UNIVERSITY FOR DEVELOPMENT STUDIES



# PHENOTYPIC AND MOLECULAR SCREENING OF THE MAGIC POPULATION FOR SOURCES OF RESISTANCE TO *Striga gesnerioides*

**GULBI NELSON**

**2019**

**UNIVERSITY FOR DEVELOPMENT STUDIES**



# PHENOTYPIC AND MOLECULAR SCREENING OF THE MAGIC POPULATION FOR SOURCES OF RESISTANCE TO *Striga gesnerioides*

**BY**

**GULBI NELSON (BSc. Agricultural Technology)**

**(UDS/MBT/0026/15)**

**THESIS SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN BIOTECHNOLOGY**

**MARCH, 2019**

## DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in the university or elsewhere. Works that were consulted have been duly acknowledged by way of references.

Candidate's signature: …….. Date: ……

Name: **GULBI NELSON**

Supervisor's Declaration

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

Supervisor's signature: ……. Date: ……

Name: **Dr. Nelson Opoku**

Co-supervisor's signature:……. Date: ……

Name: **Dr. Francis Kusi**


## ABSTRACT

Infestation caused by *Striga gesnerioides* (Willd) is a major constraint to cowpea production in the dry savanna of Northern Ghana. Among the approaches used to control the parasite, host plant resistance appears to be more efficient and economical. The objective of this study was to identify sources of resistance to *Striga gesnerioides* in three hundred RILs of the MAGIC population and their eight founder parents using conventional and molecular breeding methods. The MAGIC population were obtained from the University of California, Riverside (UCR), USA. The study also determined the mode of inheritance of MAGIC lines that were found to be resistant. The study involved field evaluation of the MAGIC population to assess their agronomic performance under rain-fed conditions and also in pots to evaluate their resistance to *Striga gesnerioides* collected from Manga in Upper East Region of Ghana. Four (4) out of the three hundred RILs of the MAGIC population and their eight founder parents screened were found to be resistant to *Striga gesnerioides*. The result showed that flowering, pods per plant and grain yield of the susceptible RILs were reduced as compared to the resistant ones. Among the three markers used (SSR1, 61RM2 and C42-2B), C42-2B had association with the trait of interest and therefore was able to distinguish resistant from susceptible lines. The SSR1 marker which is known to be a functional marker for *Striga* resistance could only amplify the resistant checks and not Suvita-2 and its resistant progenies whilst 61RM2 could not distinguish between resistant checks, resistant and susceptible MAGIC lines. The C42-2B marker had discriminating power to distinguish between resistant and susceptible. The marker amplified resistant lines at 490 bp instead of the 280 bp amplified for the resistant checks. In the $F_2$ generation, the C42-2B marker distinguished between the resistant and susceptible MAGIC lines at 490 bp. Out of 102 $F_2$ progenies of Magic 072 × Apagbaala, 51 individuals

were identified as resistant by the marker. Out of 69 $F_2$ progenies of Magic 72 × Magic 020, 42 individuals were identified as resistant by the marker. Out of 33 $F_2$ progenies from a cross of Suvita-2 × Magic 020, 14 individuals were identified as resistant by the marker. Segregation distortion were detected for the genotyped segregation populations deviating from the expected 3R:1S Mendelian ratio of the $F_2$ population. The three $F_2$ populations screened for mode of inheritance showed that single dominant gene control *Striga* resistance in the donor parents. In the $F_3$ generation, Chi-square test showed 1R:2H:1S expected genetic ratio which confirmed the 3R:1S genetic ratio at $F_2$ population which indicated that the gene that caused the resistance is controlled by a single dominant gene. The results of the study therefore revealed that selection for resistance is more effective at $F_3$ progeny testing since planting of families is done on a one progeny one *Striga* infected pot basis.



## ACKNOWLEDGEMENTS

I would like to give thanks to God Almighty for the grace and mercy granted to me to successfully complete this project.

I wish to express my sincere thanks and gratitude to my supervisors, Dr. Nelson Opoku and Dr. Francis Kusi for their time, constructive corrections, comments, suggestions and encouragement in guiding me throughout my project work.

My heartfelt gratitude goes to the University of California, Riverside (UCR), USA under the Feed the Future Innovation Lab for Climate Resilient Cowpea project at SARI headed by Dr. Francis Kusi for the sponsorship of this project.

Furthermore, I would like to thank all the lecturers of the Department of Biotechnology at the University for Development Studies for their suggestions, contributions and directions particularly during our course work.

I am grateful to the staff and students of the Savanna Agriculture Research Institute, Manga station in Bawku, especially, Patrick Ataamah, Gloria Kubi and Abeku Tetteh for the role played during the evaluation and contribution at all levels of the work.

Lastly my profound gratitude goes to all my course mates on campus especially Ballu Duwejuah who by one way or another contributed to my successful completion of my project, I say a big thank you and God richly bless you guys.

## DEDICATION

I dedicate this work to my father Dr. Justice R.A Ayam for countless encouragement and financial support and my loving mother Gulbi Alice.



# TABLE OF CONTENT

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF PLATES

## ACRONYMS

| | |
|---|---|
| AFLP | Amplified Fragment Length Polymorphism |
| BSA | Bovine Serum Albumin |
| DNA | Deoxyribonucleic acid dNTPs deoxy Nitro Triphosphate |
| $F_3$ | Third filial generation |
| $F_1$ | First filial generation |
| $F_2$ | Second filial generation |
| FAO | Food and Agricultural Organisation |
| FTA | Fast Technology Analysis |
| IITA | International Institute of Tropical Agriculture |
| MAGIC | Multi-Parent Advanced Generation Intercross |
| MoFA | Ministry of Food and Agriculture |
| PCR | Polymerase Chain Reaction |
| RFLP | Restriction Fragment Length Polymorphism |
| RILs | Recombinant Inbreed Lines |
| SARI | Savanna Agricultural Research Institute |
| SCAR | Sequences Characterised Amplified Region |
| SSR | Simple Sequence Repeat |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Cowpea is the most essential grain legume grown in Sub-Saharan Africa (Ehlers and Hall, 1997; Timko *et al.*, 2007a; Timko and Singh, 2008), and an important source of income for millions of people in West and Central Africa. It also serves as a source of minerals and protein (Badiane *et al.*, 2004; Singh *et al*., 2011; Mfeka, 2017). The fruits of cowpea at all stages of growth are consumed (for example green pods, fresh or dry seeds) and young leaves are often used for soups and stews (Quaye *et al*., 2009). In addition to its value as human food, cowpea hay is an essential source of animal fodder. Cowpea is generally tolerant to drought and it fixes nitrogen symbiotically, thereby augmenting soil fertility, especially when used in rotation with cereals (Sanginga *et al*., 2003).

Worldwide production of dried cowpeas is more than 5.4 million tons, with Africa producing nearly 5.2 million (CGIAR, 2018). Nigeria, the major producer and consumer, accounts for 61% of production in Africa and 58% worldwide (CGIAR, 2018). In Africa, it is estimated that 200 million children, women and men in West Africa consume the grain daily (CGIAR, 2018).

However, one of the major causes of food insecurity in the world is as a result of infestation caused by *Striga* which affects the production of maize (*Zea mays*), sorghum (*Sorghum bicolar*), millets (*Pennisetum typhoides*) and cowpea (*Vigna unquiculata*) (Lane and Bailey, 1992; Woomer *et al*., 2008).

*Striga*, generally known as witch weed, is a genus of parasitic plants that occur naturally in parts of Africa, Asia, and Australia (USDA, 2015). Before *Striga gesnerioides* emerges, a major irreversible damage is done (Ouédraogo *et al*., 2012). *Striga gesnerioides* causes

stunted growth, leaf necrosis, defoliation, chlorosis, senescence, no or little flowering, pods to be empty (Hibberd *et al*., 1996; Alonge 1999; Kamara *et al*., 2008), hence, reducing yield. Yield loss due to this parasite could be as high as 83% and total crop loss of susceptible cultivars have been reported (Muleba *et al*., 1997; Alonge *et al*., 2005; Parker, 2009).

*Striga* seeds are difficult to control due to long viability in the soil (Lane and Bailey, 1992; Joel *et al*. 1995). Improved cultural practices, use of chemical control and breeding using many sources of resistance are control strategies that have been developed (Berner *et al*., 1995; Boukar *et al*., 2004). However, host plant resistance appears to have the potential to vigorously and economically control the parasite since it is affordable to resource poor farmers (Singh *et al*., 1997, Omoigui *et al*., 2007, Timko *et al*., 2007b) as well as being environmentally friendly. The overall efficiency and effectiveness of cowpea improvement programs can be enhanced by the application of advanced selection and breeding tools that employ molecular markers linked to traits of interest (Hall, 2004; Hedge and Misra, 2009).

**1.2 Problem Statement**

Most cowpea plants are susceptible to *Striga* parasitism, however, some local landraces and wild accessions have been identified to have resistance to the parasite, and in most reports, resistance is a dominant characteristic, inherited in a monogenic manner (Aggarwal *et al*., 1984; Touré *et al*., 1997; Ouédraogo *et al*., 2001; Ouédraogo *et al*., 2002a; Singh, 2005; Timko *et al*., 2007a; Timko *et al*., 2007b). The identification of *Striga*-resistant germplasm is complicated due to the variable nature of the parasite with at least seven distinct races of *S. gesnerioides* (designated SG1 through SG6) now identified throughout West Africa (Lane *et al*., 1997a; Botanga and Timko, 2006). Analysis of several

advanced populations segregating for resistance to one or more of the different races of *S. gesnerioides* has resulted in the genetic mapping of several race-specific resistance (R) genes within the cowpea genome and the development of molecular markers linked to these genes (Li *et al*., 2009 and Timko *et al*., 2007a). Host plant resistance development to *S. gesnerioides* requires the application of phenotypic and genotypic protocols that are diagnostic to screen a population segregating for resistance to the parasite (Lane *et al*., 1994; Singh, 2002). The implication of this variation in resistance is that reliable screening protocols are required to identify more recombinant inbred lines for their resistance to the parasite. Therefore, the focus of the current study was to combine phenotypic and genotypic screening protocols to screen $F_8$ Multi parent advanced generation intercross (MAGIC) population of cowpea for sources of resistance to *Striga gesnerioides*.

### 1.3 Objective

The main objective of the study was to identify sources of resistance to *Striga gesnerioides* in three hundred RILs of the MAGIC population and their eight parents and specifically to:

#### 1.3.1 Specific Objectives

- Evaluate the 308 multi-parent advanced generation intercross (MAGIC) population for their agronomic performance.
- Evaluate the 308 multi-parent advanced generation intercross (MAGIC) population for their resistance to *Striga gesneriodes.*
- Determine the mode of inheritance of *Striga* resistance among the MAGIC lines that will be found to be resistant.

- To identify molecular markers associated with the source of the *Striga* resistant gene.



# CHAPTER TWO

# LITERATURE REVIEW

## 2.0 Origin, Diversity and Domestication of Cowpea

Cowpea since neolithic times is one of the most ancient human food sources and has probably been used as a crop plant (Summerfield *et al*., 1974; Coetzee, 1995; Agyeman *et al*., 2014). Cowpea precise origin has remained a controversial study to many botanists particularly the specific primary centre of cultivation in Africa, since it is the only place around the globe where the diversity of wild forms of cowpeas are found (Steele, 1979; Ba *et al.*, 2004). There are contradicting opinions supporting Africa, Asia, and South America as the center of origin of cowpea due to inadequate archaeological data (Johnson, 1970; Summerfield *et al.*, 1974; Coetzee, 1995).

The most likely origin of cowpea is Africa, as wild cowpeas only exist in Africa and Madagascar (Steele, 1976). The centre of diversity of cultivated cowpea is found in West Africa, in an area encompassing the savannah region of Nigeria, southern Niger, parts of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon (Ng and Marechal, 1985). Flight (1976) noted that carbon dating of wild cowpea remains from the Kintampo rock shelter in central Ghana discovered that, Kintampo is the oldest archaeological evidence of origin and domestication of cultivated cowpea. The archeological evidence shows the existence of gathering of cowpea by African hunters or food gatherers as early as 1500 BC.

There are diverse interpretations on the first domestication of cowpea. Some researchers are of the opinion that the crop was initially domesticated in West Africa by few farmers (Ba *et al.*, 2004). Coulibaly *et al.* (2002) on the other hand, reported North-eastern Africa as the center of domestication on the basis of molecular studies using amplified fragment

length polymorphism (AFLP). A diversity study in cowpea using randomly amplified polymorphic DNA (RAPD) markers disclosed that the wild types were more close to the domesticated cowpea in West Africa than those of South and East Africa. The distribution was suggested to be from Senegal to South Africa (Gepts, 2004).

### 2.1 Taxonomy of Cowpea

Cowpea [*Vigna unguiculata* (L.) Walp.] is an essential warm season pulse with a genome size of about 620Mb (Chen *et al*., 2007; Timko *et al*., 2008). It is highly self-pollinating diploid (2n= 2×22) species (Pasquet *et al*., 2001) in the family Leguminoseae, the order Fabaceae, sub-family Faboideae (Syn. Papillionoideae), of the tribe Phaseoleae, and genus Vigna (Timko *et al*., 2008). The unguiculata species is sub- divided into four culti-group viz: (1) *unguiculata* which is the common form, (2) *biflora* (cylinderical), (3) *sesquipedalis* or long yard-long beans characterized with very long pods and consumed as green snap beans and (4) *textilis* characterized with its long peduncles which is used for fibers (Ng and Maréchal, 1985; Padulosi and Ng 1997). Classification of wild relatives with *V. unquiculata* is more complicated with over 20 different names having been used and between 2 and 10 subgroups described (Singh *et al.*, 1997).

### 2.2 Morphology and Biology of Cowpea

Cowpea is an annual herb with varying growth forms; trailing, erect, climbing or bushy, usually indeterminate under favourable conditions. Depending on the variety of cowpea, the canopy heights can be 2-3 feet (Department of Agriculture, Forestry and Fisheries, South Africa, 2009). The nodule of the roots is smooth and spherical, about 5 mm in

diameter, numerous on the main taproot and its branches but sparse on the smaller roots (Chaturvedi *et al*., 2011).

Cowpea leaves are alternate and trifoliate with its first pair of leaves being simple and opposite. There is considerable variation in size (6-16 x 4-11 cm) with a linear, ovate shape and are usually dark green. The leaf petiole is 5-25 cm long. Striate, smooth or slightly hairy and sometimes tinged with purple are features attributed to cowpea stems. The flowers of cowpea are eye-catching, self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale blue or purple in color (Kay, 1979; Fox and Young, 1982). The flowers of cowpea in cultivated forms open at the end of the night and close in late morning with the dehiscence of the anthers taking place several hours before the flower opens. After opening once (Blooming) they wilt and collapse.

The fruit is a dehiscent pod with varying shape and length which when dry shatters. At the early stage, the pod is green usually becomes yellow, light brown, pink or purple when maturing. Pod length may differ from less than 11 cm to more than 100 cm (Rachie and Rawal, 1976). Cultivated cowpea seeds types' weigh between 80 mg and 320 mg and in shape range from kidney, ovoid, crowder, globose and rhomboid (IBPGR, 1983). The texture of cowpea seed coat varies (such as smooth, rough or wrinkled). Seed colour also varies (white, buff, green, cream, red, brown, black) (Timko and Singh, 2008). The testa may be smooth or wrinkled, white, green, buff, red, brown, black, speckled, blotched, eyed (hilum white surrounded by a dark ring) or mottled in colour. Germination is epigeal, very quick and very high in cowpea seeds.

## 2.3 Floral Biology and Breeding System

The breeding process for a crop can be influenced by its floral biology. In cowpea, the floral structure is characterised by a symmetric flower with style having a short beak (stigma) (Marechal *et al*., 1978). The flower contains ten stamens each carrying an anther sac providing pollen. Flower structure of cultivated cowpea favours self-pollination in that both sexes are in the same flower. The flower only opens after pollination and fertilization, which reduces chances for out-crossings due to foreign pollen (Marechal *et al*., 1978).

## 2.4 Climatic Condition for Cowpea Production

Cowpea is an herbaceous, warm-season annual crop cultivated between 35°N and 30°S of the equator which requires at least a minimum temperature of 18°C throughout its developmental stages and an optimum temperature of about 28°C (Craufurd *et al.,* 1997). Temperatures above 19°C accelerates germination nevertheless colder temperatures slows down the germination process (Hall *et al*., 2002). Temperature and photoperiod interact with genotype and other aspects of the environment to define yield potential of seed legumes through their effects on period of the vegetative and reproductive growth stages (Hadley *et al*., 1983; Wien and Summerfield, 1984).

An average mean temperature of 27°C is optimum for good pod formation and seed yield. Most of the genotypes of cowpea display heat-induced suppression of floral development which results in two weeks' delay in flowering when the crop is cultivated under warm environmental conditions during long days (Patel and Hall 1990). In addition, flower abortion can arise at night temperatures of above ±17°C in some cowpea genotypes. Likewise, two or more weeks of consecutive hot night during the first four weeks after germination can cause complete suppression of the first five floral buds development of

some sensitive genotypes on the main stem (Ahmad *et al.,* 1992) thereby reducing pod set, number of seeds per pod which affect seed yield. Understanding how the environmental and climatic conditions influence the development of crops is important because firstly, the time of sowing to flowering through maturity determines the duration of biomass to be accumulated and secondly the duration of the different developmental stages affects the partitioning of the biomass hence the ratio of seed to vegetative yield (Mutters *et al.,* 1989). Cowpea yields improved in regions of rainfall between 250 mm – 1000 mm per annum (Marfo and Hall, 1992).

### 2.5 Soil Fertility and pH Requirements of Cowpea

Cowpea develops well on well-drained sandy to sandy-loam soils with pH alternating from 5.5 – 6.5 (Davis *et al*., 1991; Lim, 2012; Tettey, 2017). Cowpea can be cultivated in marginal areas having low soil fertility because of its ability to fix atmospheric nitrogen through an efficient symbiotic relationship with mycorrhizae (Ghalmi *et al*., 2010).

### 2.6 Economic Importance of Cowpea

Cowpea is a multipurpose crop grown for both its grains and fodder (FAOSTAT, 2012). The versatile nature of cowpea is such that it serves as food for the people, feed for their livestock and its nitrogen fixing ability improves the soil. Cowpea has a key contribution to ensuring food security, in a sustainable environment while generating income for millions of small scale cowpea farmers in Africa (Singh *et al.,* 2003).

In the Saharan and sub - Saharan Africa where cowpea is produced, the grains serves as a rich source of protein in the diet and feed (Singh *et al.,* 2003) with about 24 % crude

protein, 53% carbohydrate and 2 % fat (FAOSTAT, 2012), A meal containing one part of cowpea and three parts of cereal is near complete. In such a diet, cowpea plays the role of a protein source that is often economical than protein from animal source (Hall, 2012).

In some parts of Africa especially Senegal, the intake of fresh 'Southern pea', prepared from cooking cowpea grains of green pods, has now become a common practice. Fresh 'southern peas' have become common in Senegal because of the introduction of extra early cowpea varieties that mature and are harvested, making the accessibility during the "hunger period" just before the harvest of cereals. About 30% of Senegal's cowpea grains in the early 2000s were consumed as fresh 'southern peas' (Hall, 2012).

Fresh cowpea leaves are consumed in sauces (Hall, 2012) in East Africa. It is a rich source of vitamins, minerals, carotenoids and phenolic compounds. These are important bio-active elements in foods that prevent occurrence of diseases like atherosclerosis and cancer (Hall, 2012).

Hay is prepared from the plants' remains after harvesting the pods. Livestock farmers normally save this hay and feed to their animals during the long dry season. The hay is also used to fatten animals for festivals and to upsurge their market value. In Niger, cowpea hay fetches about half the price of the cowpea grain (Hall, 2012).

Comparatively, cowpea is more productive on soils with little fertility and under low rainfall (Sanginga *et al.,* 2000; Abayomi *et al.,* 2008) than most tropical cereals. Cowpea is used in rotation and inter-crop with cereals in most cropping systems in Africa. It does not only fix atmospheric nitrogen and augment the soil but also suppresses some populations of nematodes and *Striga hermontica* which causes considerable yield loss to most cereals (Hall, 2012).

## 2.7 Production of Cowpea

In West Africa, cowpea cultivation is grounded mainly on small-holder subsistence farming systems in the dry regions of Africa such as the Savanna areas which receives low rainfall (IITA, 2010). The crop is ordinarily intercropped with sorghum or pearl (Ajeigbe *et al*., 2006, IITA, 2010) and also cotton. However, due to the economic importance and the demand for the crop, cowpea cultivation is moving towards a monocropping system. Cowpea is purely grown for the grains or as fodder in some parts of Europe, with the United States of America (USA) producing on a large commercial scale using a mechanised system for native consumption and export (Imrie, 2000). The sustained drought experienced in the Sahelian regions of West Africa has led farmers to endeavor into cowpea production due to its ability to tolerate low moisture levels (Duivenboo-den *et al*., 2002; Timko *et al*., 2007b). Also, the fast growing demand for cowpea in West and Central Africa has also influenced its production by farmers (Timko *et al*., 2007b; Timko and Singh, 2008).

Worldwide, cowpea is cultivated each year on about 14.5 million hectares of land (Tettey, 2017). Worldwide production of dried cowpeas is more than 5.4 million tons, with Africa producing nearly 5.2 million. Nigeria, the major producer and consumer, accounts for 61% of production in Africa and 58% worldwide (CGIAR, 2018).

In sub-Saharan Africa, approximately 38 million households made up of 194 million people cultivate the crop with productivity being stagnant over the times with total area, yield, and production growing by 4.3 %, 1.5 %, and 5.8 %, respectively (Tettey, 2017).

In Ghana, cowpea is an important component of sustainable cropping system. It is cultivated for the leaves, green pods, grain and haulm for livestock feed. Cowpea is an important source of vegetable protein and minerals for over 70% of Ghana population and

is the second most important grain legume. It is currently a food security crop (MoFA, 2010).

Cowpea is mostly grown in the savanna, coastal and transitional zones in Ghana. The vegetative parts and green pod are edible, but the dry grain is either boiled and eaten or milled in several dish preparations. Examples of cowpea dishes include; porridge and bean cake as well as processed into snacks. Boufor, Local white, Asontem, Red Nkwanta and New Era are some of the popular varieties of cowpea grown in Ghana. The seed coat can be white, red, cream, black or brown (Addo-Quaye *et al*., 2011; Akpalu, 2014).

Cowpea is known to be a drought-tolerant crop and generally cultivated in the Northern, Upper East and Upper West Regions of Ghana, providing many gains such as; domestic, economic and environmental to its inhabitants. Rains in theses region fall between May and October with an average annual rainfall between 900 and 1100mm (Akpalu, 2014). It is recommended as a protein supplement for children, pregnant and lactating mothers as a way of decreasing malnutrition in the rural and urban areas in Ghana. Cowpea additionally plays an important role by providing income for farmers. It is shade tolerant and therefore, compatible as an intercrop with maize and millet. This makes cowpea an important component of traditional intercropping systems, especially in the complex and subsistence farming systems of the dry Savanna in sub – Saharan Africa (Addo-Quaye *et al*., 2011).

Rotating or intercropping cowpea with crops such as maize, sorghum, millet and cassava contributes to the improvement of soil fertility. Sources of cowpea seeds for planting include market/traders, stored seed from own farm and from other farmers who preserve seeds for sale (MoFA, 2005).

### 2.7.1 Constraints in Cowpea Production

Cowpea has a potential yield of about 3000 kg $ha^{-1}$ however, a projected yield of between 300 to 500 kg $ha^{-1}$ is produced from farmer's field in Savannah of sub Saharan Africa (Ajeigbe *et al.*, 2006).

Cowpea is a hardy crop compared to other crops that will be unproductive when exposed to unfavourable conditions; nevertheless, production is still constrained by several biotic stress such as insect pests, disease infestations, root parasitic weeds, nematodes and abiotic stress which includes drought, low soil fertility, high salinity and post – harvest losses (Hall *et al*., 1997; Singh, 2005; Timko *et al*., 2007a). Aphids, thrips, maruca pod borer, a complex of pod sucking bugs and the storage weevil *Callosobruchus maculatus* are major insect's pest of cowpea (Singh, 2005; Timko *et al*., 2007a) and parasitic weeds such as *Striga gesnerioides* and *Alectra vogelii* are a major limitation to the production of cowpea in Africa (Timko *et al*., 2007b).

In Africa, the parasitic weed plant of most importance is the genus *Striga*. Members of this genus are obligate annual hemiparasites. The parasitic weed is chlorophyllous; but require a host to complete their life cycle (Musselman, 1987; Bisikwa *et al*., 2010). Although there are more than 35 species, only three species are renowned as economically important (Ejeta and Butler, 1993; IJLSSR, 2015). *S. hermonthica* (Del.) Benth and *S. asiatica* (L.) Kuntze are the two most prevalent and the most economically significant species that parasitize on sorghum, pearl millet, maize and rice, while *S. gesnerioides* (Willd.) Vatke attacks crops such as cowpea and peanut (Oswald, 2005; IJLSSR, 2015).

'*Striga'* is the Latin word for 'witch'. Witch weed, Buta (Kiswahili), Yan maemod (Thai) and other common names for *Striga* refer to the word 'witch'; presumably because diseased

*Striga* plants show stunted growth and an overall drought-like phenotype long before *Striga* emerge. The genus *Striga* was formerly grouped within the family Scrophulariaceae, but more recent study has placed it as a monophyletic group in the family *Orobanchaveae* (Bennett and Mathews, 2006). *Striga* belongs to the genus of the obligate hemi parasite.

### 2.8 Life Cycle of *Striga*

Life cycle of *Striga* comprises a series of growth phases that are linked to the developmental stages of the host's plant growth (Lane and Bailey, 1992; Matusova *et al*., 2005; Tignegre, 2010). There are biochemical signals that coordinate *Striga* life cycle to the host's (Matusova *et al*., 2005; Tignegre, 2010). After *Striga* seeds are formed, they need a postharvest ripening period of six to seven months upon which *Striga* completes the physiological maturing process (Thalouarn and Fer, 1993; Tignegre, 2010). Below temperatures of 25 $^{O}$C or above 35 $^{O}$C, *Striga* seeds will remain dormant (Kuiper *et al*., 1996; Tignegre, 2010). Seeds require an imbibition period or a seed conditioning phase of 10-21 days before they can germinate (Okonkwo, 1991; Lane and Bailey, 1992; Tignegre, 2010). Such conditions are usually fulfilled at the commencement of the rainy season in the semi-arid areas.

*Striga* seeds germinate within a period of two to five days if there is subsequent exposure to an exogenous stimulant produced by cowpea roots within a distance of 2 mm (Lane *et al*., 1991; Dube and Olivier, 2001; Rezig *et al*., 2016). The radicle of *Striga* grows and penetrates the host root, whereby it forms a tubercle called haustorium, which is visible on the host root surface (Tignegre, 2010). Because nutrients in seed albumen are very limited due to its small size, *Striga* radicle cannot survive more than seven days if the connection to the host is not achieved, (Berner and Williams, 1998; Tignegre, 2010). The haustorium

is an organ intended to drain nutrients, and water from the host to feed the *Striga* plant during its initial and underground development stage (Lane and Bailey, 1992; Tignegre, 2010). At this stage the impairment as a result of *Striga* attack is high in that *Striga* is a full parasite (Lane and Bailey, 1992) and depends completely on the host for its survival. The emergence above the soil happens between four to six weeks on susceptible cowpea genotypes (Tignegre, 1988). Thereafter, *Striga* grows stems and leaves and synthesizes chlorophyll (Hibberd *et al*., 1996).

*Striga gesnerioides*, unlike *S. hermonthica* is autogamous and this reduces the eventual risk of pollen flow which consequently causes the population of *S. gesnerioides* to be uniform (Botanga and Timko, 2005). The existence of races in *S. gesnerioides* is associated with a host-driven selection (Botanga and Timko, 2005) have revealed that the existence of races. The cycle from flowering to seed maturing is achieved in five to seven weeks after cowpea planting, upon which, 50,000 to 500,000 seeds per plant will be released (Lane and Bailey, 1992). The size of the seeds is microscopic (0.20 mm to 0.35 mm long), each weighing 4 to 7 µg (Dubé and Olivier, 2001). This renders *Striga* seed broadcasting easy in nature, through water, wind, animals and farming tools. Eighty percent of *S. gesnerioides* seeds are dispersed in the first 15 to 30 cm layer of the soil (Touré *et al*., 1997). The enormous amount of seeds produced, combined with the highly degraded soils in semi-arid zones and the poor access of smallholder farmers to herbicides and germination stimulants, make it difficult to eradicate *S. gesnerioides* (Tignegre, 2010).

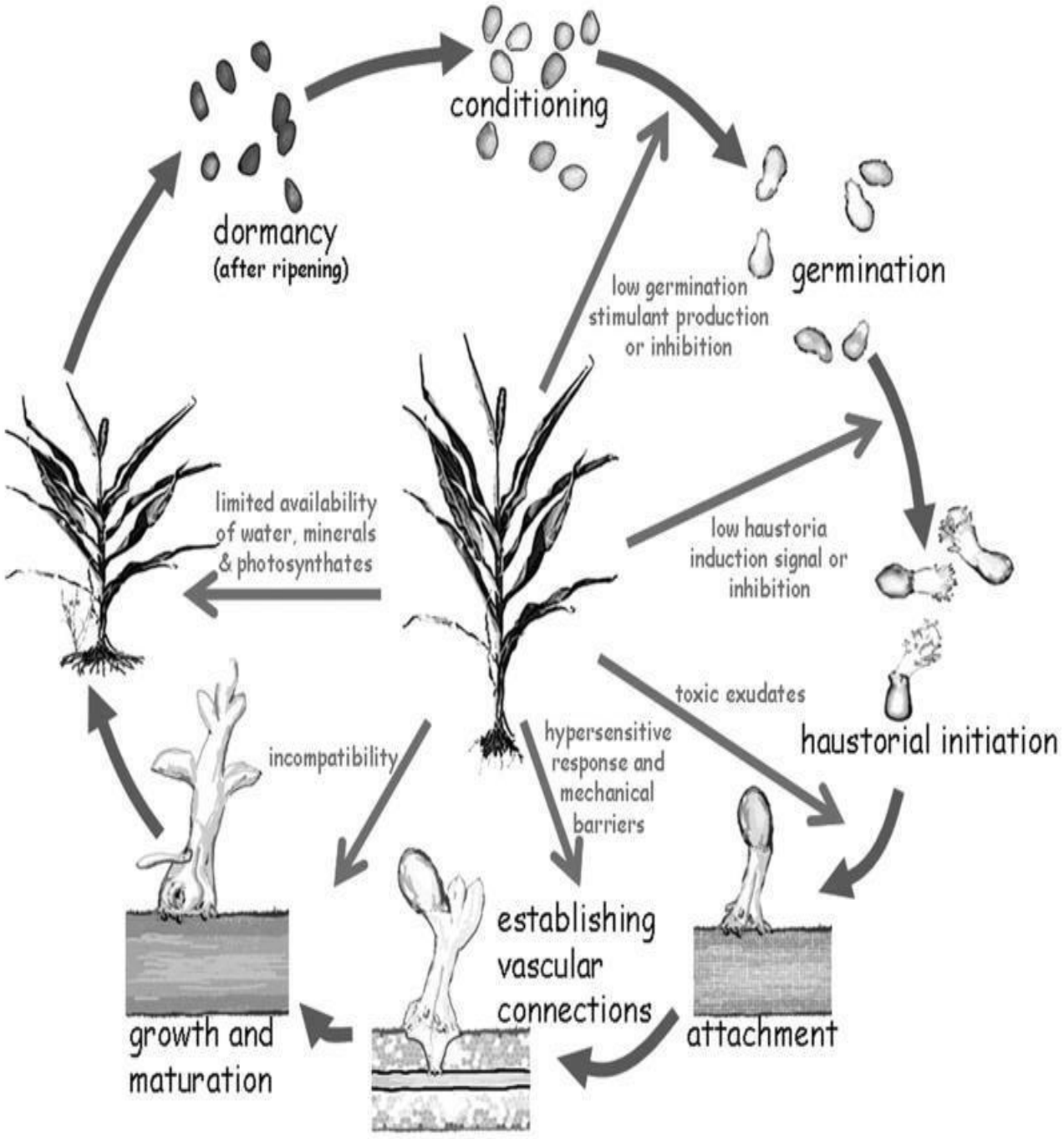


**Figure 2.1:** Life cycle of *Striga* spp (Ejeta and Butlar, 1993).

## 2.9 Economic Importance of *Striga*

In Northern Ghana, *Striga gesnerioides* characterizes a grave danger to cowpea production (Aggrarwal and Ouedranogo, 1989; Alonge *et al*., 2005). The yield of Cowpea decreases because *Striga gesnerioides* might be up to 70% depending upon the degree of harm and level of infestation (Aggrarwal and Ouedranogo, 1989; Alonge *et al*., 2005). Yield losses as an outcome of *S. gesnerioides* on susceptible cultivars could reach 100% when population was more than 10 plant for each host plant (Kamara *et al*., 2008).

Yield reduction in dry savannas of sub-Saharan Africa as brought about by *Striga gesnerioides* are valued in millions tons every year and the commonness of *Striga* pervaded soils is relentlessly (Omoigui *et al*., 2009). This is attributed to a lot of seeds produced by *Striga* plant, which can yield up to 90,000 seeds (Parker, 1991). Correspondingly, acclimatization and inactive nature of *S. gesnerioides* tolerate the seeds to stay alive in the soil for relatively a long time (20 years). The effect of *Striga* happens at diverse parts of cowpea plants, therefore influencing the physiological and biological processes of cowpea plants. It has also been reported to decrease leaf area, photosynthesis, inadequate blooming and podding, and reduced seed as the parts of cowpea (Alonge *et al*., 2004). When a field is occupied with *Striga* seeds, the seed stock will build up which sets up a state of potential yield loss in the future (Cardwell and Lane, 1995).

### 2.9.1 *Striga hermonthica*

*Striga hermonthica* (Del) Benth (a witch weed) is a flowering root parasitic plant and it is considered as a hemi-parasitic plant. *S. hermonthica* is deemed to be one of the ubiquitous parasitic weed of food crops such as rice (*Oryzasativa* L), maize (*Zeamays* L), millet (*Pennisetum giaucum*) and sorghum (*Sorghum bicolar* L. Moench) which belongs to the

family Orobanchaceae (Andrews, 1947; Tarr, 1962; Hutchinson and Dalziel, 1963; Carson,1988; Press *et al*., 2001). *S. hermonthica* is thought to have originated in the Nuba Mountains of Sudan and partly Ethiopia (Mohammed *et al*., 2001). Mali, Burkina Faso, Niger, Nigeria, Cameroon, Chad, Sudan, Ethiopia and India are countries that are severely affected with *S. hermonthica*. Crop yields may regularly be reduced by 60-70% in some regions of these countries where *Striga* is common (Ayensu *et al*., 1984).

Severe crop losses also occur widely in parts of the Gambia, Senegal, Mauritania, Togo, Ghana, Kenya, Tanzania Uganda, Botswana, Swaziland and Mozambique and more locally elsewhere in Africa, Asia, Australia and the USA (Ayensu *et al*., 1984).

### 2.9.2 *Striga asiatica*

*Striga asiatica* is the most prevailing of the 42 or so *Striga* species (Cochrane and Press, 1997). It is an annual obligate hemiparasite of monocotyledonous plants. It reproduces by seed, producing tens of thousands of minute seeds per plant (Mussleman and Parker,1981) The primary host crops of *S. asiatica* include maize (*Zea mays*), sorghum (*Sorghum* spp), rice (*Oryza sativa*), wheat and sugarcane (*Saccharum* spp). *S. asiatica* is also known to infest other grasses and some broadleaf crops (for example sunflower, tomatoes, and some legumes) and is indigenous to sub-Saharan Africa, and many countries in tropical Asia. USA (North Carolina, South Carolina), New Zealand, Papua New Guinea, and most recently Australia are countries in which *S. asiatica* has been introduced (CDFA, 2006; GISD, 2006).

### 2.9.3 *Striga gesnerioides*

*Striga gesnerioides* is one of the most important parasitic weeds in the production of cowpea (Westwood *et al*., 2010). *S. gesnerioides* is enormously widespread on the Africa continent (Mohammed *et al*., 2001). Cowpea damage due to *S. gesnerioides* can be assessed by the symptoms induced by the parasite. Alonge *et al*. (2004) reported damages such as leaf photosynthesis reduction, reduced leaf area, partial flowering, poor podding and seed development occurring at various parts of cowpea plants. In drought conditions, damages are generally intensified by the parasite (Alonge *et al*., 2004). The incidence and severity of the parasite damage depend on the cropping systems, climatic conditions, the soil type, with this severity higher on sandy soils than clay soils and the genotype involved (Cardwell and Lane, 1995).

## 2.10 Control of *Striga*

*Striga* has been difficult to eradicate because of its unique ability to adapt to the environment, and complexity of the host-parasite relationship (Botanga and Timko, 2005). In controlling *Striga*, germination stimulant can be effective in inducing suicidal germination of *Striga* (Berner *et al*., 1997; Berner and Williams, 1998). Successful control depends on eliminating the *Striga* species soil seed bank (Botanga and Timko, 2005).

Currently there is no single effective and economically feasible method in controlling *Striga* available to small holder farmers, although research effort has demonstrated that real progress can be made in reducing the devastating effects of *Striga* (Kim, 2007).

### 2.10.1 Biological Control Strategy

In biological control, *Fusarium oxysporum* is considered as one of the novel management strategies in the control of *S. hermonthica* (Sauerborn *et al.*, 2007*)*. Fungi are ideal to other microorganisms as bio-herbicides because they are usually host specific, highly aggressive, and easy to mass produce and genetically diverse (Ciotola *et al*., 2000). Field and Laboratory tests revealed that *F. oxysporum* is extremely effective in thwarting germination, growth and development of *Striga* and thus may lead to reduction of *Striga* seed bank in the soil (Ciotola *et al*., 2003).

Widespread surveys in Burkina Faso, Mali and Niger also established the occurrence of highly pathogenic and *Striga* specific isolates of *F. oxysporum* (Ciotola *et al*., 2000). Among this isolate, virulent isolate of *F. oxysporum* M12-4A provided more than 90% control of *Striga*, and a three-fold increase in sorghum biomass (Ciotola *et al*., 1996). The use of a myco-herbicide, that is F. *oxysporum* coated seeds and host plants resistance reportedly reduced *Striga* emergence by 95% and increased sorghum yield by 50% (Franke *et al*., 2006). Recent findings in Ethiopia indicated the efficacy of integrated use of *F. oxysporum* compatible and *Striga* resistant sorghum genotypes to control *Striga* (Rebeka *et al*., 2013).

In Burkina Faso, studies have shown that weevils called *Smicronyx guineanus* and *Smicronyx umbrinus* can also be used to control *Striga* in the field using their males and larvae by entering into the ovary of *Striga* inflorescence to form galls that prevented *Striga* seed production (Mahmoud *et al*., 2013). In Libya, scientist have demonstrated that a bacterium isolate, *Spirillum brasilense* from sorghum seeds was used to reduce *Striga* from 65% to 10%. It was detected that the bacterium (Spirillum brasilense) produced chemicals which interfere with stimulants from the host plants preventing the survival of *Striga* in

sorghum fields (Mahmoud *et al*., 2013). Incorporation of the isolate (Spirillum brasilense) in plots planted with sorghum resulted in 70% reduction of emerged *Striga* plants, 68% reduction in *Striga* biomass at harvest and 80% reduction in the number of flowers of *Striga* produced compared to the control treatment

(Mahmoud *et al*., 2013).

### 2.10.2 Chemical Control

Berner *et al*. (1995) in his attempt to protect cowpea seeds against *Striga*, immersed the seeds in imazaquin aqueous solution. The results of the experiment showed that cowpea plants were protected against *Striga* and improved yield of cowpea was realized. As a result of the imazaquin, the seed density of *Striga* in the soil was reduced which resulted in the mortality of *Striga* as a result of amino acid biosynthesis inhibition (Berner *et al*., 1995; Mahmoud *et al*., 2013). The use of urea and dicamba reported by Babiker *et al*. (1996) effectively controlled *Striga* between 62-92% on sorghum, whilst as much as 77-100% was achieved when a combination of chlorosulfuron and dicamba was used in *Striga* control.

### 2.10.3 Cultural Control Strategy

In cultural control, the use of trap crops such as soybean to trigger the germination of *Striga* seeds is an efficient cultural control. Soybean is not a host to *Striga*, subsequently after germination, the *Striga* seedling dies off as a result of nutrient starvation. This is known as suicidal germination (Umba *et al*., 1999; Mahmoud *et al*., 2013). The use of hand hoe to weed off *Striga* and pulling *Striga* seedlings with hands before flowering is another cultural method of controlling *Striga* (Nworgu and Olakojo, 2006; Mahmoud *et al*., 2013).

### 2.10.4 Use of Nitrogenous Fertilizers

Olakojo (2005) used six nitrogen fertilizers (Urea, CAN, NPK, $NH_4SO_4$ and Compost) on two maize varieties; *Striga* tolerant and *Striga* susceptible to study their effects in *Striga* control at Moor plantation, Ibadan. *Striga* related characters were measured. Results showed that $NH_4SO_4$ and Urea controlled *Striga* rate and subsequently improved higher grain yield under *Striga* infestation. Under artificial infestation in the same vein, NPK and Calcium Ammonium Nitrate (CAN) suppressed *Striga* and improved higher grain yield (Olakojo and Olaoye, 2005; Mahmoud *et al*., 2013).

### 2.10.5 Use of Resistant Varieties

Varietal resistance is the main control measure available for cowpea. The utmost important source of resistance is the landrace B301, originally designated for its partial resistance to *Alectra vogelii* in Botswana (Parker and Riches, 1993). In A. *vogelii*, B301 auspiciously shows high-level resistance in West Africa (based on two dominant genes) as well as to S. *gesnerioides* (based on a single dominant gene) (Atokple *et al*., 1995). The resistance, or virtual immunity, of this line has been operative against all biotypes of the parasite in West Africa excluding that occurring locally in southern Benin. Lane *et al*. (1996) describe the being of five known parasite biotypes, changing in their virulence on different 'resistant' varieties of cowpea. Suvita-2 and IT82D-849, two other resistant sources have dissimilar single dominant genes for resistance to the Mali biotype, and a different pattern of response to the five parasite biotypes (Atokple *et al*., 1995).

### 2.11 Sources of Resistance in Cowpea to *Striga gesnerioides*

In 1981, varietal differences with reverence to *Striga* infection were first noticed in Burkina Faso, and two lines, Suvita-2 and 58-5, were found to be completely resistant to the parasitic weed (IITA, 1983). Nonetheless, results of trails subsequently done on regional basis revealed that these lines were not resistant to *Striga* in Niger and Nigeria indicating strain variation of *Striga* in cowpea (Aggarwal, 1985). Several other lines were subsequently identified which had moderate to high levels of resistance to both *Striga* and *Alectra* which included IT86D-534, IT81D-994, IT86D-371, IT84D-666 (Singh and Emechebe, 1991), TVu 11788, TVu 9238, TVu 12415, TVu 12432, and TVu 12470 (Singh and Emechebe, 1991). In Zakpota in the coastal savanna of Benin Republic, several of these resistant lines were tested where severe *Striga* infestation had been reported. IT86D534, IT86D-371, and IIT84D-666 as well as all the TVu lines were susceptible to the Zakpota strain.

Suvita 2,58-57 and It81d-944 were on the other hand completely resistant indicating that the Zakpota strain was different from strains from Burkina Faso and Nigeria (Lane *et al*., 1994; Lane *et al*., 1997b).

#### 2.11.1 Genetics of Resistance to *Striga gesnerioides*

A single dominate gene, designated Rsg1 conditions resistance to *S. gesnerioides* in cowpea variety B301 (Singh and Emechebe, 1990). In 1993, Atokple *et al*. demonstrated that the genes conditioning the resistance to *Striga* in B301 and also in *Alectra* are neither allelic nor linked. Atokple *et al*. (1995) reported the results of extensive allelism tests

among cowpea lines resistant to *Striga* and *Alectra*. This work revealed that different genes are responsible for the resistance to *Striga* exhibited by B301, IT82D-849 and Suvita- 2.

An array of molecules which contrast in chemical structure and specific activity are produced by the host roots. Following germination, the host derived chemical signal from the root, known as the haustorium initiation factor, is required for the differentiation of radical into the haustorium by which the *Striga* seedlings attached to and penetrate the host roots. Once, in contact with cowpea roots, the radical's apex develops numerous hairs, which attach to host roots, When the vascular connection is established between the host and parasite, the development of the haustorium ceases, the *Striga* seedling enlarges, forming a thick mass of tissue called the tubercles. The haustorium permits the transfer of water and nutrients to the parasite. However, *Striga* penetration of host root tissue involves a combination of intrusive growth and enzymatic digestion. Studies have shown that some cowpea lines, like B301, are resistant to *Striga* due to its ability to stimulate germination of the *Striga* seeds but inhibiting hasutorial formation and growth (Godwa *et al*., 1999). *Striga* exhibits strain variation such that cultivars that are resistance in one location may be susceptible in another location (Lane *et al*., 1994).

Genetic studies have shown that three dominant nonalleic genes confer resistance to different *Striga* biotypes but the mechanism differ (Singh, 1993). Careful observation has provided evidence for at least two different mechanisms of resistance to *Striga* parasitism in cowpea (Li and Timko, 2009). One mechanism resembles the hypersensitive response (HR) detected in other plant-pathogen interactions which suggests the presence of a specific R gene-mediated response mechanism. The second type of resistance response

involve arrested development of the parasite tubercle succeeding attachment and attempted penetration of the root cortex (Li and Timko, 2009).

### 2.12 Screening Techniques for *Striga* Resistance in Cowpea

Diverse screening techniques have been applied in other to identify sources of resistance to *S. gesnerioides* in cowpea (Lane and Bailey, 1992; Muleba *et al*., 1997; Ouédraogo *et al*., 2002ab; Boukar *et al*., 2004). The screening techniques for *Striga* resistance in cowpea comprised: (1) field screening (2) pot screening where pot screening is more generally more reliable than field due to its even distribution of *Striga* races (seeds); (3)"in-vitro" screening; and (4) molecular screening technique using DNA markers associated with the resistance to *S. gesnerioides* in cowpea (Lane *et al*., 1991).

#### 2.12.1 Pot Screening Technique

Pot screening technique is an attempt to reproduce *Striga*-field conditions which is designed to ensure even infestation with *Striga* seeds, which is rarely obtained under field conditions. Musselman and Ayensu (1984) recommended 1000 *Striga* seeds per pot (8 to 10 litre content) which is an effective pot screening method for *Striga* resistance to achieve an effective screening for *S. gesnerioides*

#### 2.12.2 Field Screening Technique

Field experiments are still required in evaluating yield. In studying post-emergence *Striga* development (such as *Striga* emergence date, number of *Striga* shoots per host and vigour of *Striga*), pot and field screening is required. Improved field-testing methodologies are

required for a successful field screening (Haussman *et al*., 2000), consisting of combining suitable field layouts, with appropriate inoculation techniques (Haussman *et al*., 2000).

### 2.12.3 "in-vitro" Screening Technique

Different mechanisms of resistance can be evaluated by using petri dishes which is an "invitro" screening technique. An "in-vitro" growth media which provide optimum growing conditions for cowpea and *S. gesnerioides*, using petri dishes is made of a combination of macro and minor nutrients. Underground *Striga* development stages that are not visible under field conditions can be observed using laboratory screening (Lane *et al*., 1991).

### 2.12.4 DNA Screening Technique using Marker Assisted Selection in Breeding for *Striga* Resistance

The idea of MAS became a reality when DNA markers were observed to show variation at the DNA level (Ruane and Sonnino, 2007). Thus more polymorphisms can be revealed allowing breeders for the first time to identify large numbers of markers dispersed throughout the genome of the species of interest, using the markers to detect associations with traits of interest, independent of their stage specific expression (Ruane and Sonnino, 2007). MAS is a technique where molecular markers are used to select genotypes that carry traits of interest. The significant of MAS is that markers phenotypes can be identified at the seedling stage, eliminating the time needed for plant maturation and reduction in population size (Yu *et al*., 2000). Assessment for *Striga* resistance in the field is difficult, expensive and sometimes unreliable making MAS techniques promising.

## 2.13 Genetic Markers

Genetic differences between individual organisms or species are represented by genetic markers. Genetic markers may be referred to as gene tags when they are located in close proximity to genes. Gene tags do not affect the trait of interest because they are located only near or linked to genes controlling the trait. All genetic markers occupy specific genomic positions with chromosomes called loci. Morphological, biochemical and DNA markers are the three major types of genetic markers (Winter and Kahl, 1995; Jones *et al*., 1997).

### 2.13.1 Morphological Markers

The method is a conventional method such as determining morphological characteristics for determining genetic diversity. The method includes measuring variation in phenotypic or quantitative traits such as flower colour and growth, or quantitative agronomic traits such as yield potential (Kameswara, 2004). Expression of quantitative traits is subject to strong environmental influence which is a major disadvantage of morphological and biochemical markers (Winter and Karl, 1995). Nevertheless, despite these limitations, morphological and biochemical markers have been exceptionally useful to plant breeders (Eagles *et al.,* 2001). The method is a conventional method such as determining morphological characteristics for determining genetic diversity.

### 2.13.2 DNA Markers

The most widely used marker type is that of DNA based due to their abundance. They arise from different classes of DNA mutations such as substitution (point mutation), errors in replication of tandemly repeated DNA or rearrangement (Peterson, 1996). DNA markers may be broadly divided into three classes based on the method of their detection:

Hybridization-based Polymerase chain reaction (PCR)-based and DNA sequence-based (Winter and Kahl, 1995; Jones *et al*., 1997; Gupta *et al*., 1999; Joshi *et al*., 1999).

Restriction fragment length polymorphisms (RFLPs) were the first DNA-based genetic markers (Botstein *et al.*, 1980). In 1986, the first linkage map in a crop plant (tomato) based on RFLPs was constructed (Bernatzky and Tanksley 1986) and a complete linkage map two years later was first used by Paterson *et al*. (1988) to resolve quantitative traits into discrete Mendelian factors. Numerous DNA marker system based on PCR following the hybridization-based RFLPs were develop, such as random-amplified polymorphic DNAs (RAPDs) (Williams *et al*., 1990), amplified fragment length polymorphism (AFLPs) (Vos *et al*., 1995), microsatellites, also termed simple sequence repeats (SSRs), (Powell *et al*., 1996) or single nucleotide polymorphisms (SNPs) (Gupta *et al*., 2001).

#### 2.13.2.1 Restriction Fragment Length Polymorphism (RFLP) Markers

In the 1980s RFLP technology was first developed (Saiki *et al*., 1985) for use in human genetic applications and was later applied in plants. DNA sample is digested in RFLP analysis by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis. Unlimited number of RFLPs can be generated by digesting total DNA with specific restrictions enzymes. RFLPs are rather

small in size and co-dominant in nature. If two individuals differ by as little as a single nucleotide in the restriction site, the restriction enzyme will digest the DNA of one but not the other. Restriction fragments of different lengths are consequently generated. The analysis requires a relatively complex technique that is time consuming and expensive. However, it is still used in marker assisted selection procedures.

### 2.13.2.2 Simple Sequence Repeat (SSR) Markers

Simple Sequence Repeat, also called microsatellites, short tandem repeats (STRs) or sequence-tagged microsatellite sites (STMS), are PCR-based markers. They are random tandem repeats of short nucleotide motifs (2-6 bp / nucleotides long). Di-, tri- and tetranucleotide repeats, for example (GT)n TAT)n and (GATA)n, are broadly distributed throughout the genomes of plants and animals. The copy number of these repeats varies among individuals and is a source of polymorphism in plants.

Since, the DNA sequences flanking microsatellite regions are usually conserved, primers specific for these regions are designed for use in the PCR reaction. One of the most significant attributes of microsatellite loci is that they have high level of allelic variation, thus making them valuable genetic markers. The unique sequences bordering the SSR motifs provide templates for specific primers to amplify the SSR alleles via PCR. SSR loci are individually amplified by PCR using pairs of oligonucleotide primers specific to unique DNA sequences flanking the SSR sequence. The PCR-amplified products can be separated in high-resolution electrophoresis systems (for example AGE and PAGE) and the bands can be visually recorded by fluorescent labeling or silver-staining (Victoria, 2016).

SSR markers are characterised by their hyper-variability, reproducibility, co-dominant nature, locus-specificity, and random genome-wide distribution in most cases. The

advantages of SSR markers include that they are easily detected by PAGE and can be readily analyzed by PCR and or AGE. SSR markers can be multiplexed, have high throughput genotyping and can be automated. SSR assays require only very small DNA samples (~100 ng per individual) and low start-up costs for manual assay methods. However, SSR technique requires nucleotide information for primer design, laborintensive marker development process and high start-up costs for automated detections. Since the 1990s SSR markers have been extensively used in constructing genetic linkage maps (Song *et al*., 2010).

### 2.14 DNA Markers Linked to *Striga gesnerioides*

Studies conducted by Ouédraogo *et al*. (2001) using AFL and BS techniques identified three markers tightly linked to the resistance Rsg2 gene effective against *S. gesnerioides* race 1 from Burkina Faso, and present in IT2D-8499; and six AFLP markers linked with the resistance Rsg4 gene effective against race 3 *S. gesnerioides* from Nigeria and present in TVu 14676. E-AAC/M-CAA300 and E-ACA/M-CAT 150 markers were linked to Rsg2 and Rsg4 respectively. 61R one of the AFLP markers converted into a SCAR marker and an improved SCAR 61RM2 (Ouédraogo *et al*., 2012) both two markers reported to be associated to race 3 resistances. These two markers were dominant markers with wider applications. However, Bouker *et al*. (2004) also reported a SCAR marker for race 3 resistance that is co-dominant in nature.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Experimental Sites

The study consisted of field experiment, pot experiment, population development and genotyping with molecular markers. The field experiment, pot experiment and population development were conducted at the Manga research station of Council for Scientific and Industrial Research- Savannah Agricultural Research Institute (CSIR-SARI), Upper East Region of Ghana. The genotyping with molecular markers was conducted at the Kirk House Trust SCIO molecular lab at CSIR-SARI, Nyankpala. The studies were conducted between July, 2016 and November, 2017.

Manga is geographically located within latitude 11.02° and longitude 0.27°, with an altitude of 224 meters above sea level. The area is located within Sudan Savannah agro-ecological zone of Ghana and characterized by a unimodal rainfall pattern lasting for a period of four (4) to five (5) months from June to October usually, and a dry period lasting for 7 to 8 months. The annual rainfall of the area during the period of the experiments was approximately 1100 mm **(Table 3.3)**. The average annual temperature is about 34.86 $^{o}$C, the highest being observed from February to April 2016. The relative humidity (RH) of the location fluctuated significantly, dropping in the dry season and rising during the rainy season with an average humidity of 74.2% **(Table 3.3)**.

Soils of Manga are generally of savannah ochrosol type (Bawku municipality analytical report, 2010). Manga soils range from sandy to sandy-loam associated with hornblende and granites (Spencer and Sivakumar, 1987; Bawku municipality analytical report, 2010). Common features of the soils at Manga include low fertility, low organic matter content,

low pH and a moderately acidic upper layer easily prone to erosion. They are quite permeable with moderately good water retention (Bawku municipality analytical report, 2010).

### 3.2 Experimental Materials

Three hundred (300) RILs of the MAGIC population and their eight founder parents were used in the experiment **(Table 3.1)**. These were obtained from the University of California, Riverside (UCR), USA under the Feed the Future Innovation Lab for Climate Resilient Cowpea project at SARI. The relevant traits of the founder parents are presented in **Table3.2**.



**Table 3.1 Cowpea genotypes used for the experiment**

| Entry NO. | Genotype | Entry NO. | Genotype | Entry NO. | Genotype | Entry NO. | Genotype | Entry NO. | Genotype |
|---|---|---|---|---|---|---|---|---|---|
| 1 | IT89K-288 | 72 | M064 | 143 | M135 | 214 | M206 | 285 | M277 |
| 2 | IT845-2049 | 73 | M065 | 144 | M136 | 215 | M207 | 286 | M278 |
| 3 | CB27 | 74 | M066 | 145 | M137 | 216 | M208 | 287 | M279 |
| 4 | IT82E-18 | 75 | M067 | 146 | M138 | 217 | M209 | 288 | M280 |
| 5 | SUVITA 2 | 76 | M068 | 147 | M139 | 218 | M210 | 289 | M281 |
| 6 | ITOOK-1263 | 77 | M069 | 148 | M140 | 219 | M211 | 290 | M282 |
| 7 | IT845-2246 | 78 | M070 | 149 | M141 | 220 | M212 | 291 | M283 |
| 8 | IT93K-503-1 | 79 | M071 | 150 | M142 | 221 | M213 | 292 | M284 |
| 9 | M001 | 80 | M072 | 151 | M143 | 222 | M214 | 293 | M285 |
| 10 | M002 | 81 | M073 | 152 | M144 | 223 | M215 | 294 | M286 |
| 11 | M003 | 82 | M074 | 153 | M145 | 224 | M216 | 295 | M287 |
| 12 | M004 | 83 | M075 | 154 | M146 | 225 | M217 | 296 | M288 |
| 13 | M005 | 84 | M076 | 155 | M147 | 226 | M218 | 297 | M289 |
| 14 | M006 | 85 | M077 | 156 | M148 | 227 | M219 | 298 | M290 |
| 15 | M007 | 86 | M078 | 157 | M149 | 228 | M220 | 299 | M291 |
| 16 | M008 | 87 | M079 | 158 | M150 | 229 | M221 | 300 | M292 |
| 17 | M009 | 88 | M080 | 159 | M151 | 230 | M222 | 301 | M293 |
| 18 | M010 | 89 | M081 | 160 | M152 | 231 | M223 | 302 | M294 |
| 19 | M011 | 90 | M082 | 161 | M153 | 232 | M224 | 303 | M295 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 20 | M012 | 91 | M083 | 162 | M154 | 233 | M225 | 304 | M296 |
| 21 | M013 | 92 | M084 | 163 | M155 | 234 | M226 | 305 | M297 |
| 22 | M014 | 93 | M085 | 164 | M156 | 235 | M227 | 306 | M298 |
| 23 | M015 | 94 | M086 | 165 | M157 | 236 | M228 | 307 | M299 |
| 24 | M016 | 95 | M087 | 166 | M158 | 237 | M229 | 308 | M300 |
| 25 | M017 | 96 | M088 | 167 | M159 | 238 | M230 | | |
| 26 | M018 | 97 | M089 | 168 | M160 | 239 | M231 | | |
| 27 | M019 | 98 | M090 | 169 | M161 | 240 | M232 | | |
| 28 | M020 | 99 | M091 | 170 | M162 | 241 | M233 | | |
| 29 | M021 | 100 | M092 | 171 | M163 | 242 | M234 | | |
| 30 | M022 | 101 | M093 | 172 | M164 | 243 | M235 | | |
| 31 | M023 | 102 | M094 | 173 | M165 | 244 | M236 | | |
| 32 | M024 | 103 | M095 | 174 | M166 | 245 | M237 | | |
| 33 | M025 | 104 | M096 | 175 | M167 | 246 | M238 | | |
| 34 | M026 | 105 | M097 | 176 | M168 | 247 | M239 | | |
| 35 | M027 | 106 | M098 | 177 | M169 | 248 | M240 | | |
| 36 | M028 | 107 | M099 | 178 | M170 | 249 | M241 | | |
| 37 | M029 | 108 | M100 | 179 | M171 | 250 | M242 | | |
| 38 | M030 | 109 | M101 | 180 | M172 | 251 | M243 | | |
| 39 | M031 | 110 | M102 | 181 | M173 | 252 | M244 | | |
| 40 | M032 | 111 | M103 | 182 | M174 | 253 | M245 | | |
| 41 | M033 | 112 | M104 | 183 | M175 | 254 | M246 | | |
| 42 | M034 | 113 | M105 | 184 | M176 | 255 | M247 | | |
| 43 | M035 | 114 | M106 | 185 | M177 | 256 | M248 | | |
| 44 | M036 | 115 | M107 | 186 | M178 | 257 | M249 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 45 | M037 | 116 | M108 | 187 | M179 | 258 | M250 |
| 46 | M038 | 117 | M109 | 188 | M180 | 259 | M251 |
| 47 | M039 | 118 | M110 | 189 | M181 | 260 | M252 |
| 48 | M040 | 119 | M111 | 190 | M182 | 261 | M253 |
| 49 | M041 | 120 | M112 | 191 | M183 | 262 | M254 |
| 50 | M042 | 121 | M113 | 192 | M184 | 263 | M255 |
| 51 | M043 | 122 | M114 | 193 | M185 | 264 | M256 |
| 52 | M044 | 123 | M115 | 194 | M186 | 265 | M257 |
| 53 | M045 | 124 | M116 | 195 | M187 | 266 | M258 |
| 54 | M046 | 125 | M117 | 196 | M188 | 267 | M259 |
| 55 | M047 | 126 | M118 | 197 | M189 | 268 | M260 |
| 56 | M048 | 127 | M119 | 198 | M190 | 269 | M261 |
| 57 | M049 | 128 | M120 | 199 | M191 | 270 | M262 |
| 58 | M050 | 129 | M121 | 200 | M192 | 271 | M263 |
| 59 | M051 | 130 | M122 | 201 | M193 | 272 | M264 |
| 60 | M052 | 131 | M123 | 202 | M194 | 273 | M265 |
| 61 | M053 | 132 | M124 | 203 | M195 | 274 | M266 |
| 62 | M054 | 133 | M125 | 204 | M196 | 275 | M267 |
| 63 | M055 | 134 | M126 | 205 | M197 | 276 | M268 |
| 64 | M056 | 135 | M127 | 206 | M198 | 277 | M269 |
| 65 | M057 | 136 | M128 | 207 | M199 | 278 | M270 |
| 66 | M058 | 137 | M129 | 208 | M200 | 279 | M271 |
| 67 | M059 | 138 | M130 | 209 | M201 | 280 | M272 |
| 68 | M060 | 139 | M131 | 210 | M202 | 281 | M273 |
| 69 | M061 | 140 | M132 | 211 | M203 | 282 | M274 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 70 | M062 | 141 | M133 | 212 | M204 | 283 | M275 |
| 71 | M063 | 142 | M134 | 213 | M205 | 284 | M276 |



### 3.2 Founder Parents and Traits Relevant to Sub-Saharan Africa

**Table 3.2: Founder parents and traits relevant to sub-Saharan Africa**

| Founder parents | Desirable traits |
|---|---|
| IT89KD-288 | Root-knot Nematodes |
| IT84S249 | Aphid, Nematodes, Virus |
| CB27 | Heat, Nematodes, Fusarium |
| IT82E-18 | Broadly adapted |
| Suvita-2 | *Striga*, Drought, Macrophomina |
| IT00K-1263 | Aphid, *Striga*, Fusarium |
| IT84S-2246 | Nematodes, Aphids, Virus |
| IT93K-503-1 | Drought, *Striga*, Nematodes, Macrophomina |

### 3.3 Field Assessment of 300 RILs of MAGIC Population and their eight founder parents for their Agronomic Performance

The MAGIC population and their eight founder parents were planted in the field to assess their agronomic performance under rain-fed (between July and October) conditions. The total rainfall during the period of the field experiment was 627.4mm **(Table 3.3).** The average annual temperature was about 31.1 $^{o}$C and an average humidity of 94.33% (Manga weather station). Rainfall trend, relative humidity and temperature during evaluation of the population under field and pot experiment are also presented in **Table 3.3**

The experiment was laid out in an Augmented Latin design with a land size of 744 $m^2$ (31 m × 24 m). A planting distance of 20 cm within plants and 60 cm between rows were used. The plants were sprayed thrice with K-Optimal (Lambda-cyhalothrin 15 g/l + Acetamiprid 20 g/l) insecticides at the rate of 30 mls per 15 litres of water at the vegetative, flowering and podding phases to control insect pests. Hand weeding was done to control weeds throughout the growing period of the plants. Data taken was number of plants per stand, days to first flowering, days to 50% flowering, plant height at 50% flowering, number of peduncles, number of pods per peduncles, pod length, pod weight, seed weight, 100 seed weight and seed coat colour.

**Table 3.3 Rainfall, Temperature and relative humidity Trend during evaluation of the population under field and pot experiment (June to December, 2016)**

| Months | Rainfall (mm) | Temperature ($^{O}$C) | | Relative Humidity (%) | |
|---|---|---|---|---|---|
| | | Max | Min | Max | Min |
| June | 195 | 33.5 | 24.1 | 93 | 73 |
| July | 246.1 | 30.7 | 23.5 | 93 | 72 |
| August | 232.4 | 31.1 | 23.3 | 93 | 76 |
| September | 117.5 | 31.6 | 23.5 | 91 | 83 |
| October | 31.4 | 35.6 | 21.4 | 74 | 52 |
| November | 1.8 | 37.7 | 19.3 | 62 | 24 |
| December | 0.00 | 35.8 | 18.2 | 47 | 19 |

**Source: Weather Station (SARI) Manga-Bawku**

### 3.4 Pot Screening for *Striga gesnerioides* Resistance

The pot screening method by Botanga and Timko (2006) was employed to screen for *Striga* resistance among the MAGIC population and their eight parents under partial rain fed and irrigation conditions (between Mid-September and early December). The total rainfall during the period of the pot experiment was 482.2 mm (**Table 3.3**). The average temperature for the period was 34.5 $^{o}$C and an average humidity of 85.66% (**Table 3.3**).

Plastic pot of 23 cm diameter and depth of 21 cm was perforated and filled with sandy loam soil. One teaspoon (about 5 g) of *Striga* seeds was mixed with the soil at a depth of about 5 cm (**Plate 3.1**). Six seeds of each line were planted in each pot and thinned to four seedlings per pot two weeks after emergence. The experimental design was a completely

randomized design with 2 replications. Watering was done as and when necessary. Spraying with K-optimal (Lambda-cyhalothrin 15 g/l + Acetamiprid 20 g/l) insecticides at the rate of 30 mls per 15 litres of water was applied to control aphids, thrips, Maruca and pod sucking insects at the vegetative, flowering and podding stages of the plant. Weeds were controlled by hand pulling. Data was collected on the number of plants per pot at germination, days to 50% flowering, number of plant per pot at maturity, number of pods per plant, pod weight and seed weight.

**Plate 3.1: Infestation of pots with *Striga gesnerioides* seeds**

### 3.5 Assessment of *Striga* Attachment to Roots and *Striga* Resistance

To confirm the resistance status of plants with no *Striga* emergence, the soil mass was washed off the roots to check for the presence of *Striga* attachment to the roots. The soil mass was removed by gently agitating to loosen the soil in the pots (**Plate 3.2A**). Plants were then immersed into water and soil gently washed off the roots. Roots were then carefully examined to detect the presence or absence of *Striga* development. Lines with *Striga* emergence and those with *Striga* attached to their roots were classified as susceptible (**Plate 3.2B**) whilst those with no attachment at the roots were classified as resistant (**Plate 3.2D**).


**Plate 3.2: Washing of cowpea roots to evaluate for the presence of *Striga gesnerioides.***

**A = Checking for attachment of *Striga gesnerioides,* B = Attached Striga shoots on RIL, C = Comparison of susceptible and resistant RILs.**

### 3.6 Data Collection

Data was collected on both replicates on all plants in every pot. Data taken included Days to *Striga* emergence, Days to 50% flowering, number of pods harvested per pot, pod weight and seed weight.

#### 3.6.1 Days to *Striga* Emergence

The days taken for first *Striga* emergence after planting was recorded. After the first emergence, plots were checked for *Striga gesnerioides* emergence on daily basis (**Plate 3.3**).

**Plate 3.3**: Morphological screening for emerged *Striga*

#### 3.6.2 Days to 50% Flowering

The number of days after planting at which 50% of the plants per pot flowered was recorded as days to 50 % flowering. Data was taken every morning on a daily basis.

#### 3.6.3 Number of Pods per Plant

The total number of pods per plant at maturity were counted for each pot and averaged to get the number of pods per plant.

### 3.6.4 Pod Weight

The weight of harvested pods per plant was taken with an electronic scale after drying.

### 3.6.5 Grain Yield

The total weight of grain was taken after threshing and winnowing the harvested pods.

### 3.7 Selection of Resistant and Susceptible Parents for Hybridization

The resistant RILs (MAGIC 072, MAGIC 074, MAGIC 213) and the parent (Suvita-2) were selected and used as males for the crosses. Five susceptible genotypes were selected to serve as females based on good agronomic traits such as seed coat color, 100 seed weight(g), number of pods per plants, 50% flowering, days to maturity and yield per hectare (**Table 3.4**).

**Table 3.4: Selection of susceptible lines based on good agronomic traits**

| Susceptible lines | Seed coat colour | 100 seed weight (g) | Pod/plant | DFF | DTM | Kg/ ha |
|---|---|---|---|---|---|---|
| MAGIC 019 | Cream | 16 | 32 | 40 | 58 | 1903.04 |
| MAGIC 020 | Cream | 16.5 | 33 | 40 | 58 | 2013.343 |
| MAGIC 034 | White | 15.8 | 27 | 35 | 53 | 652.3842 |
| MAGIC 262 | White | 17 | 24 | 35 | 54 | 939.3986 |
| MAGIC 263 | White | 17 | 9 | 33 | 55 | 957.1476 |

**DFF= days to 50% flowering, DTM=days to maturity**

### 3.8 Identification of Polymorphic Markers

Three molecular markers were used to genotype the selected lines used for the crosses. The screening was done in the Kirk house Trust SCIO molecular lab at CSIR-SARI, Nyankpala. DNA was isolated from leaf samples and screen for polymorphism with three markers to ascertain which marker is closely linked to the *Striga* resistant trait. The markers used were C42-2B, SSR1, 61RM2.

### 3.9 Extraction of DNA

Young leaves from two-week old plants were collected from $F_1$ plants that were clearly labeled. A total of 189 parents consisting of 63 resistant' and 126 susceptible parents were sampled on Fast Technology Analysis (FTA) cards using the Kirkhouse Trust protocol (Kirkhouse Trust protocol, 2016).

Leaves of two-week old plants were taken from the plants and immediately cleaned with ethanol and placed over the marked circle (underside of the leaf facing down) on top of the FTA matrix card. Parafilm was overlaid on the leaf and a small porcelain pestle was used to apply moderate pounding for 15 seconds over each sample circle area to burst cell walls of the plant tissue (**Plate 3.4A**). Frequent checking was done to verify if sufficient tissue has been transferred to the matrix by checking the back of the FTA card matrix to see if plant tissue was drawn through it (**Plate 3.4B**). When plant tissue transfer was complete, the FTA was air dried for minimum of one hour at room temperature **(Plate 3.4C).**

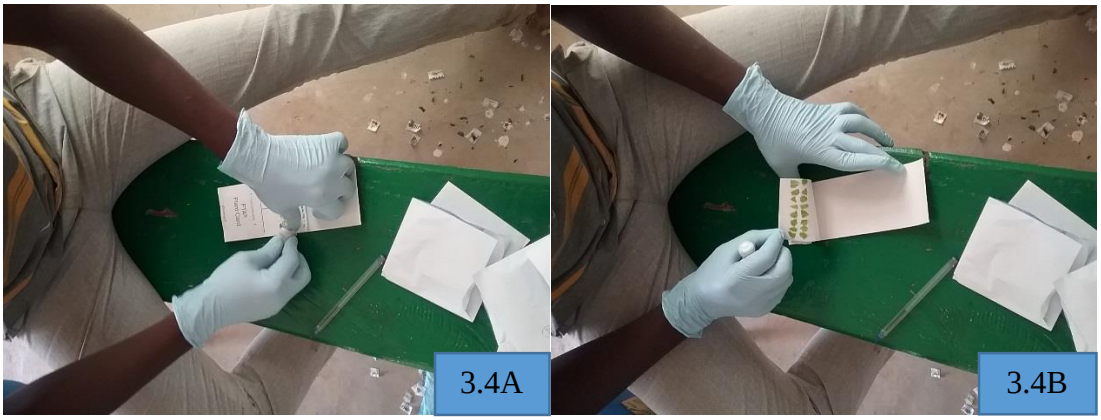



**Plate 3.4**: Sampling of DNA on FTA card matrix

The FTA matrix card was placed on FTA sample mat which was directly beneath it and a Harris 2.0 mm micro-punch was placed on the centre of the sample on the FTA card and pressed firmly with a twist to cut the disc out of the card. The disc was removed from the centre of the dried sample area into 1.5 ml micro centrifuge tube with care since the static charge that could develop on some plastic laboratory ware could repel the discs. Ethanol (70% v/v) was used to rinse the tip of the micro-punch and dried with a tissue paper between samples. Ethanol (70 % v/v) was added to each tube containing the FTA disc. The tube was then placed on a votex mixer for 5 minutes with moderate force to allow the contents to mix. This washing process was repeated using ethanol until the disc turned white. About 200 µl of FTA purification reagent was added to each tube, capped, and inverted twice before it was incubated for 4-5 minutes at room temperature. After the incubation the FTA reagent was pipetted up and down twice. To ensure that the disc remained in the tube, a pipette was used to remove and discard as much of the reagent as possible. The discs were air dry for a minimum of one hour at room temperature.

Polymerase Chain Reaction (PCR) was conducted within three hours after air drying of the disc. The discs were stored at 4 $^{o}$C or -12 $^{o}$C whenever the PCR amplification could not be conducted within three hours of disc drying. This was due to the fact that the DNA purification process removed the protective chemistry of the FTA technology.

### 3.10 Primers

Three pairs of primers known to be associated with *Striga gesnerioides* resistance in cowpea were used to amplify the DNA samples. Two sequences characterised amplified region (SCAR) primer designated as 61RM2, C42-2B and one Microsatellite primer, SSR1, were used (**Table 3.5**).

**Table 3.5: Primer sequence and their Annealing temperatures**

| Marker Name | Sequence | | Annealing Temp. (℃) |
|---|---|---|---|
| | **Forward** | **Reverse** | |
| SSR1 | CCTAAGCTTTTCTCCAACTCCA | CAAGAAGGAGGCGAAGACTG | 59 |
| C42-2B | CAGTTCCCTAATGGACAACC | CAAGCTCATCATCATCTCGATG | 58 |
| 61RM2 | GATTTGTTTGGTTTCCTTAAG | GGTTGATCTTGGAGGCATTTT | 55 |



### 3.11 PCR Amplification

A ready to go PCR bead was used. Each tube contained a single bead made up of 25 µL reaction mix. The PCR beads composed of stabilizers, dNTPs, 2.5 units puReTaq DNA polymerase, Bovine serum albumin(BSA) and reaction buffer. The pre-mix was divided into two PCR test tubes. 0.5 µL each of forward and reverse primers were added to the tube and gently mixed. After the solution appeared clear, it was fully dissolved and mixed again. The air-dried disc was gently placed in a PCR tubes which were labeled in accordance with the disc and loaded onto a thermocycler and runned in ABI 2720 thermal cycler (Applied Biosystems). PCR conditions consisted of denaturing at 94°C for 3 minutes, annealing at temperatures (**Table 3.5**) for each primer for 30 seconds and extension at 72°C for 30 seconds. This cycle was repeated 35 times and final extension at 72°C for 10 minutes. The PCR products were further run on horizontal polyacrylamide gel electrophoresis (h-PAGE) (81-2325 by Galileo Biosciences, dimension of tank: 32 cm W x 37.5 cm L x 10.5 cm H;

dimension of plate: 24.5 cm W x 27.5 cm L) to separate and resolve the bands with the protocol indicated below.

### 3.12 Casting the Gel

A 5% acrylamide gel was cast in a tray (27.5 cm 24.5 cm) with barriers to retain acrylamide and a 50 well-forming combs were inserted to create wells following manufacturer's instructions (**Table 3.6**). A lid was used to cover the tank with the gel to prevent oxygen inhibition of polymerisation. The 100 ml volume required to fill the tank was determined by weighing the tank before and after filling with water. A monomer and catalyst mix mix was poured into the tank and distributed across the whole surface, removing bubbles. The comb was inserted, the lid was laid on the tank and lowered carefully and pressed gently against the comb. This was allowed to polymerise. The whole assembly was transferred into electrophoresis tank and the comb was removed when the assembly was submerged in buffer (3-5 mm above the lid). The PCR products were loaded into the wells. During loading care was taken to avoid the 'skirt.' of polyacrylamide that might fall into the well. The lid was left in place during loading and electrophoresis. The gel was run at to at least half way to the end of the glass and a spatula was used to prise off the lid after running the gel. The gel was stained with a solution of ethidium bromide, 0.5 µg/ml for 30 minutes using the same volume used to make the gel. The gel was photographed under Ultraviolet light.

**Table 3.6: Preparation of 100 ml 5% acrylamide gel**

| Reagent | Volume |
|---|---|
| 40% acrylamide solution | 12.5 ml |
| 5X TBE Buffer | 20 ml |
| 10% Ammonium per sulphate (APS) | 0.7 ml |
| Distilled Water | 66.68 ml |
| TEMED | 0.12 ml |
| Total Volume | 100 ml |



### 3.13 Development of Crossing Block

The crossing block was designed using the North Carolina Design II. In this design, each member of a group of parents (resistant parents) used as males was mated to each member of the group of parents (susceptible parents) used as females (Acquaah, 2012). The selected parents were planted in perforated pots filled with sandy loam soil. Three pots were used for each resistant line in order to obtain more anthers for the crosses. Four seeds were planted in each pot which was later tinned to three after germination. Planting dates were staggered in order to synchronize flowering. Each resistant male was crossed to all the susceptible females **(Table 3.7).**

**Table 3.7: Crossing Block Layout**

| | Females | | | | | |
|---|---|---|---|---|---|---|
| Males | MAGIC 263 | MAGIC 019 | MAGIC 020 | MAGIC 034 | MAGIC 262 | Apagbaala |
| MAGIC 074 | X | X | X | X | X | X |
| MAGIC O72 | X | X | X | X | X | X |
| MAGIC 213 | X | X | X | X | X | X |
| Kirk house Benga 1 | X | X | X | X | X | X |
| Songotra | X | X | X | X | X | X |
| Suvita-2 | X | X | X | X | X | X |
| Wang Kae | X | X | X | X | X | X |

### 3.13.1 Crossing Procedure

All crosses were done in the screen house. Opened flowers of the donor parents were picked into labeled petri dishes early in the morning and kept in the fridge. The stored flowers were used to pollinate recipient (female) plants in the late afternoon around the hours of 5:30 to 6:30 pm. A blade was used to cut unopened flower buds just at the tip and the stamens were carefully removed leaving the pistil. The opened flowers from the fridge were also cut transversely. The upper portion of the cut flower of donor parent containing the pollen was used to cover the exposed pistil of the female bud. The covering was done such that the pollen had contact with the stigma of the pistil. The cross pollinated buds were tagged with a red tread to differentiate them from the selfed pods (**Plate 3.5**). Dried pods were harvested about three weeks after pollination.

The resistant parents (P1) was crossed to introduce traits into susceptible parents (P2). The P1 was the donor where the source of desirable traits was coming from and P2 was the recipient parent where the desirable traits was transferred to. The F1 (First filial generation) was the offspring of the resistant and susceptible parents.

$P_1$ × $P_2$

Resistant ↓ Susceptible

**F1**

**Plate 3.5**: Cross pollinated bud tagged with a red tread

### 3.14 Selfing of $F_1$ to Produce $F_2$ Seeds

The $F_1$ pods were harvested and stored in envelops. They were then dried inside a solar dryer for 24 hours. Seeds of $F_1$ crosses were planted in pots filled with sandy loam soil. Two weeks after emergence, DNA was sampled from the seedlings onto FTA cards. The FTA cards were sent to the molecular lab where the C42-2B marker was used to test the success of the crosses. $F_1$ seedlings that were identified as true hybrids by the markers were selfed to produce $F_2$ pods.

### 3.15 Phenotypic Screening of $F_2$ Population

Dried pods from $F_2$ were harvested per plant basis and placed into envelops. These were divided into two with one part threshed for planting. Threshed $F_2$ seeds were planted in pots artificially infested with *Striga* seeds collected from Manga. All pots filled with sandy loam soil were infested with one spoon full of *Striga gesnerioides* seeds. The infestation was done by removing a third of the soil content in every pot and mixed thoroughly with the *Striga* seeds and then re-poured into the main pot. Planting of $F_2$ seeds were done in a screen house. In the $8^{th}$ week after planting the roots were washed thoroughly free of soil and examined for attachment of *Striga gesnerioides* shoots and tubercles. Plants were washed by immersing into a bucket of water and gently agitated to loosen the soil mass. Plants with attachment, healthy development and emergence of *S. gesnerioides* were classified as susceptible and those that appeared free from infection, without any attachment were classified as resistant.

### 3.15.1 Genotyping $F_2$ Populations with C42 - 2B

Young leaves from two weeks old plants were collected from $F_2$ plants onto FTA matrix cards (Kirkhouse Trust protocol, 2016). A total of 102, 69, 40 and 33 $F_2$ progenies from a cross of Magic 072 × Apagbaala, Magic 072 × Magic 020, Suvita-2 × Magic 020 and Suvita-2 × Magic 263, respectively were collected onto FTA matrix cards. The marker C42-2B was used to genotype the $F_2$ individuals.

### 3.16 Progeny Testing of $F_3$ Seeds

Three $F_3$ population (Magic 072 × Apagbaala, MAGIC 072 × MAGIC 020 and Suvita-2 × MAGIC 263) were screened against *Striga gesnerioides*. One of the populations (Suvita-2 × MAGIC 020), was dropped because of insufficient *Striga* seeds. The resistant and susceptible $F_3$ seeds of the three populations were artificially screened against *Striga gesnerioides* to either confirm or reclassify $F_2$ scoring and to also classify resistant $F_3$ into homozygous and heterozygous resistant. $F_3$ seeds were grouped into families and within each family, one seed was planted per pot to make scoring for *Striga* more accurate (**Plate 3.6**). Each plastic pot was artificially infested with *Striga* seeds before the $F_3$ seeds were planted. Eight (8) weeks after planting, the families were scored. Families that showed complete resistance among its progenies were classified as homozygous resistant. Families that had both resistant and susceptible individuals were classified as heterozygous resistant. Families that showed complete susceptibility were classified as homozygous susceptible.

**Plate 3.6:** Plant per pot *Striga gesnerioides* screening of F3 families

### 3.17 Data Analysis

Data for 50% flowering, number of pods per plant and grain yield were subjected to Analysis of varience to estimate the level of significant differences using least significant difference (LSD) test at 5% (Steel *et al*., 1997). Chi-square ($\chi^2$) test was performed to test the goodness of fit to a (3:1; resistant: susceptible) ratio in the $F_2$ population and (1:2:1; resistant: heterozygous: susceptible) ratio in the $F_3$ populations.

The segregation distortion test was performed using the Chi-square goodness of fit test formula as shown below

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

Where O = observed values, E = expected values

# CHAPTER FOUR

# RESULTS

## 4.0 Introduction

This chapter presents the results on phenotypic screening of three hundred (300) MultiParent Advance Generation Inter-Cross population and their eight founder parents for sources of resistance to *Striga gesnerioides* and the mode of inheritance of the resistant RILs. The chapter also presents results on *Striga gesnerioides* linked markers to $F_1$ and $F_2$ progenies and the $F_2$ reaction to *Striga gesnerioides*. Finally, the chapter presents result on reclassification of $F_2$ progenies by screening of $F_3$ progenies against *Striga gesnerioides* and classifying resistant families into heterozygous and homozygous resistant.

## 4.1 Evaluation of 300 Multi-Parent Advanced Generation Intercross (MAGIC) Population and the 8 parents for Resistance to *Striga gesnerioides*

The results of the pot screening on three hundred RILs of the MAGIC population and their eight founder parents are presented in **Plate 4.1, Plate 4.2, Plate 4.3** and **Plate 4.4**. Four (4) out of the three hundred RILs of the MAGIC population and their eight parents were found to be resistant.

The resistant lines include MAGIC 072, MAGIC 074, MAGIC 213 and Suvita-2. The Resistant lines had normal growth and development without *Striga* emergence or attachment (**Plate 4.2** and **Plate 4.3**). In contrast, *Striga* emerged on the surface of the soil in pots of most of the susceptible lines whilst in other cases where there was no emergence, *Striga* was found attached to the roots of the susceptible lines after destructive sampling (**Plate 4.1** and **Plate 4.3**). These susceptible cowpea plants expressed varied symptoms due

to *Striga* stress. Notable symptoms include stunted growth, leaf necrosis, defoliation, chlorosis, senescence, no or little flowering and therefore no or little podding (**Plate 4.1**).

**Plate 4.1**: A susceptible RIL showing *Striga* emergence, necrotic symptoms, stunted growth, no flower formation and no podding.


**Plate 4.2**: A resistant check showing no *Striga* emergence, vigorous growth with a good number flowers and pods.

**Plate 4.3**: A resistant RIL showing no of *Striga gesnerioides* attachment

**Plate 4.4**: A susceptible RIL showing attachment of *Striga gesneriodes* at the roots.

### 4.2. Reaction of Founder Parents, Resistant Lines and Agronomic Performing Susceptible Lines to *S. gesnerioides* Infestation in Field and Pot Experiment

**Table 4.1** shows the number of days to *Striga* emergence, the number of days to 50% flowering, number of pods per plant and grain yield of the *Striga* resistant lines, some susceptible lines and their founder parents. The number of days to *Striga* emergence were recorded only on susceptible RILs whilst resistant RILs showed no emergence. A susceptible RIL, MAGIC 263 did not record *Striga* emergence due to tiny and poorly developed *Striga* shoots on the roots but was found susceptible in the 8$^{th}$ week after washing its roots. Days to *Striga* emergence ranged from 35 to 47 days after planting for susceptible RILs in the pot screening. The mean for days to *Striga* emergence was 40 recorded by MAGIC 019. Among the susceptible RILs, IT89KD-288 and IT93K-503-1 recoded the lowest days to *Striga* emergence whilst *Striga* emerged late in MAGIC 020 (**Table 4.1**).

The result showed that the resistant and susceptible lines / parents generally exhibited no significant differences for days to 50% flowering in *Striga* infested condition (**Table 4.1**). Days to 50% flowering varied from 40 to 53 and 36 to 43 for susceptible and resistant lines, respectively in *Striga* infested pot. The mean for 50% flowering was 44 and 41 days for susceptible and resistant lines, respectively. The grand mean for 50% flowering was 43 days **(Table 4.1).** Among the susceptible lines under *Striga* infested condition, IT84S-2049 was the earliest at 40 days to attain 50% flowering whilst IT93K-503-1 was late to attain 50% flowering at 53 days **(Table 4.1).** Among the resistant lines, MAGIC 074 was the earliest to attain 50% flowering at 36 days after planting whilst MAGIC 072 was late to attain 50% flowering at 43 days (**Table 4.1**).

Contrary to the *Striga* infested condition, result of field condition showed that the resistant and susceptible lines / parents generally exhibited significant differences for days to 50% flowering (**Table 4.1**). The number of days to 50% flowering varied from 33 to 49 and 38 to 45 days for susceptible and resistant lines, respectively under field condition. The mean for 50% flowering was 38 and 42 days for susceptible and resistant lines, respectively. The grand mean for 50% flowering was 40 days. Among the susceptible lines under field condition, CB27 and MAGIC 263 were the earliest at 33 days to attain 50% flowering whilst IT93K-503-1 was late to attain 50% flowering at 49 days. Among the resistant lines, MAGIC 213 was the earliest at 35 days to attain 50% flowering whilst MAGIC 074 was late to attain 50% flowering at 45 days **(Table 4.1)**.

The result showed that the resistant and susceptible lines / parents generally exhibited significant differences for the number of pods per plant under *Striga* infested conditions **(Table 4.1)**. Number of pods per plant ranged from 2 to 13 pods and 9 to 23 pods for susceptible and resistant lines, respectively under *Striga* infestation. The mean for number of pods per plant was 7 and 13 for susceptible and resistant lines, respectively. The grand mean for number of pods per plant was 10. Among the *Striga* resistant lines under, MAGIC 074 recorded 23 pods/plant, representing the highest whilst MAGIC 072 recorded the lowest of 9 pods/plant. Among the susceptible lines under artificial infested pot condition, MAGIC 020 recorded 13 pods/plant, representing the highest whilst MAGIC 019 recorded the lowest pods/plant of 2 **(Table 4.1**).

The result showed that the mean for the resistant and susceptible lines / parents generally exhibited significant differences for the number of pods per plant under field conditions **(Table 4.1**). Number of pods per plant ranged from 9 to 28 pods and 9 to 33 pods for

susceptible and resistant lines, respectively under natural field screening. The mean for number of pods per plant was 16 and 28 for susceptible and resistant lines, respectively. The grand mean for number of pods per plant was 22. Among the resistant lines, MAGIC 074 recorded 33 pods/plant, representing the highest whilst Suvita-2 recorded the lowest of 20 pods/plant. Among the susceptible lines, IT84S-2246 recorded 28 pods/plant, representing the highest whilst MAGIC 263 recorded the lowest pods/plant of 9 **(Table 4.1**).

The result showed that the resistant and susceptible lines / parents generally exhibited significant differences for grain yield under *Striga* infested condition **(Table 4.1**). The yield varied in the MAGIC population with a range of 0 to 1818.75 (mean = 586) and 575 to 700 kg/ha (mean = 636) for susceptible and resistant lines, respectively under *Striga* infested pot. The grain yield for some of the susceptible lines was high which was significantly higher than the resistant ones under *Striga* infestation (**Table 4.1**). The highest yield obtained from the *Striga* resistant lines was 700 kg/ha recorded by MAGIC 072 whilst MAGIC 074 recorded the lowest yield of 574 kg/ha. IT00K-1263, a susceptible line recorded the highest yield of 1818.75 kg/ha whilst IT89KD-288 and MAGIC 019 recorded 0 kg/ha representing the lowest yield for the susceptible lines **(Table 4.1**).

The result showed that the resistant and susceptible lines/parents generally exhibited significant differences for grain yield under *Striga* infested condition. The yield variation in the MAGIC population ranged from 30.21 to 2516.66 and 577.78 to 1196.43 kg/ha for susceptible and resistant lines, respectively under field condition (**Table 4.1).** The highest yield obtained from the resistant lines was 1196.43 kg/ha recorded by MAGIC 213 whilst Suvita-2 recorded the lowest yield. MAGIC 020, a susceptible line recorded 2516.66 kg/ha

representing the highest yield whilst IT93K-503-1 recorded 30.21 kg/ha representing the lowest yield for the susceptible lines **(Table 4.1**).

**Table 4.1**: Statistical analysis of variance for Days to 50% flowering, Number of pods per plant and Grain yield.

| | Days To 50% Flowering | | Number of pods per plant | | GRAIN weight (Kg/ha) | |
|---|---|---|---|---|---|---|
| Entry | Pot | Field | Pot | Field | POT | FIELD |
| CB27 | 41 | $33_{a}$ | $5_{a}$ | $14_{a}$ | $56.25_{a}$ | $598.48_{a}$ |
| IT00K-1263 | 44 | $42_{b}$ | $7_{b}$ | $16_{ab}$ | $1818.75_{b}$ | $1012.82_{b}$ |
| IT82E-18 | 42 | $38_{bc}$ | $7_{b}$ | $15_{ab}$ | $518.75_{bc}$ | $354.16_{bc}$ |
| IT84S-2049 | 40 | $39_{bc}$ | $8_{b}$ | $12_{acd}$ | $250_{bcd}$ | $434.52_{bc}$ |
| IT84S-2246 | 41 | $41_{bd}$ | $4_{cd}$ | $28_{bcde}$ | $975_{bcde}$ | $902.77_{bde}$ |
| IT89KD-288 | - | $57_{bcdef}$ | - | $11_{bcdf}$ | $0_{acef}$ | $275.64_{bcef}$ |
| IT93K-503-1 | $53_{a}$ | $49_{bcdefg}$ | $9_{bce}$ | $13_{acf}$ | $1375_{bcdefg}$ | $30.21_{bcdefg}$ |
| Suvita-2 | 42 | $42_{bdegh}$ | $10_{bcde}$ | $20_{bcdefgh}$ | $593.75_{bcefgh}$ | $577.78_{acdefgh}$ |
| MAGIC019 | 50 | $37_{bcfghi}$ | $2_{bcdefg}$ | $22_{bcdefgh}$ | $0_{acdefhi}$ | $2378.78_{bcdefghi}$ |
| MAGIC020 | 46 | $34_{bcdefghij}$ | $13_{bcdefgh}$ | $27_{bcdeghij}$ | $362.5_{bcdefghi}$ | $2516.66_{bcdefghij}$ |
| MAGIC034 | 45 | $42_{bdeghjk}$ | $5_{acdfghi}$ | $15_{abefgijk}$ | $112.5_{acdefghij}$ | $815.47_{bcdeghijk}$ |
| MAGIC262 | 42 | $35_{bcdefghil}$ | $5_{acdfghi}$ | $24_{bcdefghik}$ | $450_{bcefghijk}$ | $1069.44_{bdefghijkl}$ |
| MAGIC263 | 42 | $33_{acdefghijl}$ | $12_{bcdefgh}$ | $9_{bcdefhijkl}$ | $1112.5_{bcdefghijkl}$ | $723.96_{bcdefghijkm}$ |
| MAGIC072 | 43 | $45_{bcdefghijklmn}$ | $9_{bcehij}$ | $32_{bcdefghijkl}$ | $700_{bcdefghijklm}$ | $632.35_{acdefghijklm}$ |
| MAGIC074 | $36_{b}$ | $44_{bdefghjkmn}$ | $23_{bcdefghij}$ | $33_{bcdefghijkl}$ | $575_{bcefghjklmm}$ | $1174.24_{bcdefghijklno}$ |
| MAGIC213 | 42 | $38_{bcfghiklmno}$ | $9_{bcehijk}$ | $27_{bcdeghijlm}$ | $675_{bcdefghijklmmo}$ | $1196.43_{bcdefghijklmno}$ |
| Grand mean | 42.1 | 40.56 | 7.88 | 19.9 | 598.4 | 917 |
| CV (%) | 19.4 | 3.1 | 8.7 | 6.3 | 8.9 | 8 |
| LSD (5%) | 13.66 | 2.08 | 1.14 | 2.1 | 88.3 | 122.1 |

CV: Coefficient of variation; LSD: Least significant difference, Variables with different letter within the column shows significant difference

## 4.3 Molecular Analysis

### 4.3.1 Polymorphic Selection of Screening Population

The crossing blocks of the three resistant checks (Wang kae, Songotra and kirkhouse Benga) were dropped after C42-2B distinguished resistant checks and MAGIC resistant lines at different base pairs. SSR1, a functional marker amplified only the resistant checks at 150bp, whilst 61RM2 amplified resistant checks, resistant and susceptible MAGIC lines at the same base pair (400bp). MAGIC 034 and MAGIC 262 were also dropped because the polymorphic marker C42-2B amplified them at the same base pair with the resistant MAGIC lines.

### 4.3.2 DNA Marker Screening for Polymorphism

The SSR-1 marker, a functional *Striga* marker amplified at 150 bp for resistant checks but did not amplify for the resistant MAGIC RILs and parent (**Figure 4.1).**

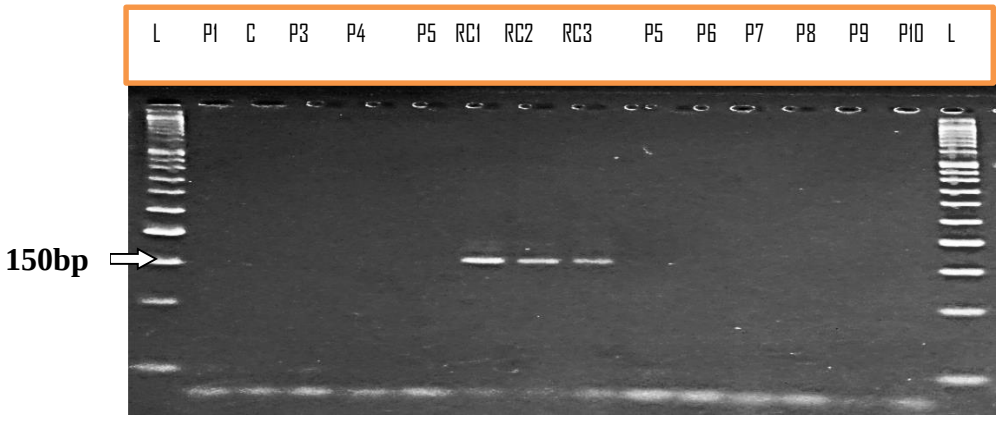


**Figure 4.1:** A 2% agarose gel electrophoretic analysis of PCR amplified product using SSR-1 marker with amplification at 150 bp for polymorphism screening of resistant and susceptible parents. Arrow pointing to amplification. L, 50 bp ladder.

The C42-2B marker with amplification at 280 bp in the resistant checks and also shows amplification at 490 bp in the *Striga* resistant test lines but completely absent in the susceptible lines with exception to P7 and P9 **(Figure 4.2).**

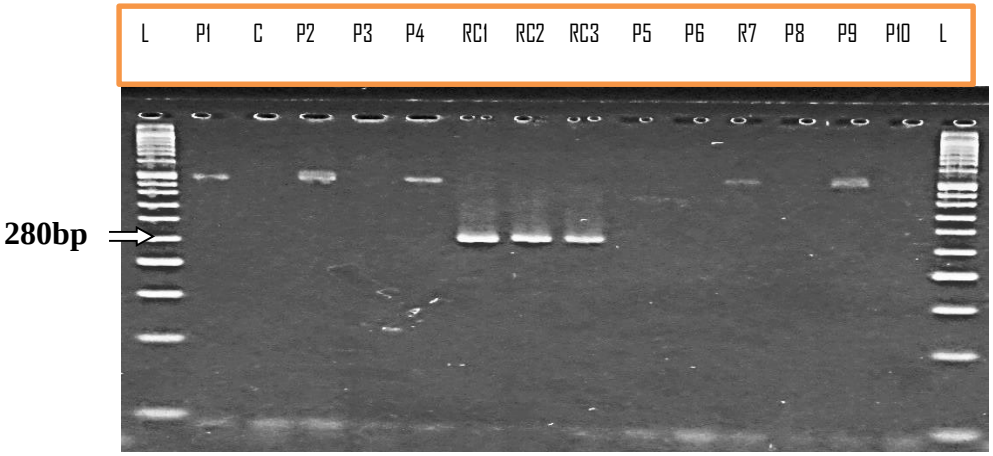


**Figure 4.2:** A 2% agarose gel electrophoretic analysis of PCR amplified product using C42-2B marker with amplification at 280 bp for polymorphism screening of resistant and susceptible parents. Arrow pointing to amplification. L, 50 bp ladder.

The 61RM2 marker with amplification at 400 bp in the resistant checks, susceptible lines and *Striga* resistant test lines except $P_1$. A 50 bp molecular ladder was run alongside the DNA samples **(Figure 4.3)**.

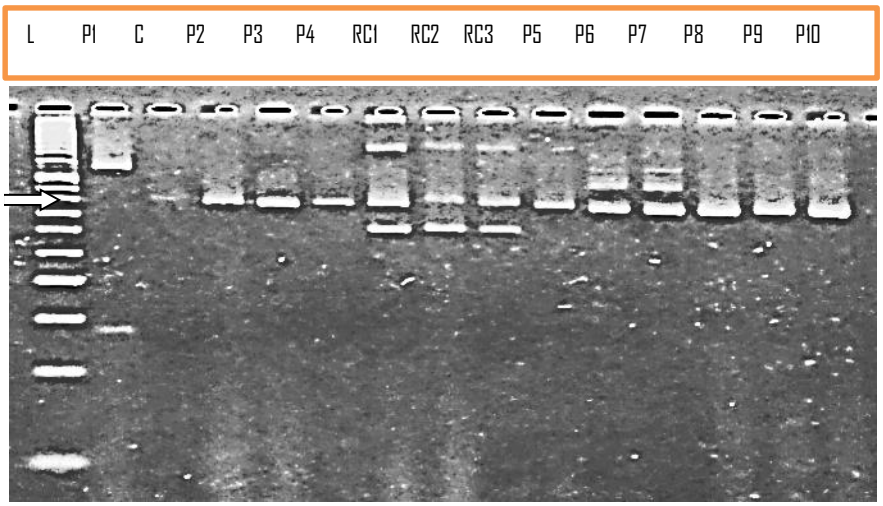


**Figure 4.3**: A 2% agarose gel electrophoretic analysis of PCR amplified product using 61RM2 marker with amplification at 400 bp for polymorphism screening of resistant and susceptible parents. Arrow pointing to amplification. L, 50 bp ladder.

$P_1$= resistant genotype (Suvita-2), $P_2$= Resistant line (Magic 072), $P_3$= Resistant line (Magic 074), $P_4$= Resistant line (Magic 213), RC1= Resistant check (Wang Kae), RC2= Resistant check (Kirkhouse Benga 1), RC3 = Resistant check (Songotra), $P_5$= Susceptible line (Magic 262), $P_6$ = Susceptible genotype (Magic Apagbaala), $P_7$ = Susceptible line (Magic 034), $P_8$= Susceptible line (Magic 019), $P_9$= Susceptible line (Magic 263), $P_{10}$= Susceptible line (Magic 200) and C= Control.

### 4.4 Hybridity confirmation F1 Progenies

A total of 20 $F_1$ progenies (comprising 5 $F_1$ progenies from a cross of Magic 072 × Magic 019, 6 $F_1$ progenies from a cross of Magic 072 × Apagbaala, 3$F_1$ from a Magic 072 × Magic 020 population, 3$F_1$ from a cross Suvita-2 × Magic 020, 2 $F_1$ progenies from a cross Suvita-2 × Magic 263, 1 $F_1$ progeny from a cross of Magic 213 × Apagbaala) were evaluated with C42-2B marker. The presence of a band indicates a successful cross whilst absence of a

band indicates unsuccessful cross. All 5 $F_1$ progenies from a cross of Magic 072 × Magic 019 genotyped, were successful. Again all 6 $F_1$ progenies from a cross of Magic 072 × Apagbaala genotyped, were successful. In Magic 072 × Magic 020 population, 2 out of 3 $F_1$ progenies genotyped were successful. All 3 $F_1$ progenies from a cross Suvita-2 × Magic 020 genotyped, were successful. Again all 2 $F_1$ progenies from a cross Suvita-2 × Magic 263 genotyped, were successful. In Magic 213 × Apagbaala population, the 1 $F_1$ progeny genotyped was not successful. The three MAGIC resistant checks genotyped (Suvita-2, Magic 072, Magic 213) had the resistant band whilst the susceptible check (Apagbaala) did not have the band (**Figure 4.4**).

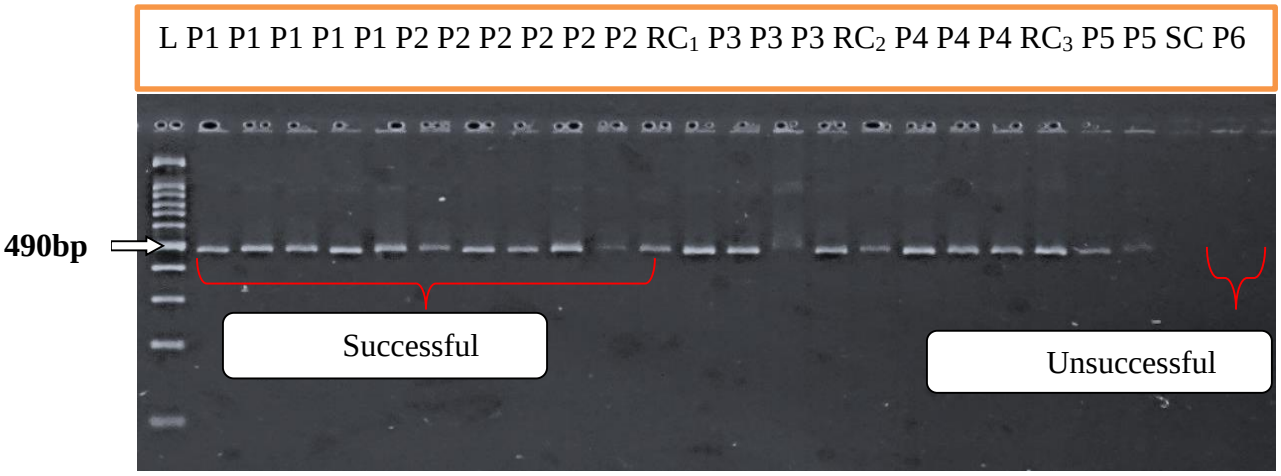


**Figure 4.4:** A 2% agarose gel electrophoretic analysis of PCR amplified product using C42-2B marker with amplification of successful parent at 490 bp and no amplification for non-successful parents. L, 100 bp ladder.

$P_1$= Magic 072 × Magic 019, $P_2$ = Magic 072 × Apagbaala, $P_3$ =Magic 072 × Magic 020, $P_4$ = Suvita 2 × Magic 020, $P_5$ = Suvita 2 × Magic 263, $P_6$ =Magic 213 × Apagbaala, $RC_1$ =

resistant check (Magic 072), $RC_2$= Resistant check (Suvita 2) and $RC_3$ = Resistant check (Magic 213) and SC = Susceptible check (Apagbaala).

### 4.5 Screening F2 Population with C42-2B Marker

Three $F_2$ populations were genotyped with the C42-2B marker to check for marker trait association. The marker showed that out of 102 $F_2$ progenies from a cross of Magic 072 × Apagbaala, 51 individuals had the resistant band whilst 51 individuals did not have the band. Three (3) progenies of resistant parent used as resistant checks all had the resistant band whilst the 3 susceptible parent used as susceptible check did not have the band. There was no amplification from the control well (**Figure 4.5**).

The presence of the alleles in **Figure 4.5** was scored 1 meaning resistant and absence of the alleles was scored 0 meaning susceptible. The product size of the marker was 280 bp but amplification of resistant correspond to the 490 bp of the resistant parents. All bands that correspond to the 490 bp were scored as resistant and those without bands were scored susceptible.

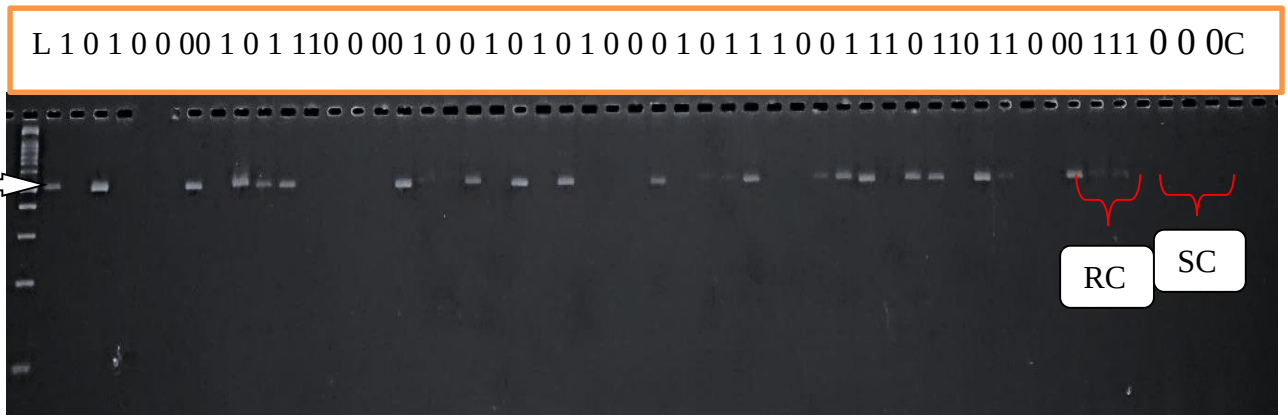


**Figure 4.5:** C42-2B marker scored on ethidium bromide stained agarose gel for Magic 072 × Apagbaala. Marker amplify a band only on resistant genotypes. Arrow pointing to band of interest at 490 bp. RP = resistant parent (Magic 072), SP = susceptible parent (Apagbaala). C= Control. The 0 and 1 represent susceptible and resistant progenies of $F_2$, respectively. L = ladder, 100 bp.

The marker showed that out of 69 $F_2$ progenies from the cross MAGIC 072 × MAGIC 020, 42 individuals had the resistant band whilst 27 individuals did not have the band. All the three (3) progenies of resistant parent used as resistant checks had the resistant band whilst the three susceptible parent used as susceptible check did not have the band. There was no amplification from the control well (**Figure 4.6**).

The presence of the alleles in **Figure 4.6** was scored 1 meaning resistant and absence of the alleles was scored 0 meaning susceptible. The product size of the marker was 280 bp but amplification of resistant correspond to the 490 bp of the resistant parents. All bands that correspond to the 490 bp were scored as resistant and those without bands were scored susceptible.


**Figure 4.6**: C42-2B marker scored on ethidium bromide stained agarose gel for Magic 072 × Magic 020. Marker amplify a band only on resistant genotypes. Arrow pointing to band of interest. RP = resistant parent (Magic 072), SC = susceptible parent (Magic 020), C= Control. The 0 and 1 represent susceptible and resistant progenies of $F_{2,}$ respectively.

The marker showed that out of 33 $F_2$ progenies from a cross of Suvita-2 × Magic 263, 19 individuals had the resistant band whilst 14 individuals did not have the band. In contrast, one out of three progenies of the resistant parent used as resistant checks had the resistant band whilst one out of the three susceptible parent used as susceptible check did not have the band (**Figure 4.7**).

The presence of the alleles in **Figure 4.7** was scored 1 meaning resistant and absence of the alleles was scored 0 meaning susceptible. The product size of the marker was 280 bp but amplification of resistant correspond to the 490 bp of the resistant parents. All bands that correspond to the 490 bp were scored as resistant and those without bands were scored susceptible.

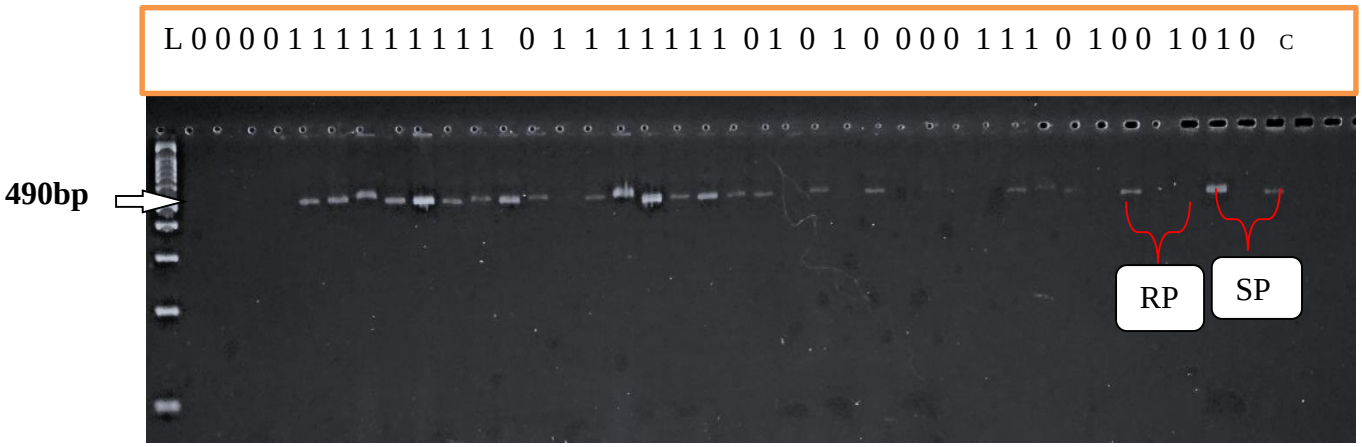


**Figure 4.7**: C42-2B marker scored on ethidium bromide stained agarose gel for Suvita-2 × Magic 263. Marker amplify a band only on resistant genotypes. Arrow pointing to band of interest. RP = resistant parent (Suvita-2× Magic 263), SP = susceptible parent (Magic 263), C = Control. The 0 and 1 represent susceptible and resistant progenies of $F_2$, respectively.

### 4.6 Phenotypic Screening and Reclassification of $F_2$ Progenies by Progeny Testing

Three $F_2$ populations were screened for their resistance to *Striga gesnerioides*. In Magic 072 × Apaagbala population, a total of 102 $F_2$ progenies were screened. In Magic 072 × Magic 020 population, a total of 65 $F_2$ progenies were screened whilst in Suvita-2 × Magic 263 population, a total of 33 $F_2$ progenies were screened.

#### 4.6.1 MAGIC 072 × APAGBAALA

The genetic ratios and chi square values for *Striga* resistance in $F_2$ and $F_{2:3}$ populations between Magic 072 and Apagbaala (**Table 4.2**). The phenotypic segregation of the $F_2$ generation was tested using the chi square for the hypothesis of 3:1 resistant to susceptible ratio (**Table 4.2**). The $F_2$ generation from Magic 072 × Apagbaala yielded 44 resistants (no *Striga* emergence and attachment) against 49 susceptible with fully emerged *Striga* and attachment (**Table 4.2**). The observed segregation ratio of resistant versus susceptible in the cross fits closely to a 1R:1S (resistant: susceptible) ($\chi^2$ =38.03, P=<0.0001) genetic ratio instead of 3:1 expected ratio for cowpea *Striga* resistance. The 3 resistant parent (Magic 072) used as checks had no *Striga* emergence or attachment whilst the susceptible parent (Apagbaala) used as checks had fully emerged *Striga* (**Table 4.2**).

The $F_3$ generation segregated into 3 categories namely 23 resistants, 48 segregating and 22 susceptible. The numbers were not significantly different from the expected 1 resistant: 2 segregating: 1 susceptible ratio at 5% significant level (**Table 4.2**).

The F2 progenies were either reclassified or confirmed using the segregation of their $F_3$ progenies (**Table 4.2**). The reclassified $F_2$ deviated from the earlier $F_2$ score by yielding 71

resistant as against 22 susceptible fitting into the expected 3R:1S ratio ($\chi^2$ = 0.0896, P = 0.7647).

**Table 4.2: Genetic ratios and Chi square values for *Striga* resistance in phenotypic $F_2$ and $F_3$ populations between MAGIC 072 and APAGBALA crosses**

| Population | expected ratio | OBSERVED SEGREGATION | | | | |
|---|---|---|---|---|---|---|
| | | resistant | Segregating | Susceptible | $\chi_2$ | p value |
| Magic 072 | 1:0 | | | | - | - |
| Apagbaala | 0:1 | | | | - | - |
| $F_2$ | 3:1 | 44 | N/A | 49 | 38.03 | <0.0001 |
| $F_3$ | 1:2:1 | 21 | 48 | 22 | 0.1183 | 0.9426 |
| $F_2$ corrected | 3:1 | 71 | N/A | 22 | 0.896 | 0.7647 |

### 4.6.2 Suvita-2 × MAGIC 263

The results of the chi square goodness of fit test for the phenotypic segregation of $F_2$ and $F_3$ populations of Suvita 2 x MAGIC 263 for 3:1 resistant to susceptible and 1:2:1 resistant: segregation: susceptible ratios respectively (**Table 4.3**). The $F_2$ generation of Suvita-2 × Magic 263 yielded 31 resistant and 7 susceptible fitting into the expected 3R:1S genetic ratio ($\chi^2$ = 0.8772, P= 0.3490).

The $F_3$ generation segregated into 10 resistant, 16 segregating and 12 susceptible. These numbers were not significantly different from the expected 1 resistant: 2 segregating: 1 susceptible ratio at 5% significant level ($\chi^2$ = 1.1579, P= 0.5605).

The $F_2$ after reclassifying yielded 26 resistants' as against 12 susceptible fitting into the expected 3R:1S ratio ($\chi^2$ = 0.0526, P = 0.8185) and thereby confirming the 3:1 segregation of the $F_2$ generation (**Table 4.3**).

**Table 4.3: Genetic ratios and Chi square values for *Striga* resistance in phenotypic $F_2$ and $F_3$ populations between Suvita-2 and MAGIC 263 crosses**

| | | OBSERVED SEGREGATION | | | | |
|---|---|---|---|---|---|---|
| Population | expected ratio | Resistant | Segregating | Susceptible | $\chi_2$ | p value |
| Suvita -2 | 1 : 0 | | | | - | - |
| Magic 263 | 0 : 1 | | | | - | - |
| $F_2$ | 3 : 1 | 31 | N/A | 7 | 0.8772 | 0.3490 |
| $F_3$ | 1 : 2 : 1 | 10 | 16 | 12 | 1.1579 | 0.5605 |
| $F_2$ corrected | 3 : 1 | 26 | N/A | 12 | 0.5260 | 0.8185 |

### 4.6.3 MAGIC 072 × MAGIC 020

**Table 4.4** shows the genetic ratios and chi square values for *Striga* resistance in $F_2$ and $F_{2:3}$ populations between Magic 072 and Magic 020. The $F_2$ generations was tested for chi square goodness of fit for the hypothesis of 3:1 resistant: susceptible segregation ratio. The $F_2$ population segregated into 34:16 resistant: susceptible. This observed ratio was a fit to the expected 3:1 ratio ($\chi^2$ = 1.3067, P = 0.2530).

The $F_{2:3}$ population segregated into 9 resistant: 30 segregating: 11 susceptible, a fit for the expected 1 resistant: 2 segregating: 1 susceptible ratio at 5% significant level ($\chi^2$ = 2.1600, P = 0.3396).

The $F_2$ after reclassification and confirmation using the $F_{2:3}$ data, yielded 40 resistant as against 10 susceptible fitting into the expected 3:1 ratio ($\chi^2$ = 0.6667, P = 0.4142) and thereby confirming the 3R: 1S segregation of the $F_2$ generation (**Table 4.4**).

**Table 4.4: Genetic ratios and chi square values for *Striga* resistance in phenotypic $F_2$ and $F_3$ populations between MAGIC 072 and MAGIC 020 crosses**

| | | OBSERVED SEGREGATION | | | | |
|---|---|---|---|---|---|---|
| Population | expected ratio | Resistant | Segregating | Susceptible | $\chi_2$ | p value |
| Magic 072 | 1 : 0 | | | | - | - |
| Magic 020 | 0 : 1 | | | | - | - |
| F2 | 3 : 1 | 34 | N/A | 16 | 1.3067 | 0.2530 |
| F3 | 1 : 2 : 1 | 9 | 30 | 11 | 2.1600 | 0.3396 |
| F corrected | 3 : 1 | 40 | N/A | 10 | 0.6667 | 0.4142 |

### 4.7 C42-2b Marker-trait Association in F2 Screening Populations

The C42-2B marker showed polymorphism in genotyping the three $F_2$ populations (Magic 072 × Apagbaala, Magic 072 × Magic 020 and Suvita-2 × Magic 263). Segregation distortion was detected for the genotyped segregation populations, deviating from the expected 3R:1S Mendelian ratio. The marker screening detected 48 (47%) out of the 93 polymorphic alleles (**Figure 4.5**) a distortion from the Chi-square test which revealed a 3R:1S (resistant: susceptible) Mendelian ratio (**Table 4.2**) for the reclassified Magic 072 × Apagbaala $F_2$ population. In Magic 072 × Magic 020 population the marker screening detected 39 (25% recombination) out of 63 polymorphic alleles (**Figure 4.6**) distorting from the Chi- square test which revealed 3R:1S Mendelian ratio (**Table 4.4**). In Suvita-2 ×

Magic 263 population, the marker screening detected 11 (43% recombination) out of 36 polymorphic alleles (**Figure 4.7**) distortion from the Chi- square test of 3R:1S Mendelian ratio (**Table 4.3**).



# CHAPTER FIVE

# DISCUSSION

*Striga* has been difficult to eradicate because of its unique ability to adapt to the environment, and complexity of the host-parasite relationship (Botanga and Timko, 2005). However, cowpea cultivars resistant to the parasite is considered the most effective and economically feasible method to control the parasite since it is affordable to resource poor-farmers (Omoigui *et al*., 2007) as well as being friendly to the environment.

Host plant resistance development to *S. gesnerioides* requires the application of phenotypic and genotypic protocols that are diagnostic to screen a population segregating for resistance to the parasite (Lane *et al*., 1994; Singh, 2002). The first identification of resistance to *S. gesnerioides* came from field experiment in Burkina Faso where the varieties Suvita-2 (previously known as Gorom Local) and 58-57 recorded zero or very low emergence of *Striga gesnerioides* (Aggarwal *et al.*, 1984).

## 5.1 Multi-Parent Advanced Generation Intercross (MAGIC) Population Cowpea Reaction to *Striga gesnerioides* under Artificial Infestation

The resistant parent Suvita-2, and it resistant progenies; MAGIC 072, MAGIC 074 and MAGIC 213 showed lack of parasite emergence and attachment.

Resistance to *Striga* of the parent and its progenies was characterized by a lack of parasite emergence and attachment on the root after washing. Contrary, susceptible RILs and parents were epitomized by several parasite attachments on the roots which indicate the supremacy of resistance over susceptible. These suggest that the resistant RILs have the ability to recognize the *Striga* parasite and activate defense response mechanism. This finding is consistent with earlier work reported by Godwa *et al*. (1999) and Omoigui *et al.*

(2015). Godwa *et al*. (1999), pointed out that the response to *Striga* varies among genotypes suggesting that differences exist in the ability of these plants to recognize the pathogen and to activate defense response mechanisms. Omoigui *et al.* (2015) pointed out that cultivars B301 and IT97K-499-35 have resistance to the prevalent race of *Striga* (SG3) from the northeast of Nigeria. Resistance to *Striga* of these lines was characterised by a lack of parasite emergence and attachment on the root.

The mechanism of resistance of these RILs to the *Striga* seeds collected from Manga, in the upper East region of Ghana is not known but could be related to resistance mechanism expressed by Suvita-2. This therefore, suggest that these RILs have similar virulence properties to the known SG1 race in Burkina, or that Suvita-2 has a resistance gene to the Manga isolate which may be different from the SG1 race. Botanga, (2005) reported that when SG1 confronts a resistant host species, such as Suvita-2, containing an active race-specific resistance gene, SG1 stimulates a hypersensitive response in the host root, its post attachment development is immediately arrested, and it quickly dies. This observation is similar to a study reported in Burkina Faso in 1981, where varietal differences with references to *Striga* infection were first noticed in two lines, Suvita-2 and 58-57, were found to be completely resistant (Aggarwal *et al*., 1984).

**5.2 Reaction of Founder Parents, Resistant Lines and Agronomic Performing Susceptible Lines to S. gesnerioides Infestation in Field and Pot Experiment** Data from this study showed that symptoms on RILs and parents as a results of *Striga gesnerioides infection* were in the form of stunted growth, leaf necrosis, chlorosis, senescence, defoliation, reduced size of young leaves, poor or no flowering and poor or no pod formation.

These observations suggest that *Striga* infestation resulted in the competition with the plant for resources, thus, affecting flowering, podding and grain yield. Similar observations have been reported by Gworgwor *et al*. (1991) and Press (1995). Alonge (1999) reported that the reduced vegetative growth of the susceptible varieties resulted in reduced leaf area, photosynthetic capacity and therefore affected flowering, podding and seed production due to serious plant-parasite competition for resources. According to Press (1995), the lower biomass accumulation by the susceptible genotypes could be the result of competition among the host and the weed for solutes, as well as carbon, water, and minor rate of photosynthesis in the leaves of *Striga* infested plant. The reduced photosynthesis might have resulted in lower number of pods per plant and translocation of photosynthate to the sink.

The resistant RILs and parent showed a relatively good growth compared to the susceptible lines and parents in the infested pots. The relatively good growth can be attributed to the ability of resistant lines in suppressing *Striga* growth and development and therefore not competing with the parasite for resources. This is in line with Gworgwor *et al*. (1991) findings on *Striga gesnerioides*. The relatively good growth and the reduced export of assimilate to the weed would have ensured sufficient biomass accumulation and seed development.

Contrary to expectation, there was upsurge in grain yield of some RILs and parents that supported *Striga gesnerioides* growth, which suggest that it was tolerant to the parasite, therefore allowing it to still perform under *Striga* infection. ITOOK-1263, IT93K-503-1, MAGIC 263 and IT84S-288 recorded high grain yields which was significantly higher than the resistant ones under *Striga* infestation. This implies that resistance to *Striga* does not

necessary translate to higher yield by some particular varieties / lines. This can be attributed to the inherent high yielding potentials of these RILs relative to the resistant lines. Ajeigbe *et al.* (2008) define tolerance as the ability of the host crop to produce acceptable yield in the presence of heavily *Striga* infestation. This finding contradicts that of Omoigui *et al.* (2007), who reported that in spite of the relatively higher number of *Striga* emerged on IT81D-994, IT99K-494-6 and Aloka loka plots, grain yield did not significantly differ from the resistant cultivars. This suggests that these cultivars are either moderately resistant or tolerant to *Striga*.

The results revealed that RILs and parents of the field experiment that were found to be susceptible under artificial infestation took less days to attain 50% flowering which is in contrast with data recorded under artificial *Striga* infestation. Susceptible RILs and parents generally yielded more which was significantly higher than the resistant ones. The results of the study also revealed that resistant RILs and parent generally recorded higher number of pods per plant but did not translate to yield. This suggests that susceptible RILs and parents escaped *Striga* infection due to the uneven distribution of *Striga* seeds in the field. This observation is in line with the findings of Kim *et al*. (2002) who reported that the major disadvantages of screening under natural infestation are the variability in *Striga* seeds dissemination and cultivars escaping infestation.

Moreover, the high performance of the susceptible lines and parents in terms of flowering and yield can be attributed to the conducive environmental condition during the time of the field experiment. The higher rainfall recorded within the period could reduce the severity of *Striga* damage by washing *Striga* seeds deeper into the soil, therefore allowing susceptible plants to escape infection. Studies had showed that higher rainfall and soil fertility status may have reduced emergence and severity of *Striga* damage on the cowpea

yields compared to low rainfall and poor soil, which recorded higher number of emerged *Striga* shoot and cause severe crop damage (Omoigui *et al*., 2007).

The results of the study also revealed that some resistant RILs and parents planted under natural condition outperformed that of the pot experiment in terms of grain yield. This observation can be attributed to favourable conditions of the environment at the time the experiment was conducted. The reduction in yield in the pot experiment compared to an increase in yield under natural condition of the same lines can be attributed to high temperature (34.5 $^{o}$C) recorded for the pot experiment. This finding is similar to that of Bagnall and King (1987), who reported that cowpea grain yield is very sensitive to conditions of the environment regardless of its hardiness. Grain yield may have been influenced by the high temperatures at flowering stage which resulted in decreased number of pods, and therefore affected the yield. It is also confirmed by Prasad *et al.* (2002), who reported that exposure to temperatures above 28 $^{o}$C also reduced photosynthesis, seed number and seed yield in kidney bean (*Phaseolus vulgaris L.).*

## 5.3 Molecular Analysis

### 5.3.1 DNA Marker Screening for Polymorphic Marker

The study showed that out of the three markers, C42-2B had associations with the resistant and susceptible genotypes. Despite its association with the trait, it amplified resistant lines and parent at 490 bp instead of the 280 bp reported for *Striga* resistance. C42-2B marker have been found to co-segregate with *S. gesnerioides* race 3 or SG3 resistance gene (Li and Timko, 2009; Omoigui *et al.*, 2009). This explains why it could not amplify Suvita-2 and its resistant progenies at the 280 bp since the study have revealed that resistance of the MAGIC lines and parent to the Manga *Striga* isolate may be resistant to SG1 race due to it

similar virulence properties. Indeed, Suvita-2 has been found to be resistant to races SG1, SG2 and SG4 (Parker and Polniaszek, 1990; Berner *et al*., 1995).

The C42-2B marker identified resistant RILs and parent with a single band whilst susceptible RILs had no band. This observation is similar to findings reported by Omoigui *et al.* (2009), the C42-2B marker identified resistant genotypes with a single band whilst the susceptible genotypes had no bands. The marker is therefore a dominant marker since it only shows presence or absence. Other markers such as SSR1, and 61RM2 showed no association with the trait, and therefore it was not able to distinguish resistant RILs from susceptible ones. The 61RM2 marker which have been reported to be effective in identifying resistance to *Striga* race 1 and 3 (Timko *et al*., 2007; Ouédraogo *et al*., 2012) was expected to distinguish between resistant from susceptible. In contrast, the marker amplified the resistant checks, resistant and susceptible MAGIC lines at the 400 bp, thereby making it non informative. The SSR1 marker which is known to be a functional marker for *Striga* resistance could only amplify the resistant checks and not Suvita-2 and its resistant progenies. The inability of the SSR1 marker to amplify Suvita-2 and its resistant progenies can be attributed to the marker not having resistant alleles responsible for coding resistance in Suvita-2 and its resistant progenies. Indeed, SSR1 has been reported to co-segregate with *Striga gesnerioides* race SG3 (Li and Timko, 2009; Omoigui *et al*., 2009). This could explain why it could not amplify Suvita-2 and its resistant progenies since the study have revealed that resistance of the MAGIC lines and parent to the Manga *Striga* isolate may also be resistant to SG1 due to it similar virulence properties. The results of the study showed that C42-2B marker was the most polymorphic among the other markers (SSR1 and 61RM2).

## 5.4 Mode of Inheritance of *Striga* Resistance

### 5.4.1 $F_2$ and $F_3$ Population

The segregation analysis of the two $F_2$ populations (Suvita-2 × MAGIC 263 and MAGIC 072 × MAGIC 020) revealed that resistance to *S. gesnerioides* is conferred by a single dominant gene action and demonstrated as monogenic resistance. The chi square values obtained from these findings for Suvita-2 × MAGIC 263 ($\chi^2$ = 0.8772, P = 0.3490) and MAGIC 072 × MAGIC 020 ($\chi^2$ =1.3067, P = 0.2530) revealed that the observed ratios showed a goodness-of-fit to Mendelian ratio of 3:1 for a single locus. This was further confirmed in the $F_3$ progeny testing by growing the entire seeds produced by the $F_2$ plants. The $F_3$ families fitted the 1R:2H:1S ratio as confirmed by the chi square test, with probability values of p = 0.5605 and p = 0.3396 for Suvita-2 × MAGIC 263 and MAGIC 072× MAGIC 020 populations, respectively. The results of the $F_3$ phenotypic generation showed few misclassifications during the scoring at the $F_2$ for resistant: susceptible for Suvita-2 × MAGIC 263 and MAGIC 072× MAGIC 020 populations.

This indicates that the selection for resistance at $F_2$ may not be sufficient. The result also revealed that the reclassified $F_2$ also confirmed the 3R:1S genetic ratio of the $F_2$ for a single dominant gene ($\chi$2 = 0.0526, P = 0.8185).

Comparable statement of single dominant gene inheritance to *S. gesnerioides* in B301 had been reported. The gene symbol Rsg1 (resistance to *S. gesnerioides*) was proposed for the populations derived from a cross involving B301 and another susceptible cultivar (Lane and Bailey, 1992; Atokple *et al.,* 1995). Studies on inheritance of *S. gesnerioides* resistance in Suvita-2 in Burkina Faso (Aggarwal *et al*., 1984) and Mali (Toure *et al*., 1997), as well

as B301 in Nigeria (Singh and Emechebe, 1990), Mali and Niger (Toure *et al*., 1997) have indicated the existence of single dominant genes controlling resistance.

In contrast to the two population, the segregation ratio in the $F_2$ population of MAGIC 072 × Apagbaala (Resistant and Susceptible) deviated from the expected 3R:1S ratio for a single dominant gene ($\chi^2$ = 38.03, P = 0.0001). The 1R:1S ratio indicates that the Striga resistance in MAGIC 072 is not controlled by a single dominant gene. This was not expected because the RIL (MAGIC 072) is an advanced $F_8$ line which is assumed to be homogenous for most of it loci. This was disconfirmed in the $F_3$ progeny testing by growing the entire seeds produced by the $F_2$ plants in families. The scoring of the $F_3$ families fitted the 1R: 2H: 1S ratio as confirmed by the chi square test, with probability values of p = 0.9426. This suggests that resistance to *S. gesnerioides* conferred in MAGIC 072 × Apagbaala $F_2$ progenies is a single dominant gene and demonstrated as monogenic resistance. The results of the $F_3$ phenotypic screening revealed huge inconsistencies of resistant and susceptible in the $F_2$ screening. The $F_2$ were therefore reclassified using the $F_3$ by rescoring susceptible $F_2$ that were found to be homozygous and heterozygous resistant in the $F_3$ whilst some resistant $F_2$ were found in the $F_3$ families as homozygous susceptible. After the reclassification of the $F_2$ using the $F_3$ screening, the segregation ratio of the reclassified $F_2$ fitted into the 3R:1S genetic ratio expected for a trait controlled by single dominant gene ($\chi^2$ = 0.0526, P = 0.8185). The inconsistency between $F_2$ and $F_3$ for susceptible: resistance could be attributed to the screening of three $F_2$ progenies in a single *Striga* infected pot and also the poor formation of *Striga* shoots on susceptible progenies. The poorly developed *Striga* shoots could easily detach from a susceptible progeny and find itself between intertwined roots of resistant and susceptible plants therefore giving a

possibility of scoring resistant progenies susceptible. The reason for the inconsistency between $F_2$ and $F_3$ for (resistant: susceptible) could also be attributed to failure to inoculate some pots with *Striga* seeds and therefore susceptible plants escaping *Striga* infection. This suggests that selection for resistance is effective in $F_3$ progeny testing since planting of families is done on a one progeny one *Striga* infected pot basis. The results suggest that the inheritance of resistance to the unclassified Manga *Striga* isolate in Ghana which have showed similar virulence properties to the known SGI race in Burkina is monogenic and dominant with segregation pattern of 3R:1S. This finding also confirmed initial inheritance studies by Atokple *et al*. 1993; and Moore *et al*. (1995); Tignegre, 2010; Asare *et al*., 2013; Omoigui *et al*., 2015 that the nature of resistance to *S. gesnerioides* race SG1, race SG2, race SG3 and race SG4 in some cowpea genotypes to be monogenic and dominant.

### 5.5 C42-2B Marker Linage to $F_2$ Phenotypic Screening

Segregation distortion varies significantly with population types (Song *et al*., 2005). Xu *et al*. (1997) found that the rate of segregation distortion was lower in $F_2$ populations compared to other populations. In contrast, the study showed large segregation distortion detected for the genotyped $F_2$ segregation populations deviating from the expected 3R:1S Mendelian ratio. The C42-2B marker showed a large proportion (47%, 25% and 43%) genetic distortion in the populations, which is a much higher frequency than was expected by chance (5%) (Perfectti and Pascuai, 1996). The C42-2B marker showed a large proportion (47%, 25% and 43%) of distortion for the reclassified MAGIC 072 × Apagbaala, MAGIC 072 × MAGIC 020 and Suvita-2 × Magic 263 population, respectively. The reason for a large proportion of distortion of marker to the phenotype can be attributed to the inability of the marker to amplify the resistant parents at the 280 bp

band. Consequently, the $F_2$ progenies amplifying at the 490 bp. The amplification at a wrong base pair for resistant parents could mean that the marker is not tightly linked to the gene of interest which can result in crossing over between marker and the gene of interest. This could have led to the high percentage of false susceptible (negative) bands scored in the $F_2$ populations. Omoigui *et al*., 2007, reported that flanking markers around the resistance gene is an important factor that can increase the efficiency of indirect selection. When a marker used for selection is not tightly linked to the gene of interest, cross over will occur between marker and the gene of interest. This will lead to a high percentage of falsepositive/negative selections in the screening process.



# CHAPTER SIX

# CONCLUSION AND RECOMMENDATIONS

## 6.1 Conclusion

The study revealed that four out of the three hundred RILs of the MAGIC population and their eight founder parents had resistance to *Striga* seeds collected from Manga. Resistance to *Striga* of this RILs and parent was characterized by a lack of parasite emergence and attachment on the root after washing. These are Suvita-2, MAGIC 072, MAGIC 074 and MAGIC 213.

The three molecular markers associated with *S. gesnerioides* used to determine polymorphism among the selected resistant and susceptible RILs and parents from the phenotypic data showed inconsistencies. The 61RM2 and SSR1 marker was not able to distinguish between resistant and susceptible. The C42-2B marker despite its inability to amplify resistant lines at the recommended 280 bp, was able to distinguish resistant from susceptible at 490 bp. Therefore, C42-2B was the most polymorphic marker among the other markers used.

The study on the gene action controlling *Striga* resistance in the cowpea RILs and parents revealed that *Striga* resistance is controlled by a single dominant gene. Two out of three $F_2$ generations (Suvita-2 × MAGIC 263, MAGIC 072 × MAGIC 020) segregated into (3R:1S). The $F_3$ generation segregated into 1R:2H:1S genetic ratio confirming that *Striga* resistance at the two $F_2$ generations that segregated into 3R:1S is controlled by a single dominant gene. The $F_3$ generation also revealed that the MAGIC 072 × Apagbaala generation is controlled by a single dominant gene since it also fitted the 3:1 genetic ratio after reclassification. The results of the study therefore revealed that selection for resistance

is more effective at $F_3$ progeny testing since planting of families is done on a one progeny one *Striga* infected pot basis. The phenotypic errors that may occur during screening at $F_2$ were resolved at the $F_3$ progeny testing stage. Findings from the study revealed that Suvita-2 and MAGIC 072 are important source of resistance needed in the cowpea improvement program to increase the sources of resistance.

### 6.2 Recommendations

There is limited sources of *Striga* resistant varieties and therefore there is the need to develop new *Striga* resistant cowpea varieties that meet end-user preference. It is recommended that the homozygous resistant $F_3$ should be further evaluated with other types of *Striga* isolate in Ghana.

It is also recommended that the resistant seeds of the $F_2$ that were dropped because of insufficient seeds should be screened at $F_3$ and those that will be identified as completely resistant should be further evaluated.



## REFERENCES

Aboyomi, Y. A., Ajibade, T. V., Sammuel, O. F. and Saadudeen, B. F. (2008). Growth and yield responses of cowpea (*Vigna ungiuculata* (L). Walp) genotypes to nitrogen fertilizer (NPK) application in the Southern Guinea Savana Zone of Nigeria. *Asian Journal of Plant Sciences*, 7**(**2): 170-176.

Acquaah, G. (2012). *Principles of plant genetics and breeding*. 2nd ed. Wiley-Blackwell, Oxford.

Addo-Quaye, A. A., Darkwa, A. A. and Ocloo, G. K. (2011). *Yield and productivity of component crops.*

Aggarwal, B. B., Moffat, B. and Harkins, R. N. (1984). Human lymphotoxin. Production by a lymphoblastoid cell line, purification, and initial characterization. *Journal Biol. Chem*., 259: 686–691.

Aggarwal, V. D. (1985). *Cowpea Striga research*. In: S.R. Singh and K.O. Rachie (Eds.) *Cowpea Research,* Production and Utilization. John Wiley & Sons Ltd, Chichester, pp. 335-340.

Aggarwal, V. D. and Ouedraogo, T. J. (1989). Estimation of cowpea yield loss from Stiga infestation. *Tropical Agriculture (Trinidad)*, 66 (7): 91-92.

Agyeman, K., Berchie, J. N., Osei-Bonsu, I., Tetteh Nartey. E. and Fordjour, J. K. (2014). Growth and yield performance of improved cowpea (*Vigna unguiculata* L.) varieties in Ghana. *Published by Science and Education Centre of North America,* 2: 44-52.

Ahmad, F. E., Hall, A. E. and DeMason, D. A. (1992). Heat injury during floral development in cowpea (*Vigna unguiculata*, Fabaceae). *Am J Bot,* 79: 784-791.

Ajeigbe, H. A., Oseni, T. O. and Singh. B. B. (2006). Effect of planting pattern, crop variety, and insecticide on the productivity of cowpea–cereal systems in Northern Guinea Savanna of Nigeria. *Journal of Food, Agriculture and Environment,* 4(1): 145–150.

Ajeigbe, H., Ihedioha, D. and Chikoye, D. (2008). Variation in physico-chemical properties of seed of selected improved varieties of cowpea as it relates to industrial utilization of the crop. *African Journal of Biotechnology,* 7 (20): 3642-3647.

Akpalu, S. E. (2014). Farmers' knowledge and cultivation of cowpea (*Vigna unguiculata* (L.) Verdc.) In: Three Communities of Bolgatanga Municipality, Upper East Region, Ghana. *British Journal of Applied Science and Technology,* 4(5): 775-792.

Alonge, S. O. (1999). *Effects of Alectra vogelii and Striga gesnerioides infestations on the growth, yield and grain chemical composition of cowpea (Vigna unguiculata (L.) Walp) varieties in the Nigerian savannah*. Ph.D. Thesis, Department of Biological Sciences, Ahmadu Bello University, Zaria, 325pp, submitted for publication.

Alonge, S. O., Lagoke, S. T. O. and Ajakaiye, C. O. (2004). Cowpea reactions to *Striga gesnerioides*: Effect on growth. *Crop Protection*, 24: 565-573.

Alonge, S. O., Lagoke, S. T. O. and Ajakaiye, C. O. (2004). *Cowpea reactions to Striga gesnerioides: Effect on growth.* Association of Official Analytical Chemist (AOAC). 12th edition. William Hortwits, Washington, DC.

Alonge, S. O., Lagoke, S. T. O. and Ajakaiye, C.O. (2005). Cowpea reactions to *Striga gesnerioides*: Effect on growth. *Crop Protection*, 24: 565-573.

Andrews, R. W. (1947). The parasitism of *Striga hermonthica* Benth on leguminous plants. *Ann. Appl. Biol.*, 34: 267-275.

Asare, A. T., Gowda, B. S., Galyuon, I. K. A., Aboagye, L. M., Takrama, J. F. and Timko, M. P. (2010). Assessment of the genetic diversity in cowpea (*Vigna unguiculata* (L.) Walp) germplasm from Ghana using simple sequence repeat markers. *Plant Genet Resour –C*, 8(2): 142–150.

Asare, A. T., Padi, F. K., Otwe, E. P. and Takrama, J. F. (2013). Responses of recombinant inbred lines of cowpea [(*Vigna unguiculata* (l.) walp] to *Striga gesnerioides* infestation in Ghana. *1st Annual International Interdisciplinary Conference, AIIC 2013, 24-26 April, Azores, Portugal.*

Atokple, I. D. K., Singh, B. B. and Emechebe, A. M. (1993). Independent inheritance of *Striga* and *Alectra* resistance in cow pea genotype B301. *Crop Science,* 33: 714715.

Atokple, I. D. K., Singh, B. B. and Emechebe, A. M. (1995). Genetics of resistance to *Striga* and *Alectra* in cowpea. *Journal of Heredity*, 86: 45-49.

Ayensu, E. S., Dogget, H., Keynes, R. D., Marton-Lefevre, J., Musselman, L. J., Parker, C. and Pickering, A. (1984). *Striga, biology and control Paris*. International Council of Scientific Unions Press.

Ba, F. S., Pasquet, R. S. and Gepts, P. (2004). Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. *Genetic Resources and Crop Evolution,* 51: 539-550.

Babiker, E. E., ElSheikh, E. A. E., Osman, A. J. and *El* Tinay, A. H. (1995). Effect of nitrogen fixation, nitrogen fertilization and viral infection- $N_2$ fixation in

*soybean*. *World Journal of Microbiology and Biotechnology*, 12(6): 639–641.

Badiane, F. A., Diouf, D., Sané, D., Diouf, O. (2004). Screening cowpea [*Vigna unguiculata* (L.) Walp.] varieties by inducing water deficit and RAPD analyses. *Afr J. Biotechnol.* 3: 174-178.

Bagnall, D. J. and King, R. W. (1987). Temperature and irradiance effects on yield in cowpea (*Vigna unguiculata*). *Field Crops Res.*, 16: 217-229.

Bennett, J. R. and Mathews, S. (2006). Phylogeny of the parasitic plant family *Orobanchaceae* inferred from phytochrome A. *Amer J Bot*, 93: 1039–1051.

Bernatzky, R. and Tanksley, S. D. (1986). Majority of random cDNA clones correspond to single loci in the tomato genome. *Mol. Gen. Genet*., 203: 8-14.

Berner, D. K. and Williams, O. A. (1998). Germination stimulation of *Striga gesnerioides* seeds by hosts and nonhosts. *Plant Disease*, 82: 1242-1247.

Berner, D. K., Kling, J. G. and Singh, B. B. (1995). *Striga* research and control: a perspective from Africa. *Plant Disease*, 79(7): 652-660.

Berner, D. K., Winslow, M. D., Awad, A. E., Cardwell, K. F., Mohan R, D. R. and Kim S. K. (1997). *Striga research methods*. The Pan African Striga Control Network (PASCON)/ International Institute for Tropical Agriculture (IITA). Ibadan, Nigeria, 80pp.

Bisikwa, J., Sekamatte, S., Kapting, I., Karuhanga, M. B., Otim, M. & Woomer, P. L. (2010). *Participatory management of Striga in cereal-based cropping systems in eastern Uganda*. Second RUFORUM Biennial Meeting 20 – 24.

Botanga, C. J. (2005). *Genetic and molecular analysis of factors defining host-specificity and virulence in Striga species (Scrophulariaceae*). Ph.D. dissertation. University of Virginia, Charlottesville, Va.

Botanga, C. J. and Timko, M. P. (2005). Genetic structure and analysis of host and nonhost Interactions of *Striga gesnerioides* (witchweed) from Central Florida. *Phytopathology*, 95: 1166-1173.

Botanga, C. J. and Timko, M. P. (2006). Phenetic relationships among different races of *Striga gesnerioides* (Willd.) Vatke from West Africa. *Genome*, 49(11): 13511365.

Botstein, D., White, R., Skolnick, M. and Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet,* 32: 314–331.

Boukar, O., Kong, L., Singh, B. B., Murdock, I. and Ohm, H. W. (2004). AFLP and AFLPderived SCAR markers associated with 41 *Striga gesnerioides* resistance in cowpea [*Vigna unguiculata*]. *Crop science,* 44: 1259-1264.

Boukar, O., Kong, L., Singh, B. B., Murdock, L. and Ohm, H. W. (2004). AFLP and AFLP-derived SCAR markers associated with *Striga gesnerioides* resistance in cowpea. *Crop Science*, 44: 1259-1264.

Cardwell, K. F. and Lane, J. A. (1995). Effect of soils, cropping system and host phenotype on incidence and severity of *Striga gesnerioides* on cowpea in West Africa. *Agriculture, Ecosystems and Environment*, 53: 253-262.

Carson, A. G. (1988). Development and testing of a control package for Striga hermonthica on small-scale holdings in the Gambia. *Tropical Pest Management*, 34: 97-101.

CDFA (Council of Development Finance Agencies) (2006). *2006 Annual Development Finance Summit - Post Page.* 100 E. Broad Street, Suite 1200, Columbus, OH 43215.

CGIAR (2018). *Grain Legumes – A CGIAR Research program 2018*. Intuition theme by CPO Themes.

Chaturvedi, S. K., Gupta, D. S. and Jain, R. (2011). *Biology of food legumes*. In: Biology and Breeding of Food Legumes. Pratap, A. and Kumar, J. (eds.), Crop Improvement Division, Indian Institute of Pulses Research, Kanpur, INDIA, pp 35-48.

Chen, X. F., Laudeman, T. W., Rushton, P. J., Thomas, T. A. and Timko, M. P. (2007). CGKB: an annotation knowledge base for cowpea (*Vigna unguiculata* L.) methylation filtered genomic gene-space sequences. *BMC Bioinformatics,* 8: 129.

Ciotola, M. A., Ditommaso, A. and Watson, A. K. (2000). Chlamydospores production, inoculation methods and pathogenicity of F. oxysporum M12-4A, a biocontrol for Striga hermonthica. *Biocontrol Science and Technology,* 10:129-145.

Ciotola, M., Hallett, S. G. and Watson, A. K. (1996). Impact of *Fusarium oxysporum* isolate M12-4A upon seed germination of *Striga hermonthica* in vitro, *proceedings of the 6th international Symposium on Parasitic Weed; 1996; Cardaba.*

Ciotola, M., Watson, A. K. and Hallett, S. G. (2003). Impacts of *Fusarium oxysporum* isolate M12-4A up on seed germination of *Striga hermonthica* in vitro. In: Moreno M.T, Cubero, J.I., and Parker C., (eds). *Proceedings of Advances in Parasitic Plant Research, Canada.* pp. 871-878.

Cochrane, V. and Press, M. C. (1997). Geographical distribution and aspects of the ecology of the hemiparasite angiosperm *Striga asiatica* (L.) Kuntze: A herbarium study. *J. Trop. Ecol., 13: 371*-380.

Coetzee, J. J. (1995). *Cowpea: A traditional crop in Africa. Africa crop info 95 Leaflet.* Vegetable and ornamental plant institute and the grain crops Institute, Agricultural Research Council, Pretoria.

Coulibaly, S., Pasquet, R. S., Papa, R. and Gepts, P. (2002). AFLP analysis of the phenetic organization and genetic diversity of *Vigna unguiculata* (L.) Walp reveals extensive gene flow between wild and domesticated types. Cowpea Research, production and utilization. John Wiley and Sons, Ltd, Chichester. *Theoretical and Applied Genetics*, 104: 358-366.

Craufurd, P. Q., Summerfield, R. J., Ellis, R. H. and Roberts. E. H. (1997). *Photoperiod, temperature, and the growth and development of cowpea*. In: eds. In : Singh B. B., Mohan Raj D. R., Dashiell K. E., Jackai L. E. N. (Eds.),

Advances in cowpea research, pp. 75 - 86. IITA and JIRCAS. Ibadan, Nigeria.

Davis, D. W., Oelke, E. A., Oplinger, E. S., Doll, J. D., Hanson, C. V., Putnam, D. H. (1991). *Cowpea: alternative field crops manual.* ttp://www.hort.purdue.edu/newcrop/ afcm/cowpea.html.

Department of Agriculture, Forestry and Fisheries, South Africa (2009). *Production guidelines for cowpea.* pp.1-24.

Diouf, D. and Hilu, K. W. *(*2005). Microsatellites and RAPD markers to study genetic relationship among cowpea breeding lines and local varieties in Senegal. *Genet*. *Res*. *Crop Evol*., 52: 1057-1067.

District Analytical report, Bawku municipality (2010). *Population and Housing census.*

Dube, M. P. and Olivier, A. (2001). *Striga gesnerioides* and its hosts, the cowpea: Interaction and methods of control. *Canadian journal of botany-revue canadienne de botanique*, 79: 1225-1240.

Duivenbooden, Van H., Abdoussalam, S. and Mohamed, A. B. (2002). Impact of climate change on agricultural production in the Sahel-Part 2. Case study for groundnut and cowpea in Niger. *Climate Change*, 54: 349–368.

Eagles, H., Bariana, H., Ogbonnaya, F., Rebetzke, G., Hollamby, G., Henry, R., Henschke, P. and Carter, M. (2001). Implementation of markers in Australian wheat breeding. *Australian Journal of Agricultural and Resources*, 52: 1349-1356.

Ehlers, J. D. and Hall, A. E. (1997). Cowpea (*Vigna unguiculata* (L.) Walp.). *Field Crops Research Improvement of Grain Legumes*, 53: 187-204.

FAOSTAT (2012). *Crops: productions quantities and covered areas*. http://faostat.fao.org /default. aspx. Consulted in January 2013.

Flight, C. (1976). *The Kintampo culture and its place in the economic prehistory of West Africa.* Pages 212-221 in Origins of African plant domestication, edited by J.R. Harlan, J.M.J. de Wet & A.B.L. Stemler. Mouton, The Hague, Netherlands.

Fox, F. W. and Young, M. E. N. (1982). *Food from the veld.* Delta Books, Johannesburg.

Franke, A. C., Ellis, J., Jones, G., Tarawali, S., Schulz, M. S., Hussaini, I., Kureh, R., White, D., Chikoye, B., Douthwaite, B. D., Oyewole, A. and Olanrewaju, S. (2006). Evaluating and scaling-up integrated *Striga hermonthica* control technologies among farmers in northern Nigeria. *Crop Prot.*, 25: 868-878.

Gepts, P. (2004). The contribution of genetic and genomic approaches to plant domestication studies. *Current opinion in plant biology*, 18: 51-59.

Ghalmi, N., Malice, M., Jacquemin, J. M., Ounane, S. M., Mekliche, L. and Baudoin, J. P. (2010). Morphological and molecular diversity within Algerian cowpea (*Vigna unguiculata* (L.) Walp.) landraces, *Genetic Resources and Crop Evolution*, 57: 371–386.

GISD (Global Invasive Species Database) (2006). *Striga asiatica* (herb).

Godwa, B. S., Riopel, J. L. and Timko, M. P. (1999). NSSRA-1: a resistance gene homolog expressed in roots of non-host plants following parasitism by *Striga asiatica* (Witchweed). *The Plant Journal*, 20(2): 217-230.

Gupta, P. K., Roy, J. K. and Prasad, M. (2001). Single nucleotide polymorphisms: a new Paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Curr Sci.*, 80(4): 524–535.

Gupta, P. K., Varshney, R. K., Sharma, P. C. and Ramesh, B. (1999). Molecular markers and their application in wheat breeding: a review. *Plant Breeding,* 118: 369-390.

Gworgwor, N. A., Bdiya, B. S. and Weber, H. C. (1991). *Screening cowpea varieties for resistance to Striga gesnerioides in the semi-arid zone of Nigeria.* In: K. Weymann., L. J. Musselman. (Eds.), Progress in Orobanche Research, Obermarchtal, Germany, 19–22 August, 1989, pp. 322–328.

Hadley, P., Roberts, E. H., Summerfield, R. H. and Minchin, F. R. (1983). A quantitative model of reproductive development in cowpea (*Vigna unguiculata* (L) Walp.) in relation to photoperiod and temperature and implications for screening germplasm. *Annals of Botany*, 51: 531–543.

Hall, A. E. (2004). Breeding for adaptation to drought and heat in cowpea. *Eur J Agron*, 21: 447–454.

Hall, A. E. (2012). *Heat stress*. In: Shabala S (ed) plant stress physiology. CABI, Cambridge, pp 118–131.

Hall, A. E., Ismail, A. M., Ehlers, J. D., Marfo, K. O., Cisse, N., Thiaw, S. and Close, T. J. (2002). *Breeding cowpeas for tolerance to temperature extremes and adaptation to drought*. In: Fatokun CA, Tarawali SA, Singh

BB, Kormawa PM, Tamo M(eds) Challenges and Opportunities for Enhancing Sustainable Cowpea Production. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp 14–21.

Hall, A. E., Singh, B. B. and Ehlers, J. D. (1997). Cowpea breeding. *Plant Breeding Review*, 15: 215-275.

Haussmann, B. I. G., Hess, D. E., Welz, H. G. and Geiger, H. H. (2000). Improved methodologies for breeding *Striga* resistant sorghums. *Field Crops Research*, 66: 195-211.

Hedge, S. V. and Mishra, K. S. (2009). Landraces of cowpea, *Vigna unguiculata* (L.) Walp. As potential sources of genes for unique characters in breeding. *Genetic Resources and Crop Evolution*, 56: 615–627.

Hibberd, J. M., Quick, W. P., Press, M. C. and Scholes, J. D. (1996). The influence of the parasitic angiosperm *Striga gesnerioides* on the growth and photosynthesis of its host, *Vigna unguiculata*. *Journal of Experimental Botany*, 47: 507-51.

Hutchinson, J. and Dalziel, J. M. (1963). *Flora of west tropical Africa*, vol 2, 2nd edition. Crown Agents. London. 515p.

IBPGR (1983). *International Board for Plant Genetic Resources: Descriptors for cowpea, Secretariat, Rome, Italy*.

IITA (2010). The quiet revolution. R4D, Review, Edition, 5, September 2010, p58.

IITA (International Institute of Tropical Agriculture) (1983). *Striga gesnerioides resistance.* In Annual Report for 1982. IITA, Ibadan, Nigeria. pp. 69-70.

Imrie, B. (2000). *Cowpea*. (Available at www.rirdc.go.au/pub/hankbook/ cowpea. pdf).

Joel, D. M., Steffen, J. C. and Matthew, D. E. (1995). Germination of weedy root parasites. In: Kigel J, Galili C (eds) Seed development and germination. Marcel Dekker Inc, New York, pp 567–597.

Johnson, D. T. (1970). The cowpea in the African areas of Rhodesia. *Rhodesia Agricultural Journal,* 67: 61-64.

Jones, N., Ougham, H. and Thomas, H. (1997). Markers and mapping: We are all geneticists now. *New Phytologist*, l137: 165-177.

Joshi, S., Ranjekar, P. and Gupta, V. (1999). Molecular markers in plant genome analysis. *Current Science*,77: 230-240.

Kamara, A. Y., Chikoye, D., Ekeleme, F., Omoigui, L. O. and Dugje, I. Y. (2008). Field performance of improved cowpea varieties under conditions of natural infestation by parasitic weed *Striga gesnerioides*. *International journal Pest Management*, 54(3): 189-195.

Kameswara, R. N. (2004). *Biotechnology for plant resources conservation and use.* Principles of seed handling in Gene banks Training course, Kampla, Uganda.

Kay, D. E. (1979). *Food legumes.* Tropical Development and Research Institute, London.

Kim, S. K. (2007). Genetics of maize tolerance of *Striga hermonthica*. *Crop Science*, 34: 900-907.

Kim, S. K., Adetimirin, V. O., Thé, C. and Dossou, R. (2002). Yield losses in maize due to *Striga hermonthica* in West and Central Africa. *International Journal of Pest Management*, 48(3): 211-217.

Kuiper, E., Verkleij, J. and Pieterse, A. (1996). *Differences in the primary dormancy pattern of Striga species: an ongoing study.* Congresos y Jornadas-Junta de Andalucia (Espana).

Lane, J. A. and Bailey, J. A. (1992). Resistance of cowpea and cereals to the parasitic angiosperm *Striga*. *Euphytica*, 63: 85-93.

Lane, J. A., Bailey J. A. and Terry, P. J. (1991). An *in-vitro* growth system for studying the parasitism of cowpea (*Vigna unguiculata)* by *Striga gesnerioides*. *Weed Research*, 31(4): 211-217.

Lane, J. A., Child, D. V., Reiss, G. C., Entcheva, V., Bailey, J. A. (1997a). *Crop resistance to parasitic plants.* In: Crute IR, Holub EB, Burdon JJ (eds) The gene-for-gene relationship in plant parasite interactions. CAB int, Wallingford, pp 81–97.

Lane, J. A., Moore, T. H. M. Child, D. V., Cardwell, K. E., Singh, B. B. and Bailey, J. A. (1994). Virulence characteristics of a new race of the parasitic angiosperm *Striga gesnerioides* from southern Benin on cowpea. *Euphytica*, 72: 183-188.

Lane, J. A., Moore, T. H. M., Child, D. V. and Bailey, J. A. (1997b). *Variation in virulence of Striga gesnerioides on cowpea: new sources of crop resistance.* Pages 225– 230 *in* Advances in cowpearesearch, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan

International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.

Lane, J. A., Moore, T. H. M., Child, D. V. and Cardwell, K. F. (1996). Characterization of virulence and geographic distribution of *Striga gesnerioides* in cowpea in West Africa. *Plant Disease,* 80(3): 299-301.

Lane, J. A., Moore, T. H. M., Cild, D. V., Cardwell, K. F., Singh, B. B. and Bailey, J. A. (1994). Virulence characteristics of a new race of the parasitic angiosperm *Striga gesnerioides* from Southern Benin on cowpea. *Euphytica*, 72: 183-188.

Lane, J., Bailey, J. and Terry, P. (1991). An "*in vitro*" growth system for studying the parasitism of cowpea (*Vigna unguiculata*) by *Striga gesnerioides*. *Weed Research*, 31**:** 211-217.

Langyintuo, A. S., Lowenberg-DeBoer, J., Faye, M., Lambert, D., Ibro, G., Moussa, B., Kergna, A., Kushwaha, S., Musa, S. and Ntoukam, G. (2003). Cowpea supply and demand in West and Central Africa. *Field Crop Research*, 82: 215-231.

Li, J. and Timko, M. P. (2009). Gene-for-gene resistance in Striga-cowpea associations. *Science,* 325: 1094.

Lim, T. K. (2012). *Vigna unguiculata* cv-gr. Biflora. In *Edible Medicinal And Non Medicinal Plants*. Springer Netherlands, pp. 967-970.

Mahmoud, E. A., Ramadan, B. S. H., Bekheet, M. A. and Gomaa, M. A. (2013). Effect of Nitrogen Fertilization and Plant Density on Productivity and Quality of Sweet Sorghum. *American-Eurasian Journal of Agriculture & Environmental Sci.,* 13(5): 654-659.

Marechal, R., Mascherpa, J. M. and Stainer, F. (1978). Etude taxonomique d'un groupecomplexed'especes des genres *Phaseolus et Vigna* (*Papillionaceae*) surla base de donneesmorphologiques et polliniques, traiteesparl'analyseinformatique. *Boissiera,* 28: 1-273.

Marfo, K. O. and Hall, A. E. (1992). Inheritance of heat tolerance during pod set in cowpea. *Crop Sci.,* 32: 912–918.

Matusova, R., Rani, K., Verstappen, F. W., Franssen, M. C., Beale, M. H. and Bouwmeester, H. J. (2005) The strigolactone germination stimulants of the plantparasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. *Plant physiology,* 139**:** 920-934.

Mfeka, N. (2017). *Morphology and mineral content of cowpea lines in response to planting date and zinc application rate*. M.Sc. Thesis Cape Peninsula University of Technology, Wellington. pp 1.

MoFA (2005). *Ministry of Food and Agriculture: Agriculture in Ghana, Facts and Figure, PPME, Accra*. P. 26.

MoFA (Ministry of Food and Agriculture) (2010). *Ministry of Food and Agriculture: Cropped area for major crops in Ghana: Statistics, Research and Info. Directorate (SRID).* Mohan Raj, K.E. Dashiell and L.E.N. Jackai (eds). IITA, Ibadan, Nigeria, pp. 1-11.

Mohamed, K. I., Musselman, L. J. and Riches, C. R. (2001). The genus *Striga* (*Scrophulariaceae*) in Africa. *Annanls of Missouri Botanical Garden,* 88: 60-103.

Moore, T. H. M., Lane, J. A., Child, D. V., Arnold, G. M., Bailey, J. A. and Hoffmann, G. (1995). New sources of resistance of cowpea (*Vigna unguiculata*) to *Striga gesnerioides*, a parasitic angiosperm. *Euphytica,* 84 (3): 165-74.

Muleba, N., Ouedraogo, J. T. and Tignegre, J. B. (1997). Cowpea yield losses attributed to *Striga* infestations. *Journal Agricultural Science,* 129: 43-48.

Musselman, L. J. (1987). *Taxonomy of witch weeds.* Pages 3-12. *In* Parasitic weeds in agriculture volume I: *Striga*. CRC Press, Inc., Boca Raton, Florida, U.S.A. 317 pp.

Musselman, L. J. and Ayensu, E. S. (1984). *Taxonomy and biosystematics of Striga*. *Striga* Biology and Control, Dakar, Sénégal, 1983. ICSU Press, Proceedings of a workshop 37-45.

Musselman, L. J. and Parker, C. (1981). Studies on indigo witchweed, the American strain of *Striga gesnerioides* (Scrophulariaceae). *Weed Science*, 594-596.

Mutters, R. G., Hall, A. E. and Patel, P. N. (1989). Photoperiod and light quality effects on cowpea floral development at high temperatures. *Crop Sci*, 29: 1501–1505.

Ng, N. Q. and Marechal, R. (1985). *Cowpea taxonomy, origin and germplasm*. In: Cowpea research, production and utilization. Singh, S. R. and Rachie, K.O. (eds.), John Wiley and Sons, Chichester, UK, pp 11-12.

Nworgu, F. C. and Olakojo, S. A. (2006). *Ethno-science in agriculture for sustainable food production and livelihood.* pp. 25-35.

Okonkwo, S. N. C. (1991). The germination of striga-A review. J. K, Ransom., L. J. Musselman., and A. D, Worsham., (ed.). *Proceeding of the 5th International Symposium of Parasitic Weeds. 24-30 June 1991, Nairobi, Kenya. 144-154*.

Olakojo, S. A. and Olaoye, G. (2005). Combining ability for grain yield, agronomic traits and *Striga lutea* tolerance of maize hybrids under artificial striga infestation. *African Journal of Biotechnology*, 4(9): 984-988.

Omoigui, I. O., Timko, M. P., Ishiyaku, F. S., Ousmane, B., Muranaka, B. S., KamaraA. Y. and Yeye, M. Y. (2009). Molecular characterization of cowpea breeding line for *Striga* resistance using FTA technology. *African Crop Science Conference, Uganda*, 9: 527-530.

Omoigui, L. O., Ishiyaku, F. S., Gowda, B. S., Kamara, A. Y. and Timko, M. P. (2015). Suitability and use of two molecular markers to track race-specific resistance University of Ghana http://ugspace.ug.edu.gh 136 *Striga gesnerioides* in cowpea (*Vigna unguiculata* (L.) Walp.). *African Journal of Biotechnology*, 14: 21792190.

Omoigui, L. O., Kamara, A. Y., Massawe, F. S., Ishiyaku, M. F., Boukar, O., Alabi, S. O. and Ekeleme, F. (2007). Evaluation of cowpea genotypes for their

reactions to *Striga gesnerioides* in the dry savanna of northern Nigeria. *African Crop Science Conference Proceedings,* 8: 273-278.

Omoigui, L. O., Timko, M. P., Ishiyaku, F. S., Ousmane, B., Muranaka, B. S., Kamara, A.Y. and Yeye, M. Y. (2009). Molecular characterization of cowpea breeding line for *Striga* resistance using FTA technology. *African Crop Science Conference, Uganda,* 9: 527-530.

Oswald, A. (2005). Striga control-technologies and their dissemination. *Crop Protection*, 24: 333–342.

Ouédraogo, J. T., Gowda, B. S., Jean, M., Close, T. J., Ehlers, J. D., Hall, A. E., Gillaspi, A. G., Roberts, P.A., Ismail, A. M., Bruening, G., Gepts, P., Timko, M. P. and Belzile, F. J. (2002a). An improved genetic map for cowpea (V*igna unguiculata* L.) combining AFLP, RFLP, RAPD. Biochemical markers, and biological resistance traits. *Genome,* 45: 175-188.

Ouédraogo, J. T., Maheshwani, V., Berner, D. K., Stpierre, C. A., Belzile, F. and Timko, M. P. (2001). Identification of AFLP markers linked Genet Resour Crop Evol to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. *Theor Appl Genet,* 102: 1029-1036.

Ouédraogo, J. T., Ouédraogo, M., Gowda, B. S. and Timko, M. P. (2012). Development of sequence characterized amplified region (SCAR) markers linked to race-specific resistance to *Striga gesnerioides* in cowpea (*Vignaunguiculata*L.). *Afr. J. Biotechnol.* 11(62): 12555-12562.

Ouédraogo, J. T., Tignegre, J. B., Timko, M. P. and Belzile, F. O. J. (2002b). AFLP markers linked to resistance against *Striga gesnerioides* race 1 in cowpea (*Vigna unguiculata*). *Genome,* 45: 787-793.

Ouédraogo, J. T., Tignegre, J.-B., Timko, M. P. and Belzile, F. O. J. (2002b). AFLP markers linked to resistance against *Striga gesnerioides* race 1 in cowpea (*Vigna unguiculata*). *Genome,* 45: 787-793.

Padulosi, S. and Ng, N. (1997). Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp. *Advances in Cowpea Research,* 1-12.

Parker, C. (1991). Protection of crops against parasitic weeds. *Crop Protection*, 10: 6-22.

Parker, C. (2009). Observations on the current status of *Orobanche* and *Striga* problems worldwide. *Pest Management Science*, 65: 453-459.

Parker, C. and Polniaszek, T. (1990). Parasitism of cowpea by *Striga gesnerioides*: variation in virulence and discovery of a new source of host resistance. *Annals of Applied Biology,* 116: 305-311.

Parker, C. and Riches, C. R. (1993). *Parasitic weeds of the World: biology and control.* CAB International, Wallingford, UK., ISBN: 9780851988733, Pages: 332.

Pasquet, R. S. (2001). Allozyme diversity of cultivated cowpea (*Vigna unguiculata* L. Walp). *Theoretical Appl. Genetics,* 101: 211-219.

Patel, P. N. and Hall, A. E. (1990). Genotypes variation and classification of cowpea for reproductive responses to high temperatures under long photoperiods. *Crop Sci.* 30: 614-621.

Paterson, A. H., Lander, E. S., Hewiit, J. D., Peterson, S., Lincoln, S. E. and Tanksley, S. D. (1988). Resolution of quantitative traits into Mendelian factors using a complete linkage map of restriction fragment length polymorphisms. *Nature*, 335: 721-726.

Perfectti, F. and Pascuai, L. (1996). Segregation distortion of isozyme loci in cherimoya (*Annona cherimola Mill*). *Theor Appl Genet.*, 93: 440-446.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (Microsatellite) markers for germplasm analysis. *Mol Breed*, 2: 225-238.

Prasad, P. V. V., Boote, K. J., Allen Jr., L. H. and Thomas, J. M. G. (2002). Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (*Phaseolus vulgaris* L.). *Global Change Biology*, 8: 710–721.

Press, M. C. (1995). How do the parasitic weeds *Striga* and *Orobancheae* influence host carbon relation? *Aspects Appl. Biol*., 42: 63-70.

Press, M. C., Scholes, J. D. and Riches, C. R. (2001). *Current status and future prospects for management of parasitic weeds (Striga* and *Orobanche).* In C.R. Riches (Ed.) The World's Worst Weeds (pp 71–90). British Crop Protection Council, Brighton, UK.

Quaye, W., Adofo, K., Madode, Y. E. and Abizari, A. R. (2009). Exploratory and multidisciplinary survey of the cowpea network in Tolon-Kumbungu district of Ghana: A food sovereignty prospective. *African J. Agric. Res.* 4: 311-320.

Rachie, K. O. and Rawal, K. M. (1976). Integrated approaches to improving cowpea (*Vigna unguiculata* (L) Walp). *IITA Technical Bulletin*, 5: 1-13.

Rebeka, G., Hussein, S., Mark, D. L., Pangirayi, T. and Nigussie, M. (2013). Evaluation of sorghum genotypes compatibility with *Fusarium oxysporum* under Striga infestation. *Crop Sci.* 53: 385-393.

Rezig, A. A. (2016). *Genotypic variability in cowpea with respect to production Striga germination stimulants.* M.Sc. Thesis. In: Plant Protection, Sudan University of Science and Technology, Sudan. pp 180.

Ruane, J. and Sonnino, A. (2007). *Marker-assisted selection as a tool for genetic improvement of crops, livestock, forestry and fish in developing countries: an overview of the issues.* In: Guimarães, E.P., Ruane, J., Scherf, B.D., Sonnino, A. and Dargie, J.D., Eds., Marker-Assisted Selection, Current Status and Future Perspectives in Crops, Livestock, Forestry and Fish, Food and Agriculture Organization of the United Nations, Rome, 4-13.

Saiki, R. K., Scharf, S. J., Fallona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. and Arnheim, N. (1985). Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*, 230(4732): 1350-1354.

Sanginga, N., Dashiell, K. E., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R. J., Tasawali, S., Asafo-Adjei, B., Menkir, A., Schulz., S., Singh, B. B., Chikoye, D., Keatinge, D. and Ortiz, R. (2003). Sustainable resource

management coupled to resilient germplasm to provide new intensive cereal grain-legume-livestock systems in the dry savannah. *Agric Ecosyst Environ*, 100: 305-314.

Sanginga, N., Lyasse, O. and Singh, B. B. (2000). Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. *Plant Soil*, 220: 119-128.

Sauerborn, J., Müller-Stöver, D. and Hershenhorn, J. (2007). The role of biological control in managing parasitic weeds. Crop Prot., 26: 246-254.

Singh, A., Baoule, A. L., Ahmed, H. G., Dikko, A. U. and Aliyu, U. (2011). Influence of phosphorus on the performance of cowpea (*Vigna unguiculata* (L.) Walp.) varieties in the Sudan savanna of Nigeria. *Agric. Sci.*, 2: 313-317.

Singh, B. and Emechebe, A. (1990). Inheritance of *Striga* resistance in cowpea genotype B301. *Crop Science*, 30: 879.

Singh, B. B. (2002). *Breeding cowpea varieties for resistance to Striga gesnerioides and Alectra vogelii.* In: Challenges and opportunities for enhancing sustainable cowpea production. Fatokum, C.A. (Ed.). pp. 154-166. IITA, Ibadan, Nigeria.

Singh, B. B. (2005). *Cowpea (Vigna unguiculata (L.) walp*. In: R.J, Singh., P.P, Jauhar (eds) Genetic Resources, Chromosome Engineering and Crop Improvement. Volume 1, CRC Press, Boca Raton, FL, USA, pp. 117–162.

Singh, B. B. and Emechebe, A. M. (1991). *Breeding for resistance to Striga and Alectra in cowpea.* In: Ransom JK, Musselman LJ, W oesham AD, Parker C(eds) *Proc 5$^{th}$IntSymp Parasitic Weeds*. CIMMYT, Mexico D.H. pp. 303-305.

Singh, B. B., Ajeigbe, H. A., Tarawali, S. A., Fernandez-Rivera, S. and Abubakar, M. (2003). Improving the production and utilization of cowpea as food and fodder. *Field Crops Research,* 84**:** 169-177.

Singh, B. B., Ehlers, J. D., Sharma, B. and Freire-Filho, F. R. (2003). *Recent progress in cowpea breeding*. In: C.A, Fatokun., S.A, Tarawali., B.B,Singh., P.M, Kormawa., Tamo, M. (eds) Challenges and Opportunities for Enhancing Sustainable Cowpea Production. Intl Inst Tropical Agric, Ibadan, Nigeria, pp. 22–40.

Singh, B. B., Mohan-Raj, D. R., Dashiel, K. E. and Jackai, L. E. N. (1997). *Advances in cowpea research-post harvest storage of cowpea in Sub-Saharan Africa.* I.I.T.A./JIRCA Publication, Ibadan, Nigeria, pp. 302-312.

Singh, B. D. (1993). *Pedigree selection*. In: *Plant breeding: Principles and methods*. Kalyani (ed). New Delhi, India, 215-226.

Song, Q. J., Shi, J. R., Singh, S., Fickus, E. W., Costa, J. M., Lewis, J., Gill, B. S., Ward, R. and Cregan, P. B. (2005). Development and mapping of microsatellite (SSR) markers in wheat. *eor Appl Genet*, 110: 550-560.

Song, Q., Jia, G., Zhu, Y., Grant, D., Nelson, R.T., Hwang, E. Y., Hyten, D. L. and Cregan, P.B. (2010). Abundance of SSR motifs and development of candidate polymorphic SSR markers (BARCSOYSSR 1.0) in soybean. *Crop Sci.*, 50: 1950-1960.

Spencer, D. S. C. and Sivakumar, M. V. K. (1987). Pearl millet in African agriculture, *Page 19-31 in Proceedings of international Pearl millet workshop, 7-11 April 1986, ICRISAT Center, India Patancheru AP 502 324, India.*

Steel, R. G. D., Torrie, J. H. and Dickey, D. A. (1997). *Principles and procedures of statistics; a biometrical approach.* 3. Boston: McGraw-Hill.

Steele, W. M. (1976). *Cowpea, Vigna unguiculata (Legumimosae-Papillionatae).* In: N.W., Simmons (ed.), Evolution of Crop Plants. Longman, London. Pp 183-185.

Steele, W. M. (1979). *Cowpeas*. In: Evolution of Crop Plants, Ed. N. W. Simmonds, Longman, London, UK.

Summerfield, R. J., Huxley, P. A. and Steel, W. (1974). Cowpea (*Vignaunguiculata* (L.) Walp.). *Field Crop Abstracts*, 27: 301-312.

Tarr, S. A. J. (1962). *Disease of sorghum, Sudan grass and broom corn*. The Commonwealth Mycological Institute, CAB international, Kew, Surry, U.K, pp. 229-302.

Tettey, C. K. (2017). *Characterization and determination of virus resistance among cowpea [Vigna unguiculata (L.) WALP.] genotypes.* A Thesis Submitted to the school of nuclear and allied sciences, University of Ghana, Legon.

Thalouarn, P. and Fer, A. (1993). *Striga*, a noxious weed of sustainable crops: Overview on recent knowledge and control methods. *Cahiers Agricultures*, 2: 167-182.

Tignegre, J. B. D. L. S. (2010). *Genetic study of cowpea (Vigna unguiculata (L.) Walp) resistance to Striga gesnerioides (Willd.) Vatke in Burkina Faso*. Ph.D thesis. School of Agricultural Sciences and Agribusiness. University of Kwazulu-Natal.

Tignegre, J. B. S. (1988). B. Sc. *Study of the resistance of selected cowpea lines to Striga gesnerioides*. Instititut du developement rural (IDR). Ouagadougou. University of Ouagadougou, Burkina Faso, 47pp.

Timko, M. P. and Singh, B. (2008). *Cowpea, a multifunctional legume*. Genomics of Tropical Crop Plants, 227-258.

Timko, M. P., Gowda, B. S., Ouedraogo, J. and Ousmane, B. (2007b). *Molecular markers for analysis of resistance to Striga gesnerioides in cowpea.* In: Integrating New Technologies for *Striga* Control: Towards Ending the Witch-hunt. Ejeta, G. and Gressell, J. (eds.), World Scientific Publishing Co. Pte Ltd, Singapore, pp In Press.

Timko, M. P., Gowda, S., Ouedraogo, J. and Ousmane, B. (2007a). *Molecular markers for analysis of resistance to Striga gesnerioides in cowpea*. In: Ejeta G, Gressell J (eds) Integrating new technologies for *Striga* control: towards ending the witchhunt. World Scientific Publishing Co. Pte Ltd, Singapore, pp 115–128.

Timko, M. P., Rushton, P. J., Laudeman, T. W., Bokowiec, M. T., Chipumuro, E., Cheung, F. (2008). Sequencing and analysis of the gene-rich space of cowpea. *BMC Genomics,* 9: 103. doi: 10.1186/1471-2164-9-103.

Toure, M., Olivier, A., Ntare, B., Lane, J. and St-Pierre, C. A. (1997). Inheritance of resistance to Striga gesnerioides biotypes from Mali and Niger in cowpea (*Vigna unguiculata* (L.) Walp.). *Euphytica*, 94: 273-278.

Umba, U., Dashiell, K., Berner, D. and Ebong, U. U. (1999). Effect of soybean cultivars on Striga emergence and yield of the subsequent maize crop. *Proceedings of Regional maize workshop 4-7th May, 1999, IITA Cotonou, Republic of Benin*. pp. 321-329.

USDA (2015). *Natural resources conservation service*. PLANTS Database, Retrieved 4 December 2015.

VICTORIA LARWEH (201). *INTROGRESSION OF STRIGA (Striga gesnerioides Willd) RESISTANCE INTO COWPEA (Vigna unguiculata L. Walp) VARIETIES.* A Thesis Submitted to the school of nuclear and allied sciences, University of Ghana, Legon.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res*., 23: 4407-4414.

Westwood, J. H., Yoder, J. I., Timko, M. P. and dePamphilis, C. W. (2010). The evolution of parasitism in plants. *Trends Plant Sci.*, 15: 227-235.

Wien, H. C. and Summerfield, R. J. (1984). *Cowpea* (*Vigna unguiculata* (L.) Walp.), p. 353–383. In: P.R. Goldsworthy and N.M. Fisher (eds.). The physiology of tropical field crops. Wiley, Chichester, England.

Williams, J. A., Scott, I. M., Atkin, *A. L.*, Brook, W. J., Russell, M. A. and Bell, J. B. (1990). Genetic and molecular analysis of $vg^{U}$ and $vg^{W}$: two dominant vg alleles associated with gene fusions in Drosophila. *Genetics*, 125: 833-844.

Winter, P. and Kahl, G. (1995). Molecular marker technologies for plant improvement. *World Journal of Microbiology and Biotechnology*, 11: 438-448.

Woomer, P. L., Bokanga, M. A. and Odhiambo, G. D. (2008). *Striga* management and the African Farmer. *Outlook Agric*, 37: 245–310.

Xu., Zhu, L., Xiao, J., Huang, N. and McCouch, S. R. (1997). Chromosomal regions associated with segregation distortion of molecular markers in F2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). *Mol. Gen. Genet.*, 253(5): 535–545.

Yu, K. F., Park, S. J. and Poysa V. (2000). Marker-assisted selection of common beans for resistance to common bacterial blight: efficacy and economics. *Plant Breeding*, 119: 411–415.