K-1042-3	1	1	1	1	1	1	1	1	0
IT93K-452-1	1	1	1	1	1	1	1	1	0
UAM09-1046-6-1	0	0	0	0	1	0	0	0	0
IT97K-499-35	0	0	0	0	0	0	0	0	0
IT00K-1217	0	0	0	0	0	0	0	0	0
UAM09-1046-6-2	0	0	0	0	0	0	0	0	0
IT98K-216-44	1	1	1	1	1	1	1	1	0
IT03K-338-1	1	0	0	0	0	0	0	0	0
UAM09-1055-6	0	0	0	0	0	0	0	0	0
IT99K-573-1-1	0	0	0	0	0	0	0	0	0
IT90K-205-8	0	0	0	0	0	0	0	0	0
TVX 3236	1	1	1	1	1	1	1	1	0

**N1 – N8 represents *S. gesnerioides* populations from eight endemic zones and N9 is control. 1 = *Striga* population is virulent on cowpea variety, cowpea variety susceptible to *Striga* population. 0 = *Striga* population not virulent on cowpea variety, cowpea variety resistant to *Striga* population.**

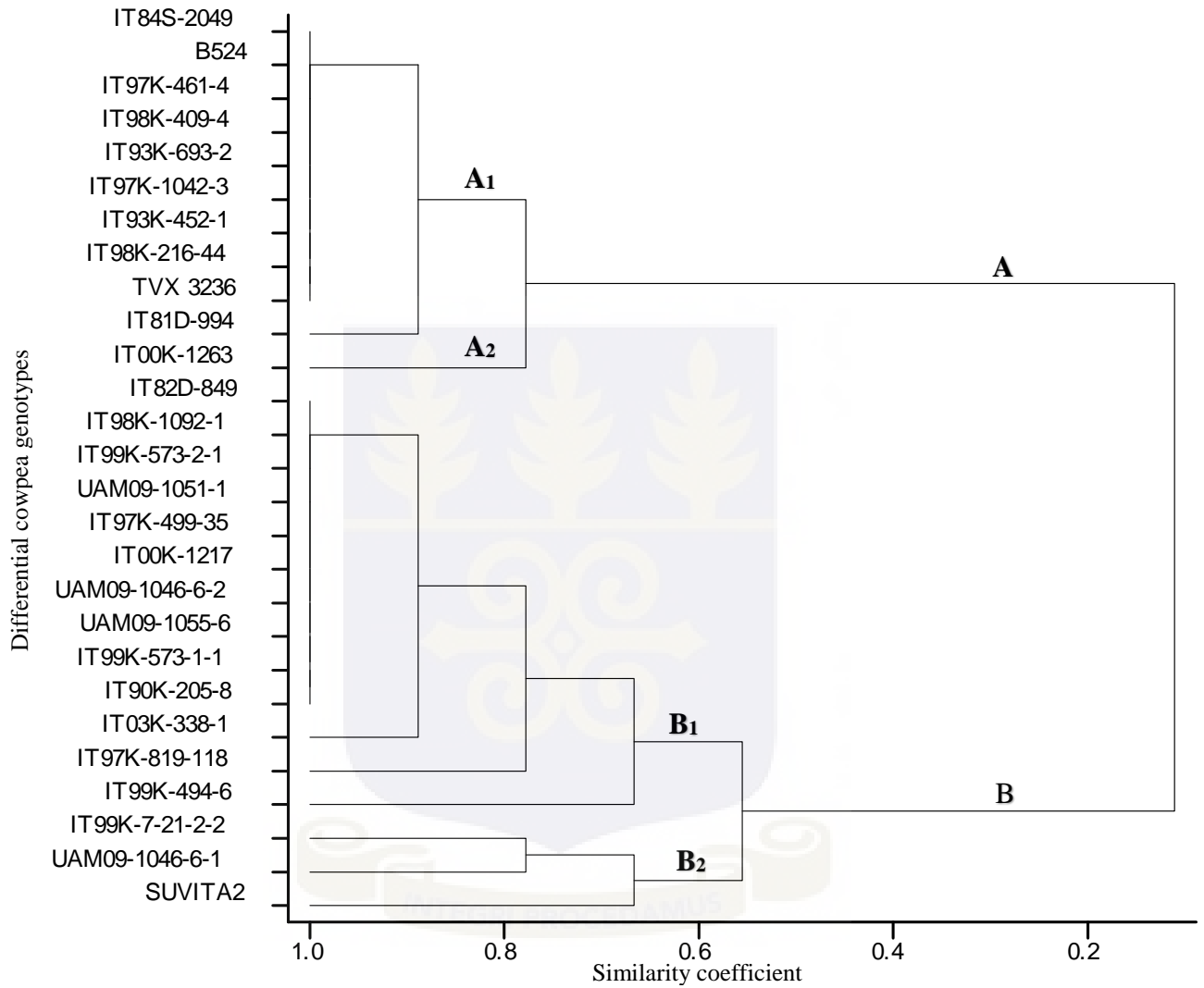
#### 4.1.5 Cluster analysis of cowpea genotypes based on their reaction to *Striga* population

The twenty-seven accessions of cowpea used in this study were characterised based on their reaction to eight populations of *Striga gesnerioides*. An Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis was carried out. This grouped the cowpea accessions into two accession clusters A and B at a similarity coefficient of about 0.15. Cluster A was sub clustered into A1 and A2 at a similarity coefficient of 0.78. The genotypes: IT84S-2049, B524, IT97K-461-4, IT98K-409-4, IT93K-693-2, IT97K-1042-3, IT93K-452-1, IT98K-216-44, TVX3236 and IT81D-994, were in cluster A1. Cluster A2 consisted of only the genotype IT00K-1263 (Figure 4:3).

Cluster B was sub clustered into two B1 and B2 at a similarity coefficient of 0.57. Cluster B2 consisted of Suvita2, UAM 09-1046-1 and IT99K-7-21-2-2. The remaining genotypes: IT82D-849, IT98K-1092-1, IT99K-573-2-1, UAM09-1051-1, IT97K-499-35, IT00K-1217, UAM09-1046-6-2, UAM09-1055-6, IT99K-573-1-1, IT90K-205-8, IT03K-338-1, IT97K-819-118 and IT99K-494-6, were clustered into B1.

At 1.00 similarity coefficient, the genotypes: IT84S-2049, B524, IT97K-461-4, IT98K-409-4, IT93K-693-2, IT97K-1042-3, IT93K-452-1, IT98K-216-44, and TVX3236, were unresolved and hence, classified as similar, based on their reaction to the populations of *Striga gesnerioides* used. The genotypes: IT82D-849, IT98K-1092-1, IT99K-573-2-1, UAM09-1051-1, IT97K-499-35, IT00K-1217, UAM09-1046-6-2, UAM09-1055-6, IT99K-573-1-1 and IT90K-205-8, were not resolved at similarity coefficient of 1.00 and clustered together as similar in terms of response to the *Striga* populations. Six genotypes were resolved at 1.00 similarity coefficient as different based of their reaction, these

genotypes included: Suvita2, UAM 09-1046-1, IT99K-7-21-2-2, IT03K-338-1, IT97K-819-118 and IT99K-494-6.

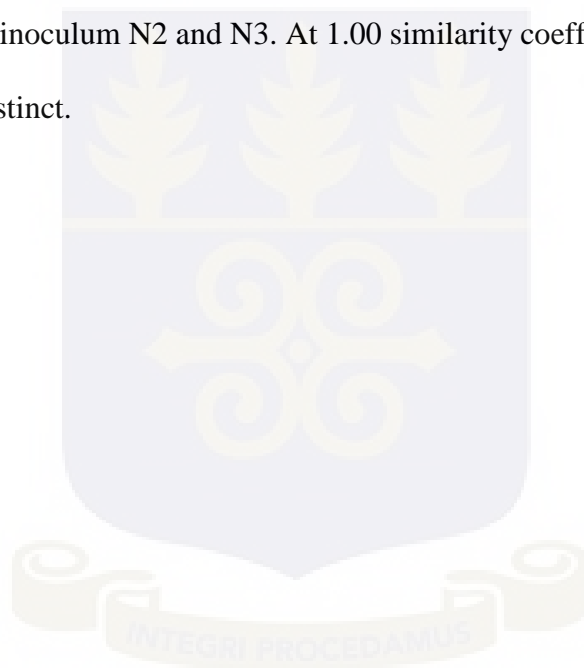


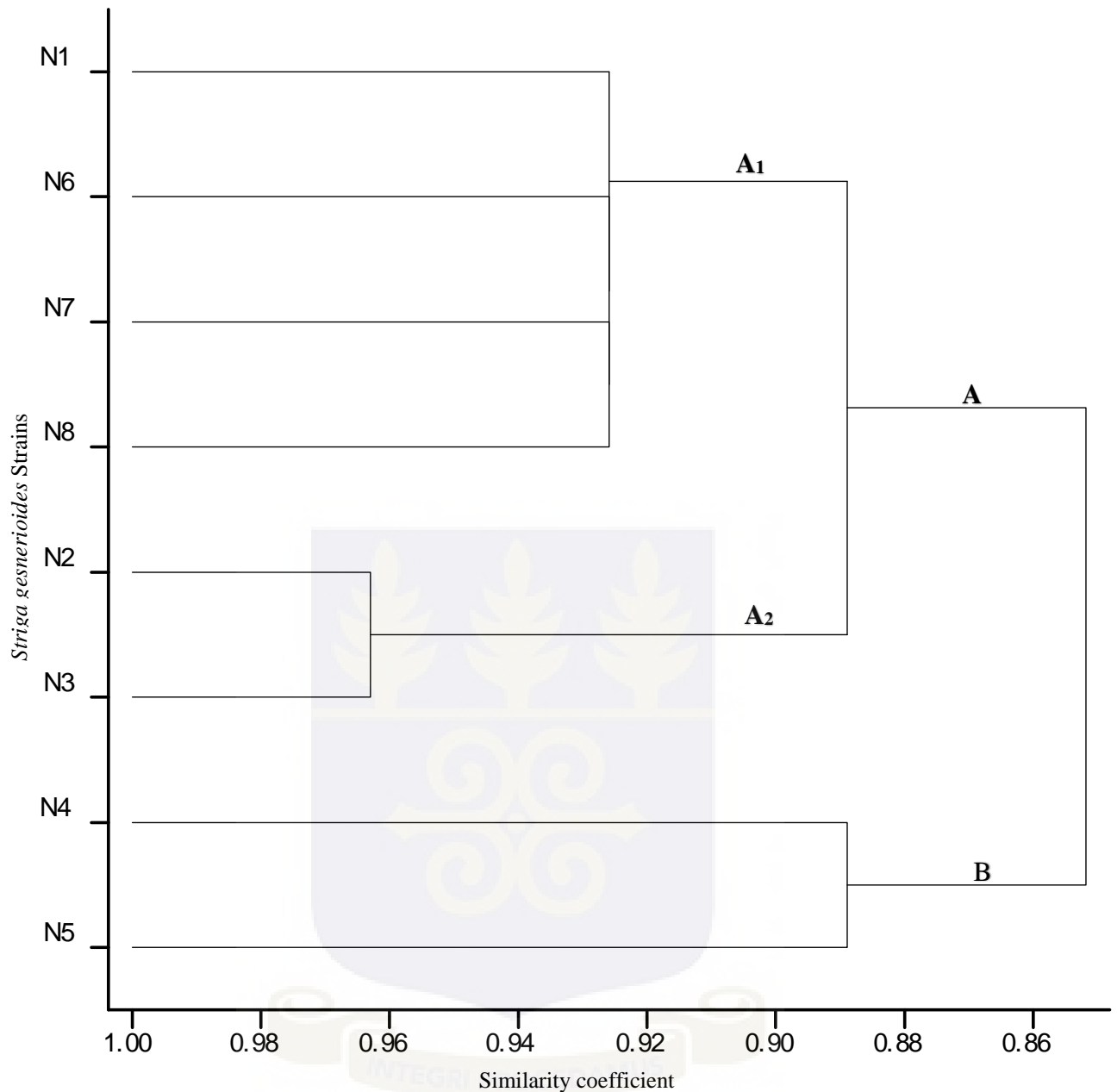
**Figure 4:3** A dendrogram of similarities among twenty-seven cowpea genotypes based on their reaction towards eight *Striga gesnerioides* population (N1-N8)

#### 4.1.6 Cluster analysis of *Striga gesnerioides* population based on their virulence

The results for the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Cluster analysis of the eight *Striga gesnerioides*(N1-N8) populations based on their virulence towards twenty-seven cowpea genotypes are presented in **Figure 4:4**.

At 0.82 similarity coefficient, two *Striga population* (inoculum) clusters, A and B were formed. Cluster B consisted of N4 and N5, whilst cluster A sub clustered into A<sub>1</sub> and A<sub>2</sub> at similarity coefficient of 0.93. Sub cluster A<sub>1</sub> consisted of N1, N6, N7 and N8; sub cluster A<sub>2</sub> consisted of the inoculum N2 and N3. At 1.00 similarity coefficient, all the inoculums were resolved as distinct.





**Figure 4:4** An Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis of eight *Striga gesnerioides* population from eight endemic zones based on their virulence on twenty-seven (27) cowpea genotypes. N1 from Bawku west, N2 from Garu, N3 from Babile, N4 from Gambaga, N5 from Manga, N6 from Lawra, N7 from Gusheigu and N8 from Walewale

#### 4.1.7 Diversity assessment of *Striga gesnerioides* using SSR markers

In all, seventy-three SSR markers were used in this study. All markers produced clear bands for determination of variability among the thirty-five (35) samples of *Striga gesnerioides*. Nineteen were monomorphic and were not informative for the current study, while the remaining 54 were polymorphic (Plate 4:4,5).



**Plate 4:4** Banding pattern for primer FA49/48 and FA3/4 on *Striga gesnerioides* sample numbered 1-35

Lane L 50 bp DNA ladder, 1-35: *Striga* samples from different endemic locations within the Northern region, Upper East region and Upper West region of Ghana, 1: Buguya kura, 2: Buguya kura \*, 3: Norilogubi, 4: Manga, 5: Yankazia, 6: Norilogubi\*, 7: Misiga, 8: Yaroyire, 9: Jagbani, 10: Tabier, 11: Tabier\*, 12: Babile, 13: Nahadaque, 14: Boko, 15: Manga, 16: Manga\*, 17:Googo\*, 18: Googo, 19: Googo\*\*, 20: Boko\*, 21: Boko, 22: Zuabuliga, 23: Manga, 24: Nankpawei, 25: Dimajan No.1, 26: Nyankpala UDS, 27: Bawku west, 28: Garu, 29: Babile, 30: Gambaga, 31: Gingande, 32: Lawra, 33: Gushiegu, 34: Walewale, 35: from a weed host. \* white flowered, \*\*taken from a resistant plot.



**Plate 4:5** Banding pattern for primer FA63/64 and FA51/56 on *Striga gesnerioides* sample numbered 1-35

Lane L 50 bp DNA ladder, 1-35: *Striga* samples from different endemic locations within the Northern region, Upper East region and Upper West region of Ghana, 1: Buguya kura, 2: Buguya kura \*, 3: Norilogubi, 4: Manga, 5: Yankazia, 6: Norilogubi\*, 7: Misiga, 8: Yaroyire, 9: Jagbani, 10: Tabier, 11: Tabier\*, 12: Babile, 13: Nahadaque, 14: Boko, 15: Manga, 16: Manga\*, 17:Googo\*, 18: Googo, 19: Googo\*\*, 20: Boko\*, 21: Boko, 22: Zuabuliga, 23: Manga, 24: Nankpawei, 25: Dimajan No.1, 26: Nyankpala UDS, 27: Bawku west, 28: Garu, 29: Babile, 30: Gambaga, 31: Gingande, 32: Lawra, 33: Gushiegu, 34: Walewale, 35: from a weed host. \* white flowered, \*\*taken from a resistant plot.

A total of 206 alleles with an average of 3.81 alleles per locus were obtained from the 54 polymorphic markers (Table 4:2). Allele number ranged from 1.00 - 10.00 per locus. The marker FA3/4 and FA37/38 had the highest number: 10 alleles. Allele frequency ranged from 0.26 – 1.00 with an average of 0.68. The highest polymorphic information content (PIC) (0.79) was obtained by the primer FA3/4 with a corresponding gene diversity of 0.82. The primer FA65/62 had the highest heterozygosity (1.00) (Table 4:2.)

**Table 4:2 Summary of genetic differentiation parameters generated by 54 polymorphic SSR markers on 27 *Striga gesnerioides* strains in Ghana**

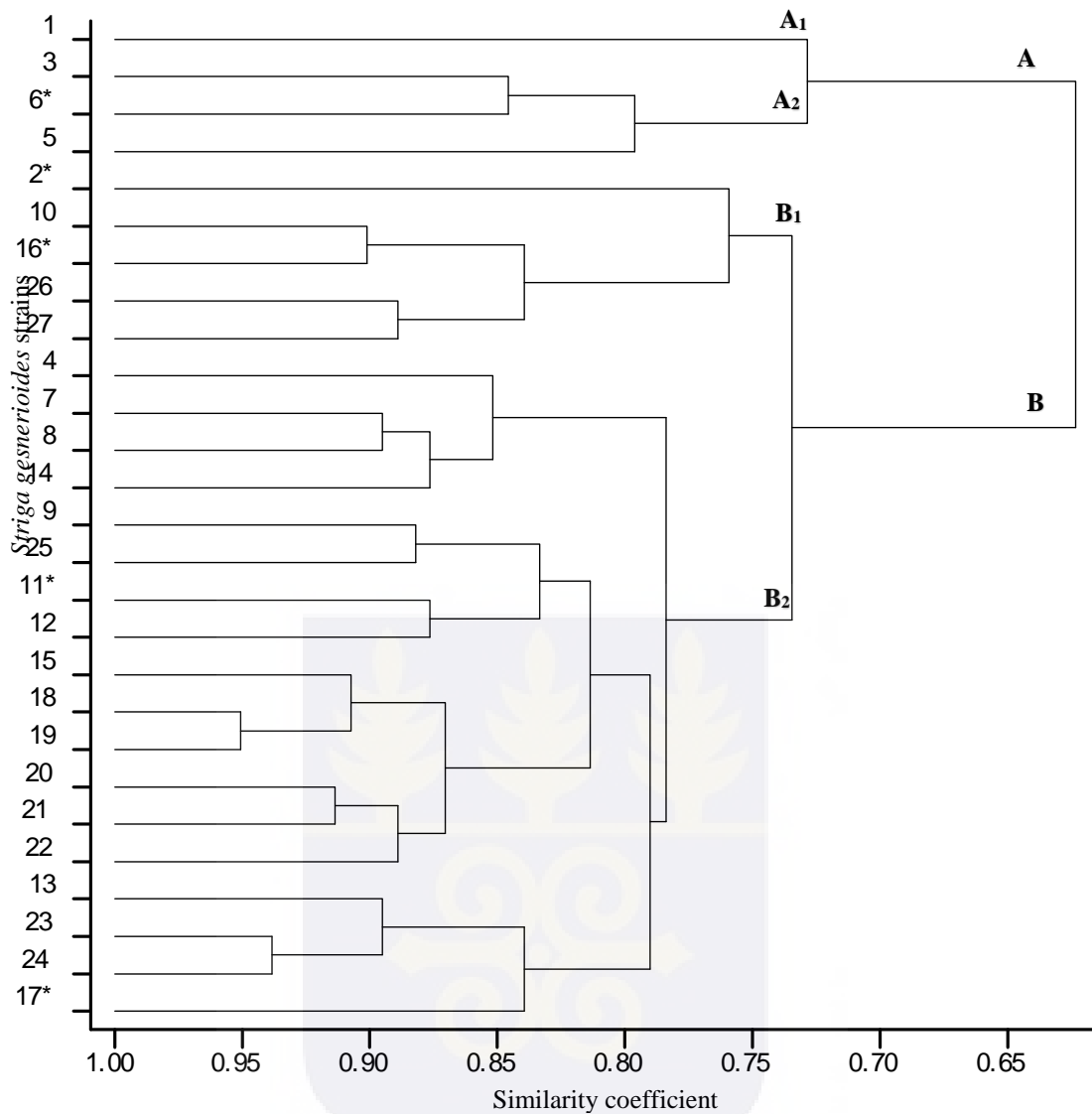
Marker	Major Allele Frequency	Allele Number	Gene Diversity	Heterozygosity	PIC
FA1/2	0.67	4.00	0.50	0.00	0.45
FA3/4	0.26	10.00	0.82	0.56	0.79
FA7/8	0.56	5.00	0.59	0.30	0.53
FA3/10	0.72	5.00	0.45	0.11	0.42
FA5/2	0.67	3.00	0.48	0.07	0.42
FA13/12	0.61	8.00	0.58	0.22	0.55
FA15/16	0.39	6.00	0.70	0.85	0.64
FA13/18	0.76	4.00	0.40	0.26	0.38
FA11/12	0.54	8.00	0.66	0.11	0.63
FA11/18	0.61	6.00	0.58	0.04	0.54
FA21/22	0.93	3.00	0.14	0.00	0.14
FA21/24	0.81	2.00	0.30	0.00	0.26
FA27/22	0.63	4.00	0.55	0.37	0.51
FA31/32	0.83	7.00	0.30	0.19	0.29
FA31/34	0.91	3.00	0.17	0.11	0.16
FA47/38	0.85	4.00	0.27	0.00	0.25
FA35/36	0.83	4.00	0.29	0.11	0.27
FA37/38	0.35	10.00	0.78	0.07	0.75
FA39/40	0.35	6.00	0.75	0.11	0.71
FA49/48	0.46	3.00	0.63	0.70	0.55
FA51/52	0.76	5.00	0.40	0.11	0.37
FA51/56	0.30	5.00	0.75	0.33	0.70
FA57/54	1.00	1.00	0.00	0.00	0.00
FA59/52	0.85	3.00	0.26	0.00	0.25
FA51/62	0.48	4.00	0.55	0.96	0.45
FA63/64	0.52	4.00	0.62	0.93	0.56
FA65/62	0.50	4.00	0.55	1.00	0.45
FA67/62	0.44	4.00	0.62	0.89	0.55
FA81/76	0.52	3.00	0.60	0.00	0.52
FA79/74	0.74	2.00	0.38	0.00	0.31
FA91/92	0.67	3.00	0.48	0.15	0.42
FA83/76	0.63	3.00	0.53	0.00	0.47
FA101/102	0.59	3.00	0.51	0.00	0.41
FA93/94	0.63	5.00	0.53	0.04	0.47
FA105/116	0.94	2.00	0.10	0.11	0.10
FA101/104	0.56	2.00	0.49	0.00	0.37
FA107/106	0.96	2.00	0.07	0.00	0.07
FA131/132	0.94	3.00	0.11	0.04	0.10
FA121/122	0.91	3.00	0.17	0.04	0.16
FA125/126	0.48	3.00	0.58	0.04	0.49
FA127/128	0.87	3.00	0.23	0.26	0.21

<b>FA129/128</b>	0.89	3.00	0.20	0.00	0.19
<b>FA131/138</b>	0.74	3.00	0.40	0.44	0.34
<b>FA123/122</b>	0.28	6.00	0.78	0.52	0.75
<b>FA139/140</b>	0.96	2.00	0.07	0.00	0.07
<b>FA133/134</b>	0.80	3.00	0.33	0.04	0.29
<b>FA141/142</b>	0.78	2.00	0.35	0.44	0.29
<b>FA149/142</b>	0.93	2.00	0.14	0.15	0.13
<b>FA155/156</b>	0.69	2.00	0.43	0.63	0.34
<b>FA157/158</b>	0.91	2.00	0.17	0.19	0.15
<b>FA159/158</b>	0.69	2.00	0.43	0.63	0.34
<b>FA151/154</b>	0.39	3.00	0.66	0.56	0.59
<b>FA97/92</b>	0.59	2.00	0.48	0.00	0.37
<b>FA143/148</b>	0.85	2.00	0.25	0.00	0.22
<b>Mean</b>	<b>0.68</b>	<b>3.81</b>	<b>0.43</b>	<b>0.23</b>	<b>0.38</b>

**PIC: Polymorphic information content**

#### 4.1.7.1 Cluster analysis based on SSR markers

The cluster analysis of the strains of *Striga gesnerioides* used in this study using 54 polymorphic SSR markers (**Figure 4.5**) showed two main clusters for *Striga* strains: cluster A and B at a coefficient of similarity 0.60. At a coefficient of similarity 0.71, cluster A, sub clustered into cluster A1, which consisted of the strain: 1; and sub cluster A2 which consisted of the strains: 3, 6 and 5. Cluster B sub clustered into B1 and B2 at a 0.73 similarity coefficient. Cluster B1 sub clustered into two: one consisting of only strain 2 and the other strains: 10, 16, 26 and 27. Cluster B2 also sub clustered into two: one cluster consisted of the strains: 4, 7, 8 and 14; with the other consisting of the rest of the strains. Strain 17 could be observed to cluster on its own.



**Figure 4:4 Cluster analysis of *Striga gesnerioides* strains based on 54 SSR markers.**

Strains 1,2,3,5,6,8,9,24 are from the Northern region of Ghana, strains 4,7,14,15,16,17,18,19,20,21,23,27 are from the Upper East region and the strains 10,11,12,13,24,25 are from the Upper West region of Ghana. \* shows white flowered *Striga* strains.

Considering regional clustering of the *Striga* strains in this work as depicted by Principal Component Analysis (PCA) (Figure 4.6), the strains from the upper east and west happened to cluster together (I) with majority of the strains from the northern region also clustering together (II). Strain 17 was separated from the rest (III). This created three strain clusters.

Flower colour differences showed no distinct clustering patterns, as the two flower colours observed clustered together. Strain 27, which was collected from the weed *Ipomoea*



#### 4.1.8 Deducing the race of *S. gesnerioides*

The race(s) of *S. gesnerioides* can be inferred from the response study and the molecular data. The response study revealed three groupings for the *Striga gesnerioides* populations (**Figure 4:3**). This grouping indicates the probable presence of at least three different races in Ghana. *Striga* populations N2 and N3 are similar from the clustering, considering the response of differential cultivars N2 and N3 can be inferred to be race 3 since majority of the differential cultivar resistant to SG3 were resistant to these two population. Populations N4 and N5 shows the possibility of races 2 and 5 from careful examination of the differential cultivars. Populations 1, 6, 7 and 8 also show a mixture of races 2, 3 and 5 as these populations could not be inferred as a single race but several (**Table 4:3**). Since these populations covered a wide geographical area the observed mixture might be as a result of mixed populations.

The molecular data also reveals similar inference, populations from the northern region clustered together forming a distinct strain on their own. Populations from the Upper East region form three groupings of only Upper East strains and the Upper West region formed two clusters of only Upper West strain. The remaining clustered together consisting of population from two regions and all three regions (**Figure 4:3**). The existence of multiple races is therefore evident from the molecular and response study.

**Table 4:3 Differential cowpea cultivars and their responses to different *Striga* populations**

ENTRY	<i>S. gesnerioides</i> populations							
	N1	N2	N3	N4	N5	N6	N7	N8
<b>IT99K-494-6</b> (3)	0	0	0	1	0	0	0	0
<b>IT00K-1263</b> (3)	1	1	1	0	1	1	1	1
<b>IT99K-7-21-2-2</b> (3)	0	1	0	1	1	0	0	0
<b>SUVITA2</b> (1,2,4,5,6)	0	1	1	0	0	0	0	0
<b>IT81D-994</b> (1,2,5)	1	1	0	1	1	0	1	1
<b>UAM09-1046-6-1</b> (3)	0	0	0	0	1	0	0	0
<b>IT03K-338-1</b> (3)	1	0	0	0	0	0	0	0



## CHAPTER FIVE

### 5.1 DISCUSSION

#### 5.2 *Striga* Hot spots in Ghana

Ghana has neighbors Togo and Benin to the east and Burkina Faso and Nigeria to the north. Most of the countries bordering Ghana have serious problems with the parasitic weed, *Striga gesnerioides*. Different races of *Striga gesnerioides* are found within these surrounding countries. The tiny seed size of the parasitic weed enhances dispersal; hence the seeds are most likely to have spread across west African countries. This is evident in the mixed races across affected countries. Ghana will not be an exception in the presence of mixed races.

The *Striga* hot spots recorded in this study though not exhaustive, shows a denser distribution from the Upper East region and Upper West regions and sparsely in the Northern region. The continuous cropping of farmers in these areas with little inputs depletes soil nutrients and this enhances *Striga gesnerioides* colonization thereby increasing its population and spread. According to Spallek *et al.* (2013), the spread of parasitic weed is agricultural in nature and under environmental influences. The soils in these three regions are also very low in nutrients with poor rainfall pattern (Ministry of Food and Agriculture Ghana, 2010). This favours *Striga* growth and colonization. Researches have reported on high *Striga* infestation in areas of poor soils and rainfall (Estep *et al.*, 2011, 2012; Lenzemo *et al.*, 2009). A similar situation was observed in this study.

### 5.3 Morphological variation and host range of *Striga gesnerioides*

Morphological variations are the earliest form of noticeable variations that are observed in every species that exhibits different morphotypes. These variations have been reported to exist in the genus *Striga*, with variations occurring in flower colour between species and within species. According to Musselman (1980), flower colour in the genus *Striga* varies from red, purple to white. Spallek *et al.* (2013), also reported on variation in flower colour in *Striga asiatica*. The findings of this study confirm results of previous research. In this study two morphotypes were found to exist for *Striga gesnerioides* on cowpea, the white flower and purple flower *Striga gesnerioides* (Plate 4:1). No research has linked the difference in flower colour and virulence within a species of *Striga*. From personal communication with farmers the purple flowered *Striga gesnerioides*, is more virulent because it was said to attack much earlier and growth is more vigorous. The ratio of purple to white flowered observed was 3:1, which shows that purple is predominant over white in the areas covered by this study. This accounts for the reason why most of the *Striga gesnerioides* samples across the three northern regions were purple flowered (Figure 4:1). Thus, the purple flowered *S. gesnerioides* can be said to be more adapted than the white flowered ones and probably more virulent.

According to Westwood *et al.* (2012) and Spallek *et al.* (2013), *Striga gesnerioides* parasitizes a range of crops including cowpea, tobacco and other leguminous weeds. Parker & Riches (1994) and Parker (2012) reported on *Striga gesnerioides* parasitizing the leguminous weed *Tephrosia pedicellata*. This was also earlier reported by Musselman (1980). This assertion was supported by this study, as *Striga gesnerioides* was found

parasitising the leguminous weed *Tephrosia pedicellata* (Plate 4:2), but the parasite was green on some and purple on others.

*Striga gesnerioides* was also found in this study to parasitize the weed *Ipomoea tribola* (Plate 4:3), which is not a leguminous weed. Musselman (1980), reported on sweet potato (*Ipomoea batatas*) as a host to *Striga gesnerioides* but no report on a weed as a host to *Striga gesnerioides* within the genus *Ipomoea*. This study makes a first report of the weed *Ipomoea tribola* as a host to this parasite. Since the parasite uses these weeds as alternative hosts, it makes its eradication very difficult as the parasite in the presence of resistant varieties survive on these alternative hosts to evolve more virulent strains to parasitize the primary host (cowpea). Hence resistance breeding must also target diversity between the parasite on the weed and that on the cowpea for relatedness.

#### **5.4 Response of cowpea cultivars on different populations of *Striga gesnerioides***

The response of cowpea genotypes used in this study confirms the differential virulence of *Striga gesnerioides*. The genotypes were grouped into three; resistant to all strains, susceptible to all strains and those with differential responses. Genotypes resistant to all strains might have resistance genes for all the strains used in this study and those susceptible to all have none. However, of importance in determining if there exist different strains of the parasitic weed in Ghana or not are those with differential responses. This confirms the existence of different races in Ghana. Two or more strains of *Striga gesnerioides* have been reported in some countries in west African. Race 4 and 4z in Benin (Timko *et al.*, 2007), there has also been reports of races 5 and 2 in Burkina Faso and two races in Mali (Ouédraogo *et al.*, 2001), Nigeria harbours race 3, 2 and 5 (Timko, 2016).

From the response study, the probable races in Ghana are 3, 2 and 5, however, these needs to be confirmed. For breeding activities for improvement of cowpea to *Striga* resistance, those cultivars resistant to all strains of *Striga* used will be of great importance.

### **5.5 Genetic diversity in *Striga gesnerioides***

This study recorded a high allelic polymorphism of 74% among the populations using the 74 SSR primers. This reflects the informative nature of the designed primers and is indicative of the germane use of these primers in diversity studies. The genetic dissimilarity generated by the polymorphic SSR markers ranged from 0.65 – 1.00. This indicates a moderate genetic variation among the populations, which is congruent with reports by Musselman (1980).

The genetic diversity observed in this study was in relation to geographical distribution, such that *Striga* populations from the Northern region clustered together whilst those from the Upper East and West regions also clustered together. Within the cluster for the upper east and west were some sub clusters for Upper East and Upper West separately. This makes a grouping of at least three clusters. This indicates that at least three strains of *Striga* on cowpea probably exist in Ghana, probably races 3, 2 and 5. This needs to be confirmed through further studies. This also confirms results obtained from the response studies pointing to existence of multiple strains in Ghana. The parasite can be said to adapt to a location through mutations and changes in climatic and environmental condition prevailing in that location might cause a change in the parasite independent of what the others are exposed to at different locations.

The observed morphological variation in flower colour did not correlate with the genetic diversity as the two flower colour did not cluster separately. Flower colour thus cannot be used to differentiate between the different strains of *Striga gesnerioides* within a location.



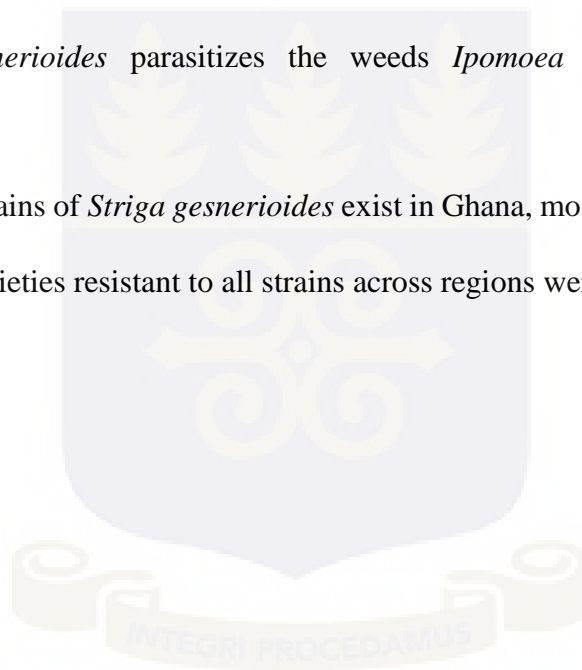
## CHAPTER SIX

### 6.1 CONCLUSION AND RECOMMENDATION

#### 6.1.1 CONCLUSION

From this study the following conclusions can be drawn:

- *Striga gesnerioides* is widely distributed in the three Northern (Northern, Upper East and Upper West) regions of Ghana.
- The major form of *Striga gesnerioides* in Ghana is the purple flowered type.
- *Striga gesnerioides* parasitizes the weeds *Ipomoea tribola* and *Tephrosia pedicellata*
- Multiple strains of *Striga gesnerioides* exist in Ghana, most likely races 3, 2 and 5.
- Cowpea varieties resistant to all strains across regions were also identified



### 6.1.2 RECOMMENDATION

To confirm the races of *Striga gesnerioides* in Ghana, further work needs to be done using the molecular markers from this study. Different races of *Striga* known in West Africa can be used together with strains from Ghana to confirm how these races clusters with the different races.

Researchers should consider cowpea lines that were resistant to all strains of *Striga* used in this work in developing *Striga* resistant cowpea varieties in Ghana.

Improved cowpea lines should be tested in the *Striga gesnerioides* endemic zones as there is a possibility of different strains of the parasite at these zones.

The number per location sampled for this work, thus. 35 in total for the three regions is not representative enough to represent an accurate distribution of *Striga gesnerioides* in Ghana. Hence, more cowpea growing areas should be surveyed in future research to give an accurate distribution of the parasite in Ghana.

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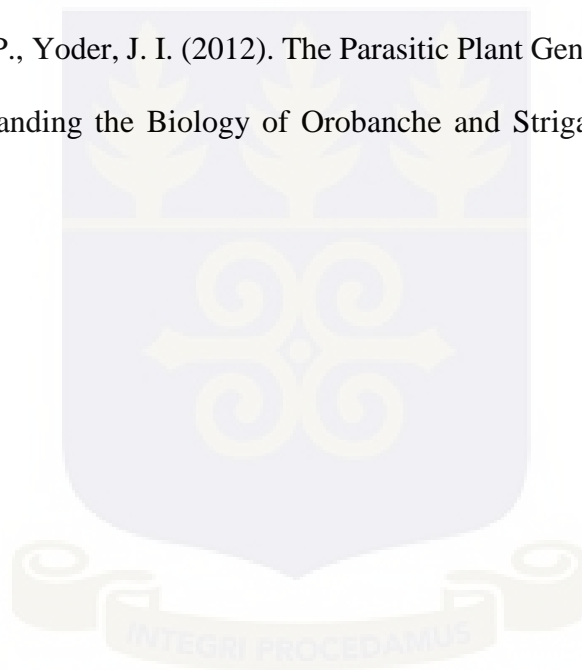
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## APPENDIX

### APPENDIX A

A1: List of cowpea genotypes used in this studies.

Sample ID	Genotype	Race Responds
1	IT84S-2049	susceptible check
2	B524	3
3	IT97K-461-4	susceptible check
4	IT98K-409-4	3
5	IT93K-693-2	1
6	IT98K-205-10	3
7	IT82D-849	1, 2, 3, 5
8	IT99K-494-6	3
9	IT98K-1092-1	3
10	IT00K-1263	3
11	IT97K-819-118	3
12	IT99K-7-21-2-2	3
13	IT98K-615-6-1	3
14	IT99K-573-2-1	3
15	SUVITA2	1, 2, 4, 4z, 5, 6
16	UAM09-1051-1	3
17	IT81D-994	1, 2, 5
18	IT97K-1042-3	3
19	IT93K-452-1	3
20	UAM09-1046-6-1	3
21	IT97K-499-35	Resistant check
22	IT00K-1217	3
23	UAM09-1046-6-2	3
24	IT98K-216-44	3
25	IT03K-338-1	3
26	UAM09-1055-6	3
27	IT99K-573-1-1	3
28	IT90K-205-8	3
29	TVX 3236	Susceptible check

A2: *Striga gesnerioides* endemic zones in Ghana

Region	Endemic Zone
Upper East	Manga
	Googo
	Garu
Upper West	Lawra
	Babilla
Northern	Gushiegu
	Walewale
	Gambaga

A3: Drum method of soil sterilisation



A4: List of locations for *Striga gesnerioides* sample collection

<b>ID</b>	<b>Name of location</b>	<b>District</b>	<b>Region</b>
1	Buguya-kura	West Mamprusi	Northern
2	Norilogubi	East Mamprusi	Northern
3	Yankazia	East Mamprusi	Northern
4	Barinya	East Mamprusi	Northern
5	Misiga	Bawku Municipal	Upper East
6	Yaro yire	East Mamprusi	Northern
7	Jagbani	West Mamprusi	Northern
8	Tabier	Laura	Upper West
9	Babile	Laura	Upper West
10	Nahadague	Sisala East	Upper West
11	Manga	Binduri	Upper East
12	Googo	Bawku West	Upper East
13	Boko	Binduri	Upper East
14	Zuabuliga	Pusiga	Upper East
15	Nankpawei	Sisala East	Upper West
16	Dimajan	Sisala East	Upper West
17	Nyankpala-UDS	Tolon kunbumgu	Northern
18	Weed ( <i>Ipomoea tribola</i> )	Binduri	Upper East

A5: Gene sequences used for the primer design and their corresponding GenBank ID

ID	GenBank ID	Sequence Region
1	EU140575	<i>Striga gesnerioides</i> isolate <i>Striga02</i> internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
2	AF489963.1	<i>Striga gesnerioides</i> maturase K (matK) gene, partial cds; chloroplast gene for chloroplast product
3	EU140574	<i>Striga gesnerioides</i> isolate <i>Striga01</i> ribosomal protein subunit 2 gene, partial cds; chloroplast
4	AF026839.1	<i>Striga gesnerioides</i> ribulose 1,5-bisphosphate carboxylase large subunit (rbcL) gene, partial cds
5	JX091339.1	<i>Striga gesnerioides</i> maturase K (matK) pseudogene, complete sequence; chloroplast
6	KC480440	<i>Striga gesnerioides</i> voucher Steiner s.n. (NBG) ribosomal protein subunit 2 (rps2) gene, partial cds; chloroplast
7	KC480406.1	<i>Striga gesnerioides</i> voucher Steiner s.n. (NBG) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
8	KC480404.1	<i>Striga gesnerioides</i> voucher Jury 19619 (RNG) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
9	KC542275.1	<i>Striga gesnerioides</i> voucher Jury 19619 (RNG) phytochrome B (phyB) gene, partial cds
10	U48747.1	<i>Striga gesnerioides</i> ribosomal protein subunit 2 (rps2) gene, chloroplast gene encoding chloroplast protein, partial cds
11	FR728646.1	<i>Striga gesnerioides</i> chloroplast partial tRNA-Lys gene intron and matK gene for maturase K, isolate LE471
12	AY911257.1	<i>Striga gesnerioides</i> internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
13	AB542056.1	<i>Striga gesnerioides</i> mRNA for hypothetical protein, partial cds
14	EU259234.1	<i>Striga gesnerioides</i> rpl16 gene, intron; chloroplast
15	AM234026.1	<i>Striga gesnerioides</i> partial phyA gene for phytochrome A, clone 1626_2b_3
16	EU264195.1	<i>Striga gesnerioides</i> trnT-trnL intergenic spacer and tRNA-Leu (trnL) gene, partial sequence; chloroplast

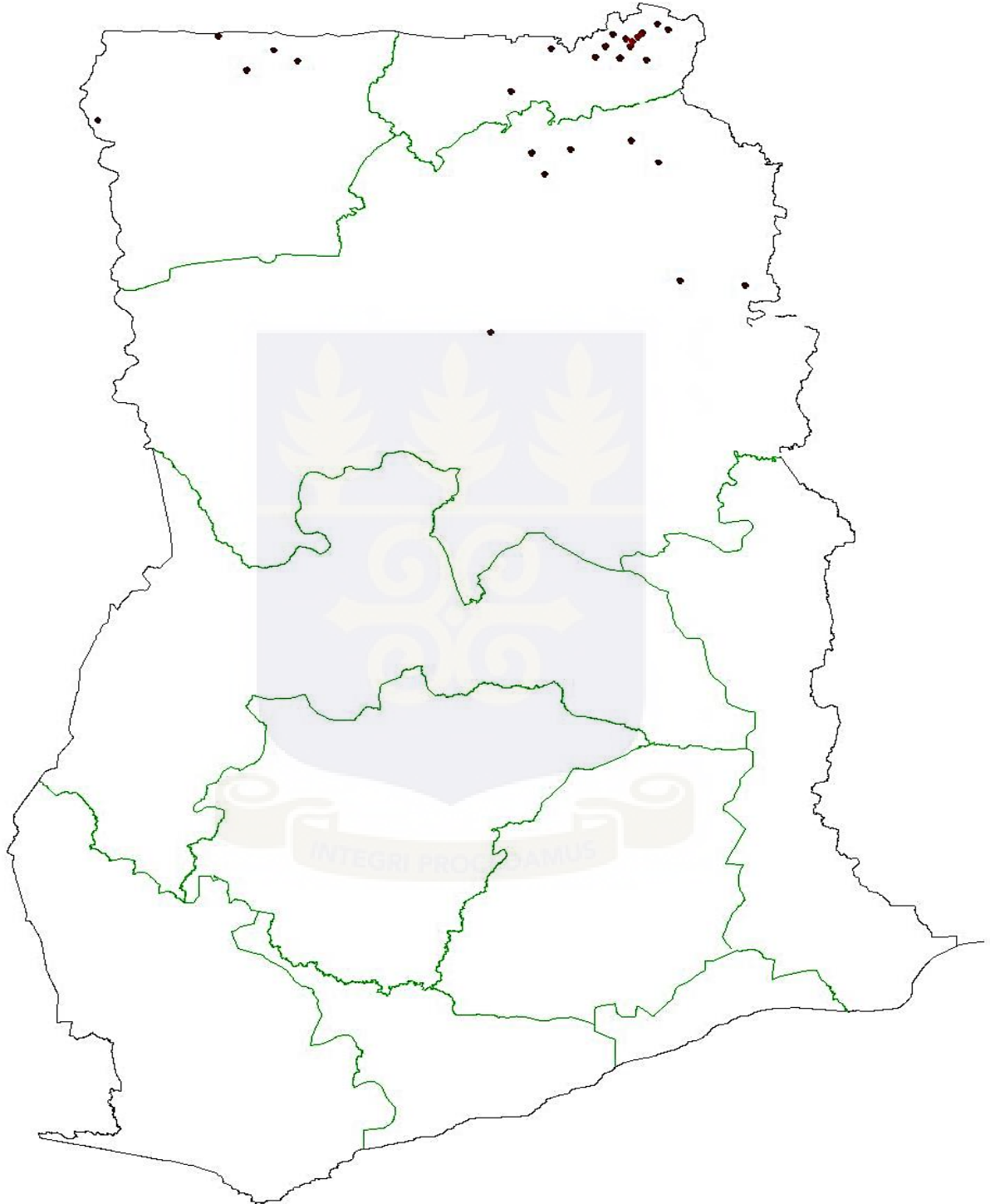
A6: List of SSR primers

Primer Name	Forward	Reverse	Expected Product size (bp)
FA1/2	GCCGGAACAAAGACTCGATC	TCACCAGCCTCCAAGTTGAT	244
FA3/4	TAGGTTGGAAGTGGGACCAC	TCTGTACAGGAGGAGGTCGA	218
FA5/6	TGTCACCAGAAGAGCCGAAT	TCACCAGCCTCCAAGTTGAT	193
FA7/8	GACCTCCCAAACAACACCAC	GTGGTCCCAGTTCCAACCTA	231
FA9/10	TAGGTTGGAAGTGGGACCAC	CTAACACCGAGAGCAGCAAC	157
FA11/12	GCTCTACGCCTGGAAGATCT	CAACGCATAAATGGCTGGGA	248
FA13/14	TAAAACCTTTCCAAGGCCCGC	CAACGCATAAATGGCTGGGA	207
FA15/16	CTTCGCGGTGGACTTGATT	ATTTCTTCGCATGTACCCGC	173
FA17/18	GCTCTACGCCTGGAAGATCT	AAATCAAGTCCACCGCGAAG	203
FA19/20	TAAAACCTTTCCAAGGCCCGC	AAATCAAGTCCACCGCGAAG	162
FA21/22	CTGGTTGCGTCATTGGCTAA	TCCGAGCCAAAGTTCTAGCA	217
FA23/24	CTGGTTGCGTCATTGGCTAA	CCGAGCCAAAGTTCTAGCAC	216
FA25/26	TTCCTCTGGTTGCGTCATTG	TCCGAGCCAAAGTTCTAGCA	222
FA27/28	GCATCCCATTAGTAAGCCGG	TCCGAGCCAAAGTTCTAGCA	173
FA29/30	GGCATCCCATTAGTAAGCCG	TCCGAGCCAAAGTTCTAGCA	174
FA31/32	CCTAGTCATGTCCGTCGTC	CGACGTGAATGGAGAACACC	194
FA33/34	CCTAGTCATGTCCGTCGTC	CCGACGTGAATGGAGAACAC	195
FA35/36	GAGTGTGCCATGGAGAGACT	CCACCCCAACAGCCAGTATA	242
FA37/38	GCGCACTACAGTCGTACAAG	CTCCCCATGGTCGTCTTCAT	167
FA39/40	TTAGGGGATGCCTTCTGTGG	CGCCTTACCACCTCTAGAA	189
FA41/45	CCTAGTCATGTCCGTCGTC	CGACGTGAATGGAGAACACC	194
FA43/44	CCTAGTCATGTCCGTCGTC	CCGACGTGAATGGAGAACAC	195
FA45/46	TTAGGGGATGCCTTCTGTGG	CGCCTTACCACCTCTAGAA	189
FA47/48	ACTTCGAGCCTGTTAAGCCT	CTCCCCATGGTCGTCTTCAT	212
FA49/50	TTCGAGCCTGTTAAGCCTCA	CTCCCCATGGTCGTCTTCAT	210
FA51/52	CATGCGCCAAGGAAAACAGA	GATGGTTCACGGGATTCTGC	184
FA53/54	GCAGAATCCCGTGAACCATC	GGGTGCCTCGTGAATTCATT	194
FA55/56	CATGCGCCAAGGAAAACAGA	ACTCGATGGTTCACGGGATT	188
FA57/58	AATCCCGTGAACCATCGAGT	GGGTGCCTCGTGAATTCATT	190
FA59/60	ATGCGCCAAGGAAAACAGAT	GATGGTTCACGGGATTCTGC	183
FA61/62	CGGCTGTGGTGAAAAGACAA	AATCTGCAAGGTCAGGGTCA	192
FA63/64	CGGCGGGATGTTAACGAATT	CCGCCCAGATATGTTTGCAA	159
FA65/66	ACAAAGGGCGGGAAGACTAA	AATCTGCAAGGTCAGGGTCA	233
FA67/68	AACAAAGGGCGGGAAGACTA	AATCTGCAAGGTCAGGGTCA	234
FA69/70	CGGCGGGATGTTAACGAATT	CGCAGCGCCGTATATTCTTT	231
FA71/72	CGCCAAGGAAAACGGATAGG	GATGGTTCACGGGATTCTGC	180
FA73/74	CGCCAAGGAAAACGGATAGG	ACTCGATGGTTCACGGGATT	184
FA75/76	CGCCAAGGAAAACGGATAGG	TCGATGGTTCACGGGATTCT	182

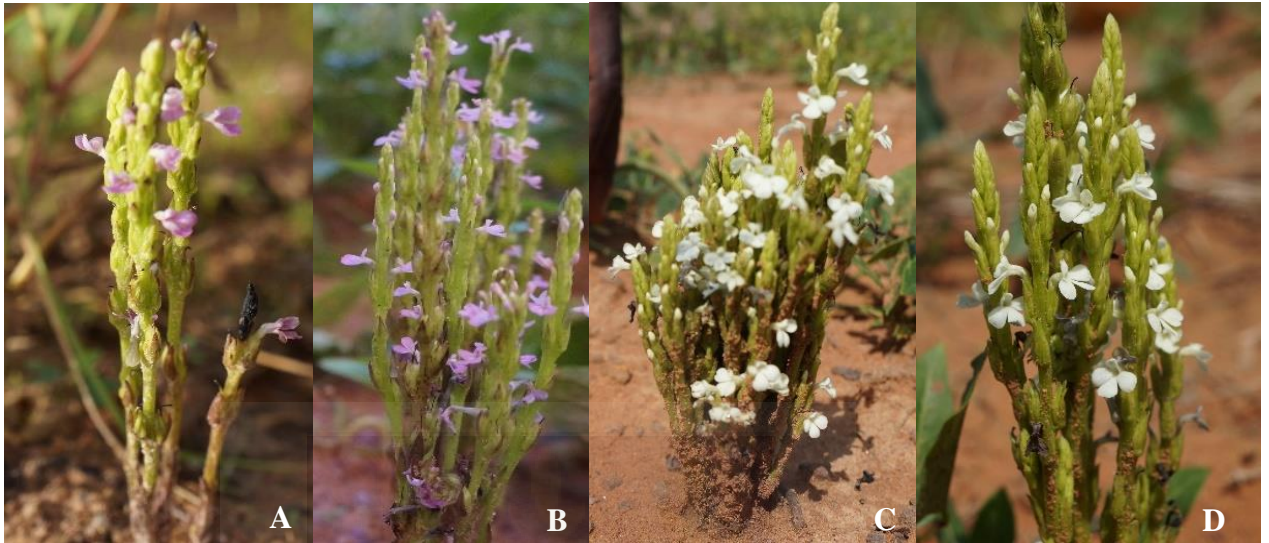
FA77/78	AGGAAAACGGATAGGAGCGT	GATGGTTCACGGGATTCTGC	175
FA79/80	AGGAAAACGGATAGGAGCGT	ACTCGATGGTTCACGGGATT	179
FA81/82	GTGGTGGGAACGATGAAACC	CGGGCGAATATTTACTCTTGCA	171
FA83/84	GGTGGGAACGATGAAACCTG	CGGGCGAATATTTACTCTTGCA	169
FA85/86	TGGTGGGAACGATGAAACCT	CGGGCGAATATTTACTCTTGCA	170
FA87/88	GTGGTGGGAACGATGAAACC	TGCAATCTCTATTTTCAGCCGT	153
FA89/90	GGTGGGAACGATGAAACCTG	TGCAATCTCTATTTTCAGCCGT	151
FA91/92	TGGATTCACACCCCTTACACT	ACATACCATTTTCCCACGCA	216
FA93/94	CCTTGCGTGGGAAAATGGTA	GGATTACTATCGCGAAAAGGGG	163
FA95/96	TGGATTCACACCCCTTACACT	ACATACCATTTTCCCACGCA	216
FA97/98	TGTTTTGGATTACACCCCT	ACATACCATTTTCCCACGCA	221
FA99/100	TGGATTCACACCCCTTACAC	ACATACCATTTTCCCACGCA	216
FA101/102	TGATTTCCCAGATCTCCCGG	GTGCCTGCAGTTTTTCATTGC	172
FA103/104	TGATTTCCCAGATCTCCCGG	CGTGCCTGCAGTTTTTCATTG	173
FA105/106	GCATCCCATTAGTAAGCCGG	TCCGAGCCAAAGTTCTAGCA	173
FA107/108	GGCATCCCATTAGTAAGCCG	TCCGAGCCAAAGTTCTAGCA	174
FA109/110	GATTTCCCAGATCTCCCGGA	GTGCCTGCAGTTTTTCATTGC	171
FA111/112	GCATCCCATTAGTAAGCCGG	TCCGAGCCAAAGTTCTAGCA	173
FA113/114	GGCATCCCATTAGTAAGCCG	TCCGAGCCAAAGTTCTAGCA	174
FA115/116	GCATCCCATTAGTAAGCCGG	CCGAGCCAAAGTTCTAGCAC	172
FA117/118	GGCATCCCATTAGTAAGCCG	CCGAGCCAAAGTTCTAGCAC	173
FA119/120	CCCATTAGTAAGCCGGTTCG	TCCGAGCCAAAGTTCTAGCA	169
FA121/122	CCGAACCTGGTGTCCCTAGT	AACGTGGAAAAGCACCTGTC	241
FA123/124	ACCGAACCTGGTGTCCCTAG	AACGTGGAAAAGCACCTGTC	242
FA125/126	AGCAACTCCGCAACTTGAAG	TCCACACCTTCTTAGCAGCA	216
FA127/128	AGAAGCAACTCCGCAACTTG	TCCACACCTTCTTAGCAGCA	219
FA129/130	CAAGAAGCAACTCCGCAACT	TCCACACCTTCTTAGCAGCA	221
FA131/132	CGCCAAGGAAAACGGATAGG	GATGGTTCACGGGATTCTGC	174
FA133/134	CTGCGGAAGGATCATTGTGCG	CCTATCCGTTTTCTTGCG	158
FA135/136	CGCCAAGGAAAACGGATAGG	ACTCGATGGTTCACGGGATT	178
FA137/148	CGCCAAGGAAAACGGATAGG	TCGATGGTTCACGGGATTCT	176
FA139/140	AGGAAAACGGATAGGAGCGT	GATGGTTCACGGGATTCTGC	169
FA141/142	GCAGAATCCCGTGAACCATC	CCCAACACGACAGGAAGAGA	224
FA143/144	GCAGAATCCCGTGAACCATC	TCCCAACACGACAGGAAGAG	225
FA145/146	GCAGAATCCCGTGAACCATC	CTCCCAACACGACAGGAAGA	226
FA147/148	GCAGAATCCCGTGAACCATC	TGTTGGGTCTAAGGGTACGC	250
FA149/150	AATCCCGTGAACCATCGAGT	CCCAACACGACAGGAAGAGA	220
FA151/152	GCAGGAGTTCATTTTGGGCA	GTTTTCGCCTACTTGCTGCA	163
FA153/154	GCAGGAGTTCATTTTGGGCA	TGTTTTCGCCTACTTGCTGC	164
FA155/156	ATCCGAAGCCTGCAATTTGG	CTTTTCGGAAGGCGGCTTAG	246
FA157/158	GCAATAAAGGCTCGGTGTCA	TTGTCTTTTACCACAGCCG	171
FA159/160	TGCAATAAAGGCTCGGTGTC	TTGTCTTTTACCACAGCCG	172

## APPENDIX B

B1: Map showing *Striga gesnerioides* hot spots in Ghana



B2: Flower colour variation of *Striga gesnerioides* in Ghana



B3: *Striga gesnerioides* on *Tephrosia pedicellata*



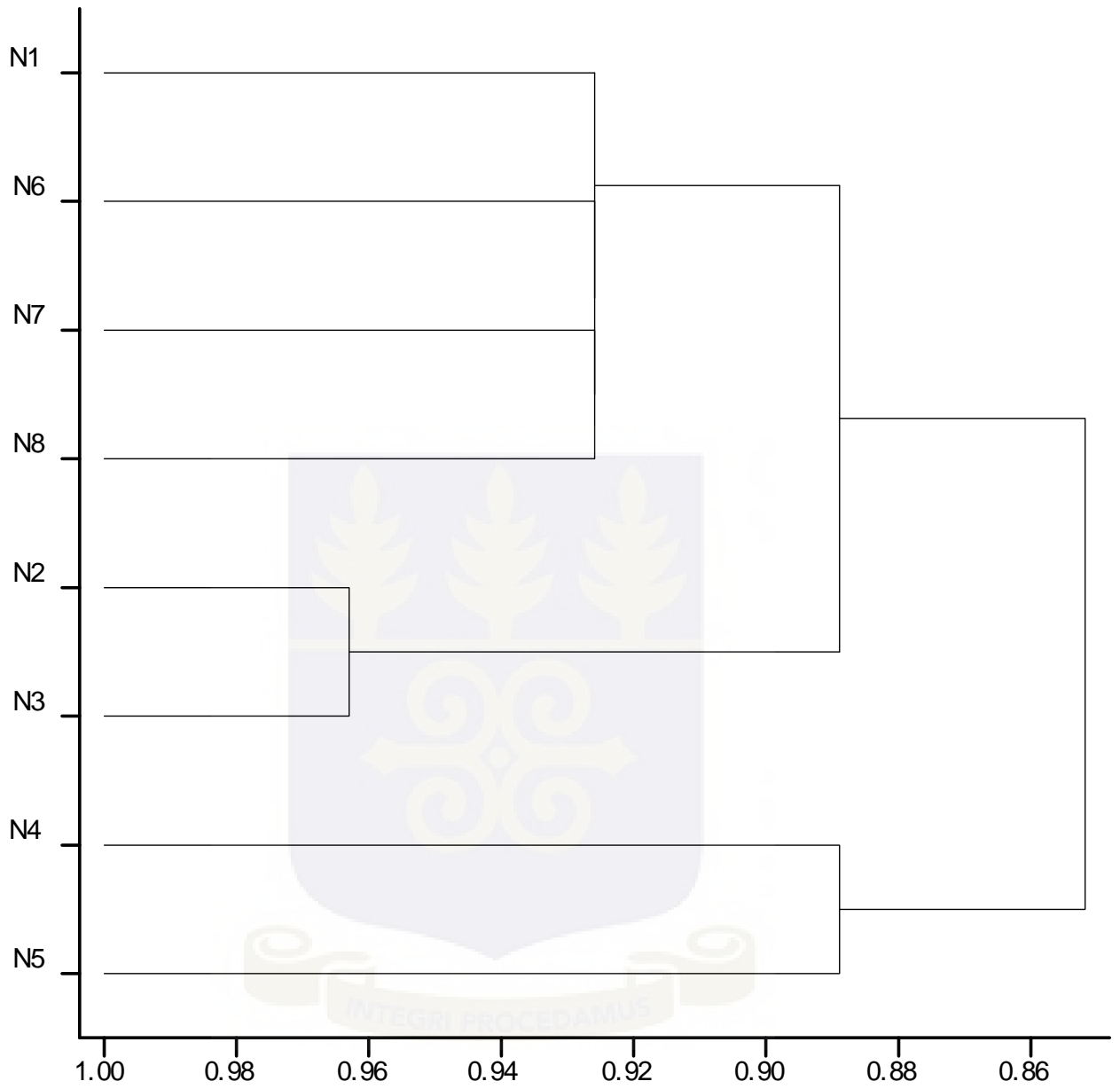
B4: *Striga gesnerioides* on *Ipomoea tribola*



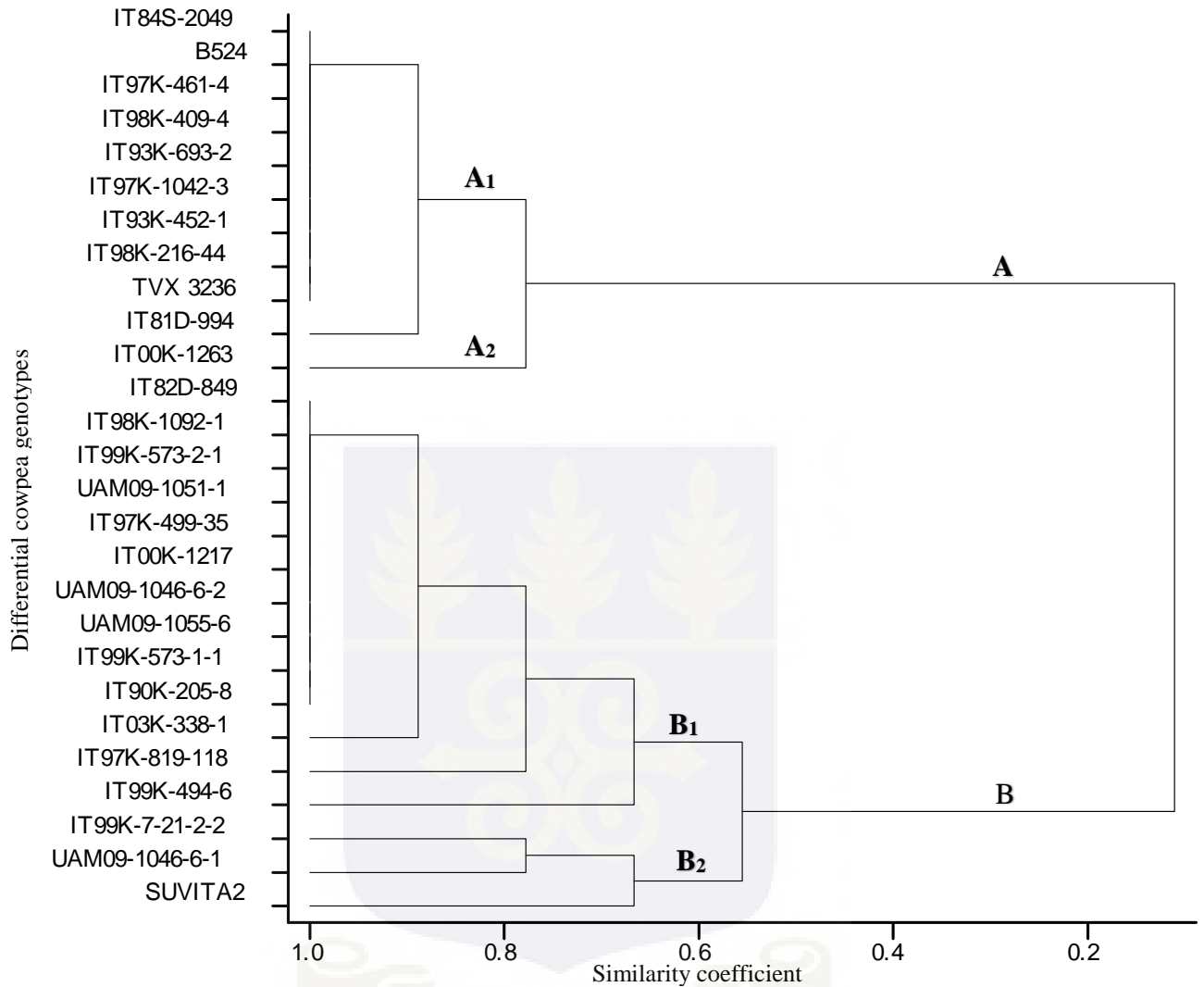
B5: Response of selected cowpea genotypes on different population of *Striga* in Ghana.

ENTRY	Strains of <i>Striga gesnerioides</i>								
	N1	N2	N3	N4	N5	N6	N7	N8	N9
IT84S-2049	1	1	1	1	1	1	1	1	0
B524	1	1	1	1	1	1	1	1	0
IT97K-461-4	1	1	1	1	1	1	1	1	0
IT98K-409-4	1	1	1	1	1	1	1	1	0
IT93K-693-2	1	1	1	1	1	1	1	1	0
IT82D-849	0	0	0	0	0	0	0	0	0
IT99K-494-6	0	0	0	1	0	0	0	1	0
IT98K-1092-1	0	0	0	0	0	0	0	0	0
IT00K-1263	1	1	1	0	1	1	1	1	0
IT97K-819-118	0	0	0	0	0	1	0	0	0
IT99K-7-21-2-2	0	1	0	1	1	0	0	0	0
IT99K-573-2-1	0	0	0	0	0	0	0	0	0
SUVITA2	0	1	1	0	0	0	0	0	0
UAM09-1051-1	0	0	0	0	0	0	0	0	0
IT81D-994	1	1	0	1	1	0	1	1	0
IT97K-1042-3	1	1	1	1	1	1	1	1	0
IT93K-452-1	1	1	1	1	1	1	1	1	0
UAM09-1046-6-1	0	0	0	0	1	0	0	0	0
IT97K-499-35	0	0	0	0	0	0	0	0	0
IT00K-1217	0	0	0	0	0	0	0	0	0
UAM09-1046-6-2	0	0	0	0	0	0	0	0	0
IT98K-216-44	1	1	1	1	1	1	1	1	0
IT03K-338-1	1	0	0	0	0	0	0	0	0
UAM09-1055-6	0	0	0	0	0	0	0	0	0
IT99K-573-1-1	0	0	0	0	0	0	0	0	0
IT90K-205-8	0	0	0	0	0	0	0	0	0
TVX 3236	1	1	1	1	1	1	1	1	0

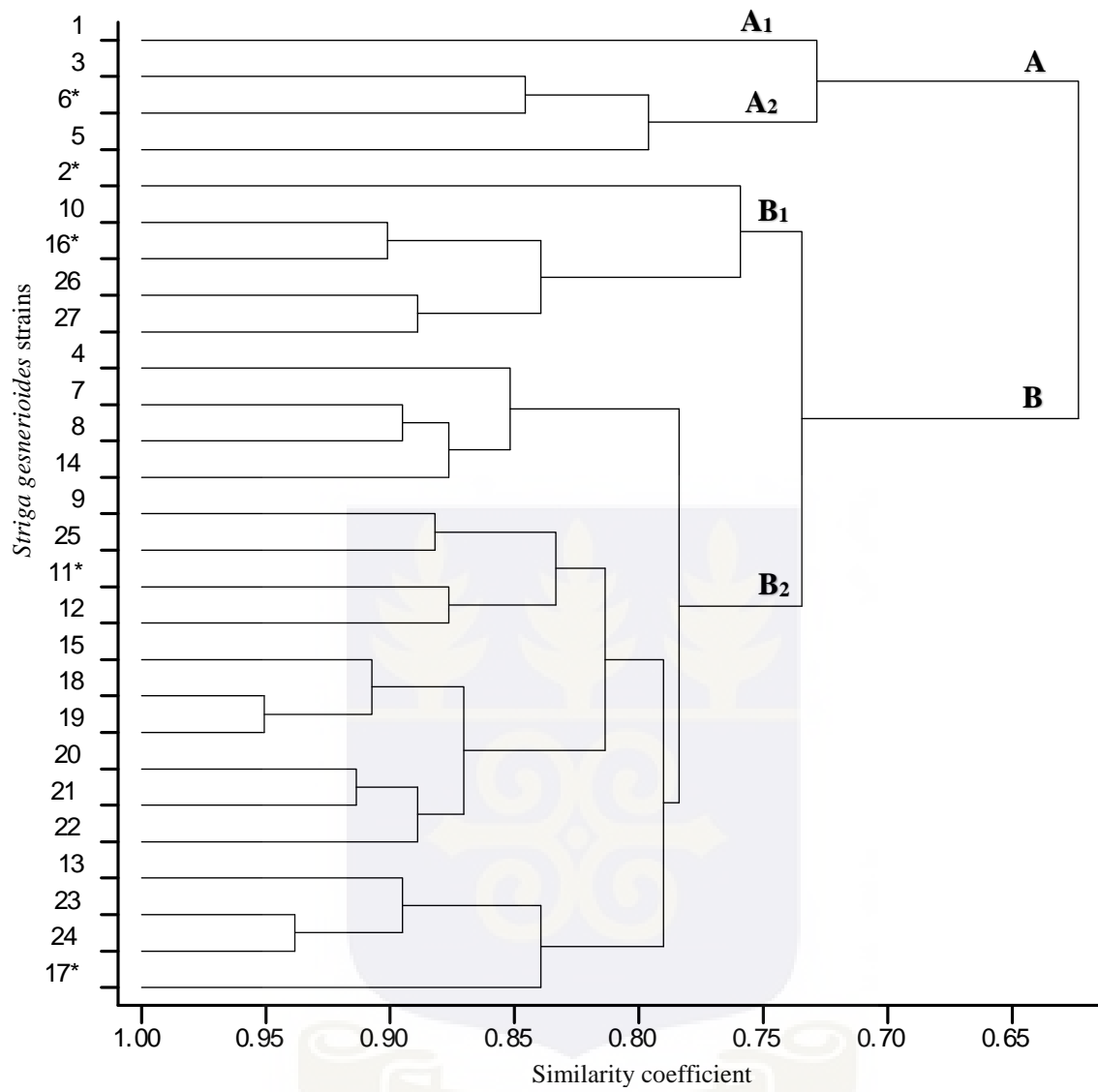
B6: Cluster analysis of *Striga* population based on virulence



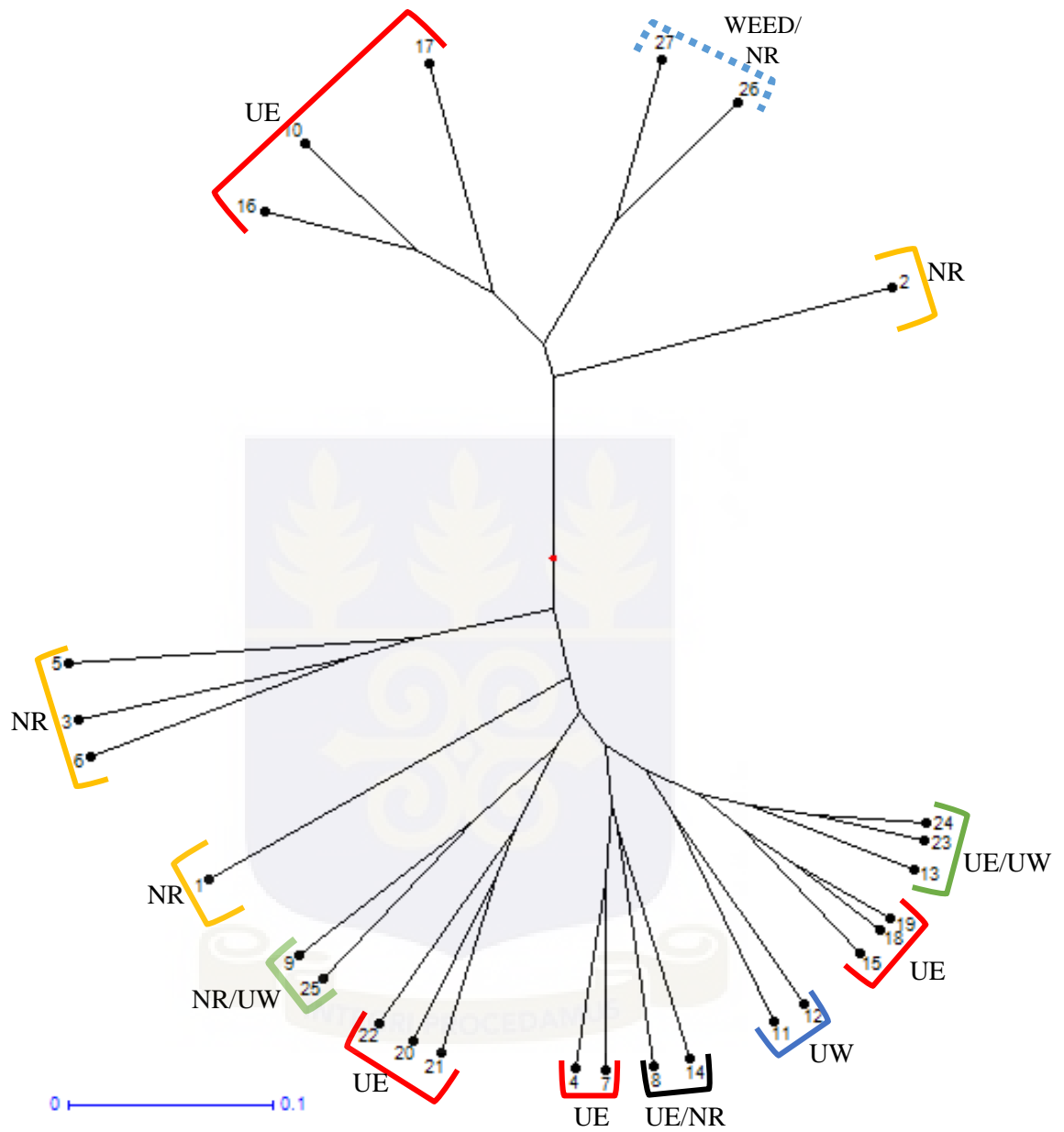
B7: Custer analysis of cowpea genotypes based on their response



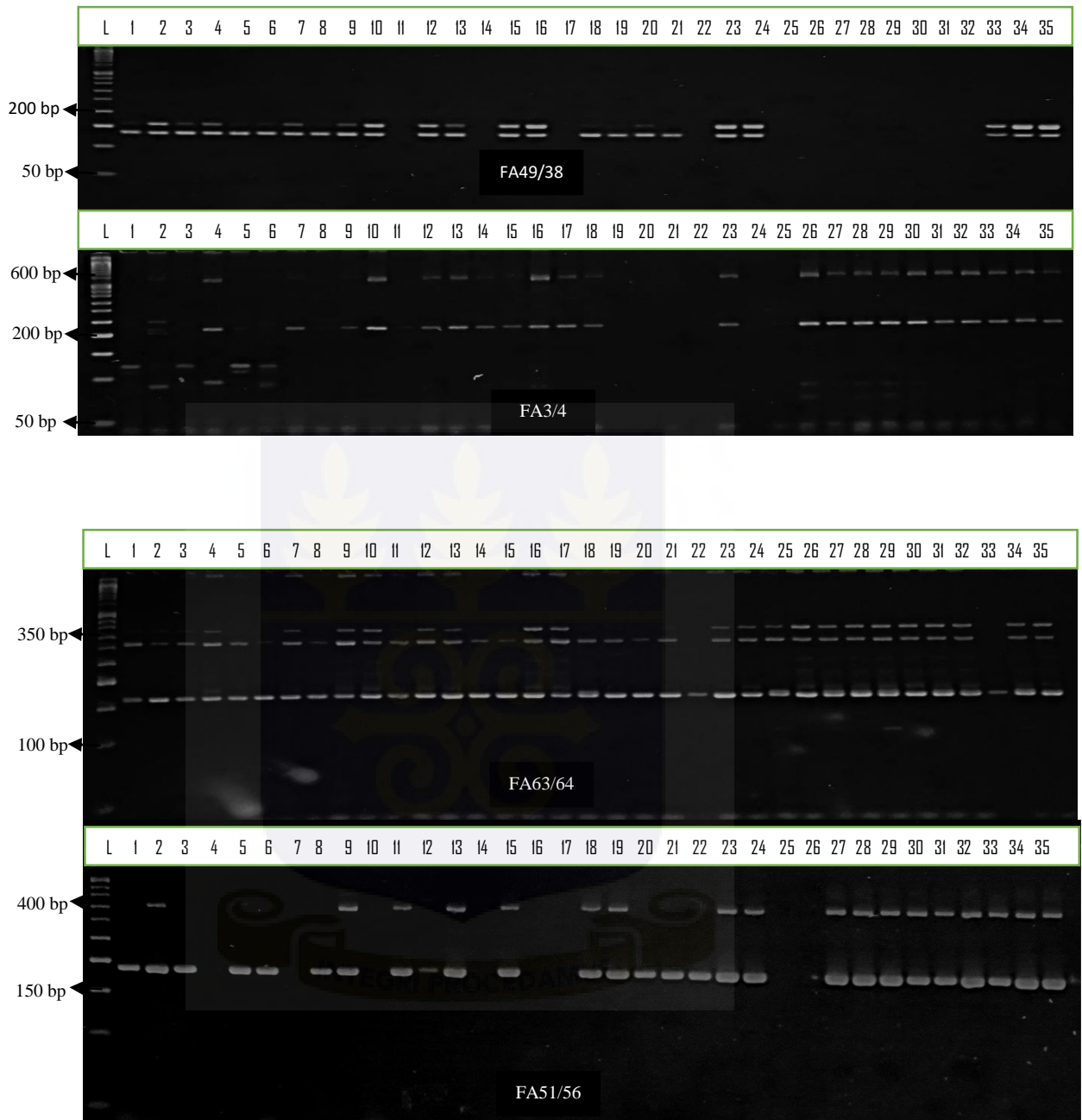
B8: Molecular Cluster analysis of *Striga gesnerioides*, dendrogram



B9: Molecular cluster analysis of *Striga gesnerioides*, tree diagram



B9: Banding pattern of some polymorphic markers used.



B12: Genetic differentiation parameters of *Striga gesnerioides* populations

<b>Marker</b>	<b>Major Allele Frequency</b>	<b>Allele Number</b>	<b>Gene Diversity</b>	<b>Heterozygosity</b>	<b>PIC</b>
<b>FA1/2</b>	0.67	4.00	0.50	0.00	0.45
<b>FA3/4</b>	0.26	10.00	0.82	0.56	0.79
<b>FA7/8</b>	0.56	5.00	0.59	0.30	0.53
<b>FA3/10</b>	0.72	5.00	0.45	0.11	0.42
<b>FA5/2</b>	0.67	3.00	0.48	0.07	0.42
<b>FA13/12</b>	0.61	8.00	0.58	0.22	0.55
<b>FA15/16</b>	0.39	6.00	0.70	0.85	0.64
<b>FA13/18</b>	0.76	4.00	0.40	0.26	0.38
<b>FA11/12</b>	0.54	8.00	0.66	0.11	0.63
<b>FA11/18</b>	0.61	6.00	0.58	0.04	0.54
<b>FA21/22</b>	0.93	3.00	0.14	0.00	0.14
<b>FA21/24</b>	0.81	2.00	0.30	0.00	0.26
<b>FA27/22</b>	0.63	4.00	0.55	0.37	0.51
<b>FA31/32</b>	0.83	7.00	0.30	0.19	0.29
<b>FA31/34</b>	0.91	3.00	0.17	0.11	0.16
<b>FA47/38</b>	0.85	4.00	0.27	0.00	0.25
<b>FA35/36</b>	0.83	4.00	0.29	0.11	0.27
<b>FA37/38</b>	0.35	10.00	0.78	0.07	0.75
<b>FA39/40</b>	0.35	6.00	0.75	0.11	0.71
<b>FA49/48</b>	0.46	3.00	0.63	0.70	0.55
<b>FA51/52</b>	0.76	5.00	0.40	0.11	0.37
<b>FA51/56</b>	0.30	5.00	0.75	0.33	0.70
<b>FA57/54</b>	1.00	1.00	0.00	0.00	0.00
<b>FA59/52</b>	0.85	3.00	0.26	0.00	0.25
<b>FA51/62</b>	0.48	4.00	0.55	0.96	0.45
<b>FA63/64</b>	0.52	4.00	0.62	0.93	0.56
<b>FA65/62</b>	0.50	4.00	0.55	1.00	0.45
<b>FA67/62</b>	0.44	4.00	0.62	0.89	0.55
<b>FA81/76</b>	0.52	3.00	0.60	0.00	0.52

<b>FA79/74</b>	0.74	2.00	0.38	0.00	0.31
<b>FA91/92</b>	0.67	3.00	0.48	0.15	0.42
<b>FA83/76</b>	0.63	3.00	0.53	0.00	0.47
<b>FA101/102</b>	0.59	3.00	0.51	0.00	0.41
<b>FA93/94</b>	0.63	5.00	0.53	0.04	0.47
<b>FA105/116</b>	0.94	2.00	0.10	0.11	0.10
<b>FA101/104</b>	0.56	2.00	0.49	0.00	0.37
<b>FA107/106</b>	0.96	2.00	0.07	0.00	0.07
<b>FA131/132</b>	0.94	3.00	0.11	0.04	0.10
<b>FA121/122</b>	0.91	3.00	0.17	0.04	0.16
<b>FA125/126</b>	0.48	3.00	0.58	0.04	0.49
<b>FA127/128</b>	0.87	3.00	0.23	0.26	0.21
<b>FA129/128</b>	0.89	3.00	0.20	0.00	0.19
<b>FA131/138</b>	0.74	3.00	0.40	0.44	0.34
<b>FA123/122</b>	0.28	6.00	0.78	0.52	0.75
<b>FA139/140</b>	0.96	2.00	0.07	0.00	0.07
<b>FA133/134</b>	0.80	3.00	0.33	0.04	0.29
<b>FA141/142</b>	0.78	2.00	0.35	0.44	0.29
<b>FA149/142</b>	0.93	2.00	0.14	0.15	0.13
<b>FA155/156</b>	0.69	2.00	0.43	0.63	0.34
<b>FA157/158</b>	0.91	2.00	0.17	0.19	0.15
<b>FA159/158</b>	0.69	2.00	0.43	0.63	0.34
<b>FA151/154</b>	0.39	3.00	0.66	0.56	0.59
<b>FA97/92</b>	0.59	2.00	0.48	0.00	0.37
<b>FA143/148</b>	0.85	2.00	0.25	0.00	0.22
<b>Mean</b>	<b>0.68</b>	<b>3.81</b>	<b>0.43</b>	<b>0.23</b>	<b>0.38</b>

**B13. Randomisation and layout of screen house experiment**

RANDOMIZATION AND LAYOUT

=====

EXPERIMENTAL DESIGN = SPLIT-PLOT

REPLICATIONS = 3

TREATMENTS = 29 x 9

\*\*\*\* MAINPLOT \*\*\*\*

VARIETY (V) = 29 levels

VARIETY (1) = V1

VARIETY (2) = V2

VARIETY (3) = V3

VARIETY (4) = V4

VARIETY (5) = V5

VARIETY (6) = V6

VARIETY (7) = V7

VARIETY (8) = V8

VARIETY (9) = V9

VARIETY (10) = V10

VARIETY (11) = V11

VARIETY (12) = V12

VARIETY (13) = V13

VARIETY (14) = V14

VARIETY (15) = V15

VARIETY (16) = V16



VARIETY (17) = V17

VARIETY (18) = V18

VARIETY (19) = V19

VARIETY (20) = V20

VARIETY (21) = V21

VARIETY (22) = V22

VARIETY (23) = V23

VARIETY (24) = V24

VARIETY (25) = V25

VARIETY (26) = V26

VARIETY (27) = V27

VARIETY (28) = V28

VARIETY (29) = V29

\*\*\*\* SUBPLOT \*\*\*\*

STRAIN (ST) = 9 levels

STRAIN (1) = ST1

STRAIN (2) = ST2

STRAIN (3) = ST3

STRAIN (4) = ST4

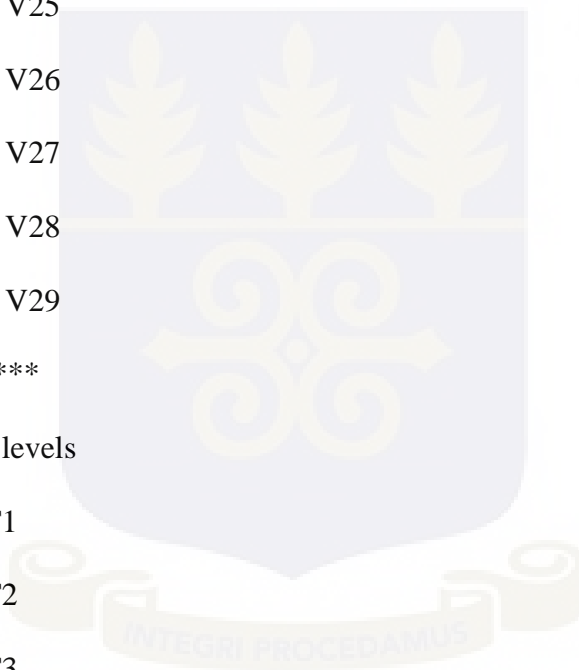
STRAIN (5) = ST5

STRAIN (6) = ST6

STRAIN (7) = ST7

STRAIN (8) = ST8

STRAIN (9) = ST9



=====

Experimental layout: (SPLIT-PLOT)

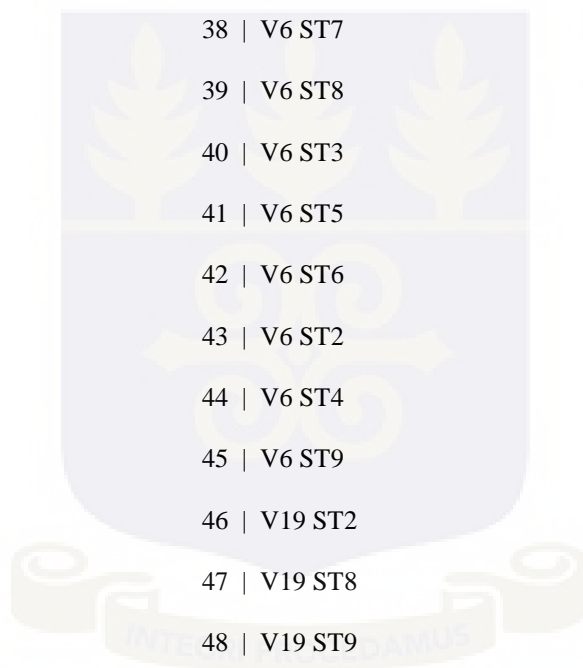
The following field layout applies to all replications:

(Note: layout is not drawn to scale)

1	2	3	4	5	6	7	8	9	10	11	12	13
14	15	16	17	18	19	20	21	22	23	24	25	26
27	28	29	30	31	32	33	34	35	36	37	38	39
40	41	42	43	44	45	46	47	48	49	50	51	52
53	54	55	56	57	58	59	60	61	62	63	64	65
66	67	68	69	70	71	72	73	74	75	76	77	78
79	80	81	82	83	84	85	86	87	88	89	90	91
92	93	94	95	96	97	98	99	100	101	102	103	104
105	106	107	108	109	110	111	112	113	114	115	116	117
118	119	120	121	122	123	124	125	126	127	128	129	130
131	132	133	134	135	136	137	138	139	140	141	142	143
144	145	146	147	148	149	150	151	152	153	154	155	156
157	158	159	160	161	162	163	164	165	166	167	168	169
170	171	172	173	174	175	176	177	178	179	180	181	182
183	184	185	186	187	188	189	190	191	192	193	194	195
196	197	198	199	200	201	202	203	204	205	206	207	208
209	210	211	212	213	214	215	216	217	218	219	220	221
222	223	224	225	226	227	228	229	230	231	232	233	234
235	236	237	238	239	240	241	242	243	244	245	246	247
248	249	250	251	252	253	254	255	256	257	258	25	

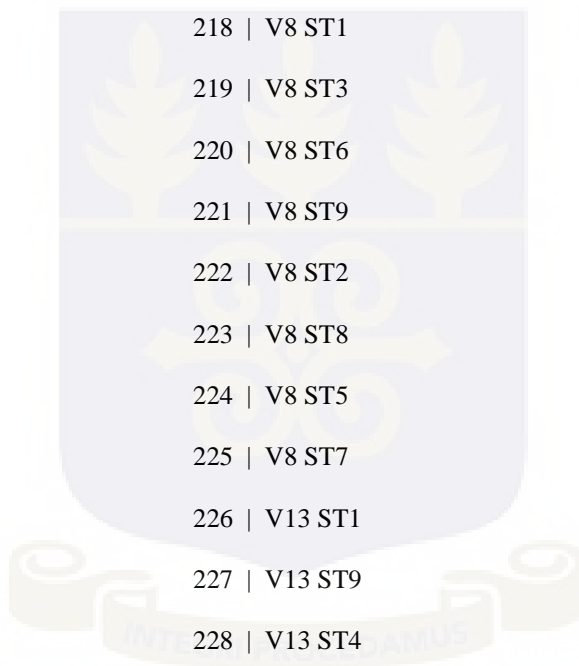


REPLICATION NO. 1	28   V15 ST7	58   V3 ST2
-----	29   V15 ST6	59   V3 ST4
PLOT NO.   TREATMENT ID	30   V15 ST1	60   V3 ST9
1   V23 ST6	31   V15 ST5	61   V3 ST7
2   V23 ST3	32   V15 ST8	62   V3 ST3
3   V23 ST9	33   V15 ST4	63   V3 ST8
4   V23 ST1	34   V15 ST3	64   V27 ST1
5   V23 ST5	35   V15 ST2	65   V27 ST7
6   V23 ST8	36   V15 ST9	66   V27 ST3
7   V23 ST4	37   V6 ST1	67   V27 ST9
8   V23 ST7	38   V6 ST7	68   V27 ST6
9   V23 ST2	39   V6 ST8	69   V27 ST8
10   V24 ST5	40   V6 ST3	70   V27 ST5
11   V24 ST3	41   V6 ST5	71   V27 ST2
12   V24 ST4	42   V6 ST6	72   V27 ST4
13   V24 ST8	43   V6 ST2	73   V12 ST9
14   V24 ST9	44   V6 ST4	74   V12 ST4
15   V24 ST6	45   V6 ST9	75   V12 ST6
16   V24 ST1	46   V19 ST2	76   V12 ST3
17   V24 ST2	47   V19 ST8	77   V12 ST5
18   V24 ST7	48   V19 ST9	78   V12 ST7
19   V17 ST1	49   V19 ST4	79   V12 ST1
20   V17 ST5	50   V19 ST5	80   V12 ST8
21   V17 ST6	51   V19 ST7	81   V12 ST2
22   V17 ST7	52   V19 ST6	82   V4 ST2
23   V17 ST3	53   V19 ST3	83   V4 ST7
24   V17 ST4	54   V19 ST1	84   V4 ST6
25   V17 ST8	55   V3 ST1	85   V4 ST5
26   V17 ST9	56   V3 ST6	86   V4 ST4
27   V17 ST2	57   V3 ST5	87   V4 ST9

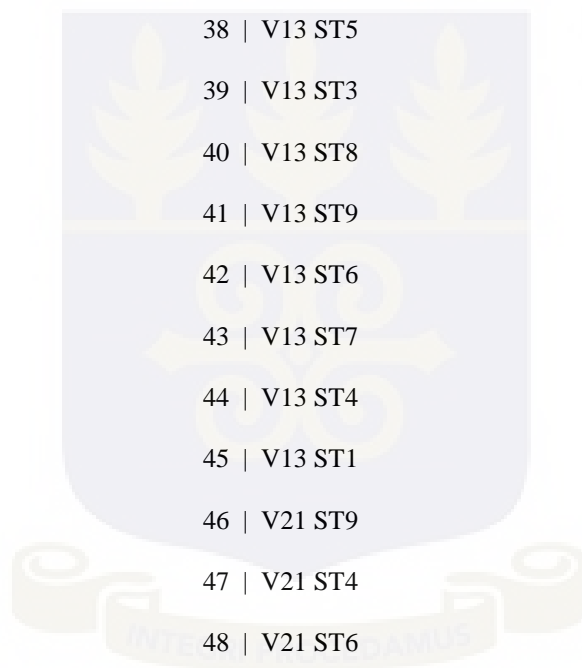


88   V4 ST3	118   V22 ST8	148   V10 ST8
89   V4 ST8	119   V22 ST9	149   V10 ST9
90   V4 ST1	120   V22 ST7	150   V10 ST3
91   V16 ST9	121   V22 ST6	151   V10 ST2
92   V16 ST7	122   V22 ST3	152   V10 ST6
93   V16 ST1	123   V22 ST4	153   V10 ST4
94   V16 ST2	124   V22 ST1	154   V18 ST9
95   V16 ST5	125   V22 ST5	155   V18 ST1
96   V16 ST8	126   V22 ST2	156   V18 ST4
97   V16 ST6	127   V7 ST5	157   V18 ST8
98   V16 ST3	128   V7 ST6	158   V18 ST6
99   V16 ST4	129   V7 ST2	159   V18 ST3
100   V20 ST9	130   V7 ST7	160   V18 ST7
101   V20 ST7	131   V7 ST4	161   V18 ST2
102   V20 ST8	132   V7 ST9	162   V18 ST5
103   V20 ST5	133   V7 ST3	163   V29 ST3
104   V20 ST1	134   V7 ST8	164   V29 ST2
105   V20 ST3	135   V7 ST1	165   V29 ST6
106   V20 ST4	136   V28 ST6	166   V29 ST4
107   V20 ST6	137   V28 ST7	167   V29 ST9
108   V20 ST2	138   V28 ST1	168   V29 ST7
109   V21 ST4	139   V28 ST2	169   V29 ST1
110   V21 ST6	140   V28 ST3	170   V29 ST8
111   V21 ST7	141   V28 ST4	171   V29 ST5
112   V21 ST9	142   V28 ST5	172   V25 ST2
113   V21 ST2	143   V28 ST8	173   V25 ST6
114   V21 ST1	144   V28 ST9	174   V25 ST7
115   V21 ST5	145   V10 ST7	175   V25 ST3
116   V21 ST8	146   V10 ST1	176   V25 ST4
117   V21 ST3	147   V10 ST5	177   V25 ST5

178   V25 ST8	208   V5 ST8	238   V26 ST7
179   V25 ST9	209   V5 ST3	239   V26 ST1
180   V25 ST1	210   V5 ST4	240   V26 ST4
181   V1 ST2	211   V5 ST9	241   V26 ST3
182   V1 ST5	212   V5 ST1	242   V26 ST6
183   V1 ST7	213   V5 ST7	243   V26 ST9
184   V1 ST4	214   V5 ST6	244   V14 ST8
185   V1 ST1	215   V5 ST2	245   V14 ST1
186   V1 ST3	216   V5 ST5	246   V14 ST6
187   V1 ST8	217   V8 ST4	247   V14 ST2
188   V1 ST6	218   V8 ST1	248   V14 ST3
189   V1 ST9	219   V8 ST3	249   V14 ST4
190   V2 ST5	220   V8 ST6	250   V14 ST7
191   V2 ST2	221   V8 ST9	251   V14 ST5
192   V2 ST8	222   V8 ST2	252   V14 ST9
193   V2 ST7	223   V8 ST8	253   V11 ST4
194   V2 ST9	224   V8 ST5	254   V11 ST3
195   V2 ST4	225   V8 ST7	255   V11 ST1
196   V2 ST3	226   V13 ST1	256   V11 ST6
197   V2 ST1	227   V13 ST9	257   V11 ST9
198   V2 ST6	228   V13 ST4	258   V11 ST2
199   V9 ST9	229   V13 ST6	259   V11 ST8
200   V9 ST7	230   V13 ST7	260   V11 ST5
201   V9 ST2	231   V13 ST8	261   V11 ST7
202   V9 ST3	232   V13 ST3	
203   V9 ST8	233   V13 ST2	
204   V9 ST5	234   V13 ST5	
205   V9 ST6	235   V26 ST5	
206   V9 ST1	236   V26 ST2	
207   V9 ST4	237   V26 ST8	

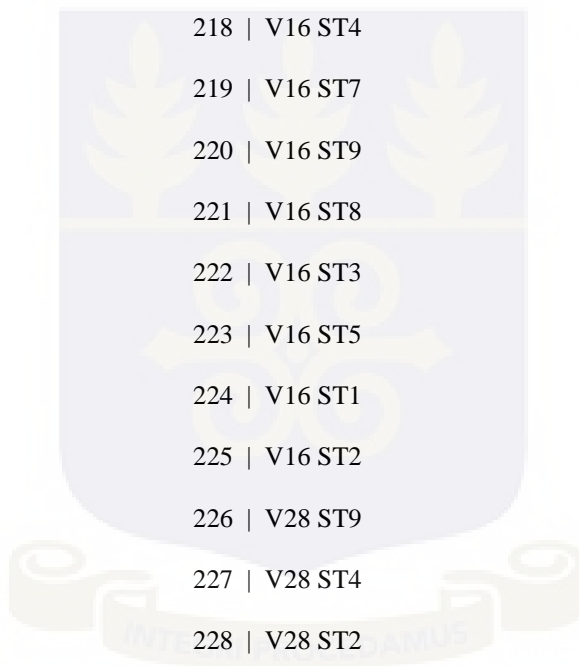


REPLICATION NO. 2	28   V6 ST4	58   V23 ST1
-----	29   V6 ST7	59   V23 ST7
PLOT NO.   TREATMENT ID	30   V6 ST1	60   V23 ST4
1   V25 ST6	31   V6 ST5	61   V23 ST8
2   V25 ST8	32   V6 ST8	62   V23 ST2
3   V25 ST7	33   V6 ST3	63   V23 ST5
4   V25 ST3	34   V6 ST2	64   V7 ST2
5   V25 ST9	35   V6 ST9	65   V7 ST6
6   V25 ST4	36   V6 ST6	66   V7 ST5
7   V25 ST5	37   V13 ST2	67   V7 ST3
8   V25 ST1	38   V13 ST5	68   V7 ST1
9   V25 ST2	39   V13 ST3	69   V7 ST7
10   V19 ST1	40   V13 ST8	70   V7 ST8
11   V19 ST4	41   V13 ST9	71   V7 ST4
12   V19 ST8	42   V13 ST6	72   V7 ST9
13   V19 ST2	43   V13 ST7	73   V12 ST7
14   V19 ST7	44   V13 ST4	74   V12 ST4
15   V19 ST6	45   V13 ST1	75   V12 ST1
16   V19 ST9	46   V21 ST9	76   V12 ST8
17   V19 ST5	47   V21 ST4	77   V12 ST5
18   V19 ST3	48   V21 ST6	78   V12 ST2
19   V5 ST7	49   V21 ST1	79   V12 ST3
20   V5 ST8	50   V21 ST3	80   V12 ST6
21   V5 ST6	51   V21 ST2	81   V12 ST9
22   V5 ST3	52   V21 ST5	82   V24 ST8
23   V5 ST1	53   V21 ST7	83   V24 ST3
24   V5 ST9	54   V21 ST8	84   V24 ST5
25   V5 ST4	55   V23 ST6	85   V24 ST6
26   V5 ST5	56   V23 ST3	86   V24 ST9
27   V5 ST2	57   V23 ST9	87   V24 ST1



88   V24 ST4	118   V20 ST3	148   V26 ST2
89   V24 ST7	119   V20 ST6	149   V26 ST7
90   V24 ST2	120   V20 ST5	150   V26 ST8
91   V3 ST3	121   V20 ST2	151   V26 ST3
92   V3 ST4	122   V20 ST1	152   V26 ST9
93   V3 ST8	123   V20 ST4	153   V26 ST1
94   V3 ST2	124   V20 ST7	154   V22 ST3
95   V3 ST5	125   V20 ST9	155   V22 ST7
96   V3 ST7	126   V20 ST8	156   V22 ST4
97   V3 ST6	127   V8 ST6	157   V22 ST2
98   V3 ST9	128   V8 ST1	158   V22 ST6
99   V3 ST1	129   V8 ST8	159   V22 ST9
100   V11 ST8	130   V8 ST3	160   V22 ST1
101   V11 ST4	131   V8 ST7	161   V22 ST8
102   V11 ST1	132   V8 ST2	162   V22 ST5
103   V11 ST9	133   V8 ST9	163   V10 ST3
104   V11 ST7	134   V8 ST5	164   V10 ST6
105   V11 ST3	135   V8 ST4	165   V10 ST8
106   V11 ST2	136   V14 ST2	166   V10 ST2
107   V11 ST5	137   V14 ST7	167   V10 ST7
108   V11 ST6	138   V14 ST3	168   V10 ST5
109   V18 ST2	139   V14 ST1	169   V10 ST9
110   V18 ST1	140   V14 ST4	170   V10 ST1
111   V18 ST4	141   V14 ST8	171   V10 ST4
112   V18 ST7	142   V14 ST5	172   V1 ST4
113   V18 ST6	143   V14 ST6	173   V1 ST1
114   V18 ST5	144   V14 ST9	174   V1 ST8
115   V18 ST8	145   V26 ST4	175   V1 ST7
116   V18 ST3	146   V26 ST6	176   V1 ST9
117   V18 ST9	147   V26 ST5	177   V1 ST6

178   V1 ST5	208   V2 ST1	238   V27 ST6
179   V1 ST3	209   V2 ST4	239   V27 ST4
180   V1 ST2	210   V2 ST5	240   V27 ST1
181   V17 ST5	211   V2 ST9	241   V27 ST2
182   V17 ST4	212   V2 ST2	242   V27 ST7
183   V17 ST9	213   V2 ST7	243   V27 ST9
184   V17 ST6	214   V2 ST8	244   V29 ST6
185   V17 ST3	215   V2 ST3	245   V29 ST1
186   V17 ST1	216   V2 ST6	246   V29 ST3
187   V17 ST8	217   V16 ST6	247   V29 ST7
188   V17 ST2	218   V16 ST4	248   V29 ST4
189   V17 ST7	219   V16 ST7	249   V29 ST2
190   V15 ST5	220   V16 ST9	250   V29 ST5
191   V15 ST3	221   V16 ST8	251   V29 ST9
192   V15 ST1	222   V16 ST3	252   V29 ST8
193   V15 ST2	223   V16 ST5	253   V9 ST4
194   V15 ST6	224   V16 ST1	254   V9 ST8
195   V15 ST8	225   V16 ST2	255   V9 ST1
196   V15 ST7	226   V28 ST9	256   V9 ST9
197   V15 ST9	227   V28 ST4	257   V9 ST7
198   V15 ST4	228   V28 ST2	258   V9 ST3
199   V4 ST4	229   V28 ST7	259   V9 ST2
200   V4 ST3	230   V28 ST5	260   V9 ST5
201   V4 ST2	231   V28 ST8	261   V9 ST6
202   V4 ST5	232   V28 ST1	
203   V4 ST6	233   V28 ST3	
204   V4 ST8	234   V28 ST6	
205   V4 ST9	235   V27 ST3	
206   V4 ST7	236   V27 ST5	
207   V4 ST1	237   V27 ST8	



REPLICATION NO. 3	28   V18 ST2	58   V9 ST2
-----	29   V18 ST4	59   V9 ST9
PLOT NO.   TREATMENT ID	30   V18 ST1	60   V9 ST4
1   V26 ST8	31   V18 ST5	61   V9 ST1
2   V26 ST6	32   V18 ST3	62   V9 ST5
3   V26 ST9	33   V18 ST9	63   V9 ST6
4   V26 ST2	34   V18 ST6	64   V15 ST4
5   V26 ST1	35   V18 ST8	65   V15 ST2
6   V26 ST7	36   V18 ST7	66   V15 ST1
7   V26 ST4	37   V10 ST3	67   V15 ST3
8   V26 ST3	38   V10 ST4	68   V15 ST5
9   V26 ST5	39   V10 ST8	69   V15 ST9
10   V22 ST8	40   V10 ST2	70   V15 ST7
11   V22 ST2	41   V10 ST1	71   V15 ST6
12   V22 ST3	42   V10 ST7	72   V15 ST8
13   V22 ST7	43   V10 ST6	73   V25 ST5
14   V22 ST9	44   V10 ST9	74   V25 ST3
15   V22 ST1	45   V10 ST5	75   V25 ST9
16   V22 ST4	46   V7 ST8	76   V25 ST1
17   V22 ST5	47   V7 ST4	77   V25 ST6
18   V22 ST6	48   V7 ST7	78   V25 ST7
19   V11 ST5	49   V7 ST1	79   V25 ST8
20   V11 ST2	50   V7 ST6	80   V25 ST2
21   V11 ST6	51   V7 ST3	81   V25 ST4
22   V11 ST7	52   V7 ST5	82   V28 ST5
23   V11 ST3	53   V7 ST9	83   V28 ST1
24   V11 ST4	54   V7 ST2	84   V28 ST7
25   V11 ST8	55   V9 ST3	85   V28 ST8
26   V11 ST1	56   V9 ST7	86   V28 ST3
27   V11 ST9	57   V9 ST8	87   V28 ST2



88   V28 ST9	118   V1 ST2	148   V16 ST3
89   V28 ST4	119   V1 ST4	149   V16 ST1
90   V28 ST6	120   V1 ST9	150   V16 ST2
91   V29 ST5	121   V1 ST1	151   V16 ST9
92   V29 ST1	122   V1 ST7	152   V16 ST7
93   V29 ST3	123   V1 ST6	153   V16 ST6
94   V29 ST4	124   V1 ST8	154   V6 ST4
95   V29 ST7	125   V1 ST3	155   V6 ST9
96   V29 ST8	126   V1 ST5	156   V6 ST8
97   V29 ST6	127   V13 ST9	157   V6 ST6
98   V29 ST2	128   V13 ST3	158   V6 ST1
99   V29 ST9	129   V13 ST1	159   V6 ST7
100   V14 ST8	130   V13 ST8	160   V6 ST2
101   V14 ST6	131   V13 ST7	161   V6 ST3
102   V14 ST5	132   V13 ST5	162   V6 ST5
103   V14 ST1	133   V13 ST6	163   V4 ST6
104   V14 ST7	134   V13 ST2	164   V4 ST5
105   V14 ST4	135   V13 ST4	165   V4 ST4
106   V14 ST9	136   V19 ST3	166   V4 ST2
107   V14 ST2	137   V19 ST2	167   V4 ST9
108   V14 ST3	138   V19 ST1	168   V4 ST7
109   V17 ST6	139   V19 ST9	169   V4 ST8
110   V17 ST8	140   V19 ST8	170   V4 ST3
111   V17 ST3	141   V19 ST7	171   V4 ST1
112   V17 ST4	142   V19 ST4	172   V21 ST9
113   V17 ST7	143   V19 ST5	173   V21 ST7
114   V17 ST5	144   V19 ST6	174   V21 ST5
115   V17 ST1	145   V16 ST5	175   V21 ST4
116   V17 ST9	146   V16 ST4	176   V21 ST6
117   V17 ST2	147   V16 ST8	177   V21 ST2



178   V21 ST3	208   V5 ST5	238   V12 ST8
179   V21 ST8	209   V5 ST2	239   V12 ST1
180   V21 ST1	210   V5 ST8	240   V12 ST7
181   V23 ST7	211   V5 ST3	241   V12 ST4
182   V23 ST8	212   V5 ST1	242   V12 ST9
183   V23 ST6	213   V5 ST9	243   V12 ST5
184   V23 ST2	214   V5 ST6	244   V20 ST9
185   V23 ST1	215   V5 ST4	245   V20 ST8
186   V23 ST3	216   V5 ST7	246   V20 ST5
187   V23 ST9	217   V2 ST2	247   V20 ST4
188   V23 ST5	218   V2 ST4	248   V20 ST7
189   V23 ST4	219   V2 ST5	249   V20 ST1
190   V24 ST6	220   V2 ST6	250   V20 ST3
191   V24 ST7	221   V2 ST8	251   V20 ST2
192   V24 ST3	222   V2 ST3	252   V20 ST6
193   V24 ST4	223   V2 ST1	253   V3 ST3
194   V24 ST2	224   V2 ST7	254   V3 ST4
195   V24 ST1	225   V2 ST9	255   V3 ST6
196   V24 ST5	226   V8 ST2	256   V3 ST7
197   V24 ST9	227   V8 ST9	257   V3 ST2
198   V24 ST8	228   V8 ST4	258   V3 ST9
199   V27 ST5	229   V8 ST7	259   V3 ST5
200   V27 ST8	230   V8 ST6	260   V3 ST1
201   V27 ST7	231   V8 ST5	261   V3 ST
202   V27 ST6	232   V8 ST1	
203   V27 ST2	233   V8 ST8	
204   V27 ST9	234   V8 ST3	
205   V27 ST4	235   V12 ST2	
206   V27 ST1	236   V12 ST3	
207   V27 ST3	237   V12 ST6	

