INTROGRESSION OF STRIGA (*Striga gesnerioides* Willd) RESISTANCE INTO COWPEA (*Vigna unguiculata* L. Walp) VARIETIES

BY

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BSc. (HONS) GENERAL AGRICULTURE

NOVEMBER, 2016

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

DEPARTMENT OF CROP AND SOIL SCIENCES

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A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF PHILOSOPHY

IN

AGRONOMY (PLANT BREEDING)

NOVEMBER, 2016

DECLARATION

I hereby declare that this thesis is my original work and that, it has not been submitted either in part or whole for any other degree elsewhere. References to other published works have been duly acknowledged.

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DEDICATION

Dedicated to Dr. James Yaw Asibuo, and my sons, Derrick and Derrickson Gasoo.

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ABSTRACT

The parasitic weed, *Striga gesnerioides* (Willd) Vatke is one of the most important constraints to cowpea production in the dry savanna (Derived Savanna, Southern Guinea Savanna and Northern Guinea Savanna) of Northern Ghana. Yield losses due to *S. gesnerioides* range from 83 to 100%. No single method however, seems to be fully adequate in the control of this parasite. Host plant resistance, appears to have merit in effectively and economically controlling the parasite in that it is affordable to farmers. The objective of this study was to introgress *Striga* resistance into existing farmerpreferred cowpea varieties. Two resistant genotypes IT99K-573-1-1 and GH3684 were crossed to two susceptible varieties "Hewale" and "Asomdwee" respectively. The chisquare test was used to test the goodness-of-fit of the observed ratios to the expected genetic ratio in F_2 segregating populations. The results of the cross of genetic of inheritance demonstrated 3R:1S ratio indicating single dominant gene action (monogenic inheritance). The result of the inheritance study indicated that the environment had great influence on a number of agronomic traits. The broad sense heritability for susceptible and resistant were high (63% and 78% respectively). Narrow sense heritability were low for some of the traits which is an indication that environmental factors (*Striga*) influenced cowpea production in this study. Three simple sequence repeat (SSR) markers SSR-1, C42-2B and 61RM2 associated with *Striga* resistance were used to screen 93 F_2 progenies. The study showed that the three markers had discriminating power to distinguish between the resistant and susceptible genotypes and with presence of bands in resistant genotypes. The allele frequency for marker SSR-1 was 65% and 61RM2 was 73%, suggesting that these markers are highly repeatable within the population. Yield loss due to *Striga* infestation was estimated to be (78.22 to 87.17%). Other yield component including pods per plant, 100 seed weight, fodder yield, pod length as well as the number of seeds per pod of the susceptible genotypes were affected. There was significant correlation between percentage yield reduction and percentage reduction in various yield components indicating that *Striga* infestation was responsible for the overall yield reduction. At present very limited sources of *Striga* resistant varieties are available, therefore there is the need to develop new *Striga* resistant cowpea varieties that meet end-user preference. Promising lines will be screened with more *Striga* resistant markers to determine their level of genetic status.

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LIST OF ABBREVIATIONS

ASL	Above Sea Level
AFLP	Amplified Fragment Length Polymorphism
bp	Base pair
BC	Backcross
Bt	*Bacillus thuringiensis*
CM	Centimetre
CRD	Completely Randomised Design
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
WECARD	West and Central Africa Council for Agricultural Development
DNA	Deoxyribonucleic acid
dNTPs	deoxy Nitro Triphosphate
FAO	Food and Agriculture Organisation
UNESCO	United Nations Education Scientific and Cultural Organisation
F_1	First filial generation
GDP	Gross Domestic Product
GE	Genetic Engineering
GEOs	Genetically Engineered Organisms
GMOs	Genetically Modified Organisms
H^2b	Broad sense heritability
HCl	Hydrogen chloride
H^2n	Narrow sense heritability
IITA	International Institute of Tropical Agriculture
KCl	Potassium chloride
KNUST	Kwame Nkrumah University of Science and Technology
MAS	Marker Assisted Selection

$MgCl_2$	Magnesium chloride
MGDW	Molecular Grade Distilled Water
mM	Molar mass
MoFA	Ministry of Food and Agriculture
Mt	Metric tonnes
NH_4	Sodium chloride
PCR	Polymerase Chain Reaction
PGRRI	Plant Genetic Resource Research Institute
QTL	Quantitative Trait Loci
RAPD	Restriction Amplified Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SARI	Savanna Agricultural Research Institute
Sg	*Striga gesnerioides*
SSR	Simple Sequence Repeat
SNPs	Single Nucleotide Polymorphisms
STMs	Sequence Tagged Microsatellite Sites
STRs	Short Tandem Repeats
SRID	Statistics Research Information Directorate
TAE	Tris Acetic acid EDTA
Vp	Variance of F_2
VE	Environmental Variance
WAP	Week after Planting

CHAPTER ONE

1.0 INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is considered the most essential leguminous grain in the dry Savannas of tropical Africa. It is also known as the black- eyed pea or southern pea and is cultivated in a range of ecologies and cropping systems in the tropics. It originated from the semi-arid areas of West Africa and has been cultivated for human consumption for more than 4,000 years. (Tweneboah, 2000). The name cowpea probably originated from the fact that the plant was an important source of hay for cattle in the southern United States of America and in other parts of the world (Timko *et al*. 2007). Some important local names for cowpea include “Beng”in Dagari, “Ayi” in Ewe, and “caupi” in Brazil.

Cowpea is a member of the Phaseoleae tribe of the Leguminosae family (Timko *et al* 2007). It plays a critical role in the lives of millions of people in Africa and other parts of the developing world, where it is a major source of dietary protein that nutritionally complements staple low protein cereals and tuber crops. The high protein content present a major advantage in the use of cowpea as nutritional products, for infants and children and could compensate for the large proportion of carbohydrate often ingested in African diets (Lambot, 2002).

In Ghana, cowpea is an important source of vegetable protein and minerals for over 70% of the population and it is the second most important grain legume after groundnut in terms of production and utilisation (SRID-MOFA 2008). Notwithstanding its significance as human food, cowpea fodder is an imperative source of animal feed (Tarawali *et al*., 2002). Legume haulm provides an especially basic function in nourishing livestock during the harmmattan season in various West African countries

(Tarawali *et a*l., 1997, 2002, Tarawali 1997). Cowpea is a valuable and dependable commodity that generates income for farmers and helps to restore soil fertility for succeeding cereal crops growing in rotation with it.

Cowpea is an important crop in Ghana due to its contribution to national GDP, farmers incomes, food and nutrition security and sustainable agriculture (CORAF/WECARD Cowpea Report, 2011). The per capita consumption of cowpea in Ghana is about 9kg each year (Coulibaley *et al*., 2010). Ghana still import 3.380 metric tonnes of cowpea grains which augment the country production of 219,300 metric tonnes in 2010 (Egbadzor *et al*., 2013).

Regardless of the significance of black-eyed pea in West Africa, its production is still impeded by a myriad of abiotic factors. Biotic components, for example, pests and diseases, and parasitic weeds cause serious threat to cowpea production. The parasitic angiosperm *Striga gesnerioides* (Willd) is one of the significant limitations to cowpea cultivation particularly, in the Guinea Savanna agro-ecology. The parasitic weed *S. gesnerioides* is an obligate root-parasitic blossoming plant of the *Scrophulariaceae* family. Complete crop loss has been reported in susceptible cowpea genotypes following severe *S. gesnerioides* infestation (Muranaka *et al*., 2011). It is believed that the fast spread of this parasitic weed and huge yield decrease would constitute an extreme danger to cowpea production. For the resourced-poor farmers, developing *S. gesnerioides*-resistant cowpea genotypes in blend with fitting management practices are most conservative and effective choices to forestall yield loss brought on by this parasite which seeds are found in plenitude in plagued fields. The utilization of *Striga* resistant cultivars lessens the parasite's seed multiplication and this exhausts the *Striga* seed bank (Badu-Apraku and Lum, 2007; Haussmann *et al*., 2004).

Over the years, the CSIR-Crops Research Institute has released cowpea varieties which are being grown all over the country. Examples of such varieties "Asomdwee" and "Hewale". These varieties are known to be early maturing, high yielding and farmer preferred. They are also known to be adapted to Forest transition, Coastal and Savanna agro-ecologies but are susceptible to *S. gesnerioides*. These varieties are tolerant to other biotic and abiotic stresses and have consumer acceptability. However, the cultivation of these two varieties is a problem in the Savanna areas where *S. gesnerioides* is prevalent. The Savanna zones including Derived Savanna, Southern

Guinea Savanna and Northern Guinea Savanna of Northern Ghana, constitute about 41% of Ghana's landmass and major cowpea growing areas. Therefore, there is the need to address this *Striga* problem by developing resistant or tolerant varieties. This study sought to transfer *Striga* resistance into the background of two existing farmer- preferred cowpea varieties ("Asomdwe" and "Hewale") using conventional and molecular breeding tools.

The main objective of the study was to introgress *Striga* resistance into two improved cowpea varieties using molecular breeding tools.

Specific objectives were to:

i. determine gene action controlling *Striga gesnerioides* resistance, ii. identify F_2 progenies that may be resistant to *S. gesnerioides* using SSR and

SCAR markers associated with *S. gesnerioides* resistance, iii. confirm *Striga* resistance in selected lines through inoculation in pot experiments, and iv. determine the yield loss due to *S. geesnerioides* in cowpea.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Distribution of Cowpea

According to Ng and Padulosi (1988), West Africa is home to different varieties of cultivated legumes and probable domesticated by farmers in this region (Ba *et al*., 2004). India seems to be the origin of hereditary diversification of cowpeas and it is likely that the legume was initially familiar with India in the Neolithic era (Pant *et al*., 1982). The point of divergence of varying characteristics of wild *Vigna* species in Southeastern Africa (Ng and Padulosi 1988; Padulosi *et al.*,1997. Some confirm that domestication happened in Northeastern Africa, taking into account investigations of amplified fragment length polymorphism (AFLP) (Coulibaley *et al*., 2002).

The native cowpea *Vigna unguiculata* ssp. *Unguiculata var.spontanea* is deemed to be the precursor to cultivated southern pea (Pasquet, 1999). *Vigna unguiculata* ssp. Dekindtiana is thought to be immediate progenitor of developed cowpea as strains from this variety can be hybridized with cultivated cowpea (Ehler and Hall, 1997). A cross between the native and domesticated cowpeas gives rise to "weedy" hybrids in some parts of West Africa. Many studies have surveyed the hereditary variability using isozymes (Vaillancourt *et al*., 1993; Panella and Gepts, 1992), protein diversity of seed storage (Panella *et al.,* 1993) and chloroplast DNA, the domesticated cowpea has been found to have a restricted hereditary base, indicating the cowpea experienced a 'genetic bottleneck' during cultivation (Vaillancourt and Weeden, 1992).

About 67% of the cultivation and more than 75% of the range under cultivation is spread over vast Sudan Savanna and Sahelian zones of sub-Saharan Africa. This stretches out from Senegal toward the east through Nigeria and Niger to the Sudan, in

Kenya and Tanzania, and from Angola transversely over Botswana to Mozambique (Timko *et al*., 2007). Greater amounts of black-eyed pea are also produced in South America (generally in the semi-dry north eastern Brazil).

The global production of dried cowpea in 2010 was 5.5 million metric tons (www.cgiar.org). It was reported that Nigeria, being the largest producer of cowpea in the world accounted for 2.2 million tonnes of dried grain in 2010 (www.cgiar.org). The average yield per hectare of cowpea in Nigeria was only 417kg per hectare (Abiola *et al.*, 2010). Niger is the second producer, followed by Burkina Faso, Myanmar, Cameroon and Mali (ww.cgiar.org). However, an average of 143,000 metric tonnes produced annually on about 156,000 hectares making Ghana the fifth highest producer of cowpea in Africa. (Boukar *et al*., 2010).

2.2 Morphological Characteristics of Cowpea

Black-eyed pea is a herbaceous warm-season crop that is comparable in appearance to basic bean with the exception that leaves are most part darker green, shinier, and less pubescent. Cowpea is more colourful in appearance than regular beans with better thrive root structure and thicker stems and branches (Timko *et al*., 2007). Plant development propensity is straight, semi-erect, prostrate (trailing), or climbing based on the genotype, even though photoperiod and external factors can alter the stature of black-eyed pea. Many black-eyed pea genotypes bear uncertain branches and stem apices. Fast blossoming black-eyed pea genotypes mature and produce yield in 60 days, while longer season genotypes may require more than 150 days completing its life cycle depending upon photoperiod (Timko *et al*., 2007). Blossoms emerge on racemes on 15 to 40mm branches that rise up out of the leaf axils. A few pods on a branch are expected, and regularly four or more pods are carried on a branch if external factors are favourable. The proximity of these long branches is a characteristic trait of cowpea, and this quality additionally encourages harvesting by hand. Black-eyed pea seed can weigh around 8 and

32 mg and appear round or kidney-shaped. At the point when seed development is confined by the pod the seed turn out to be continuously more globular. Pods are round and hollow and might be bended or erect, with about 8 and 15 seeds in a pod. The seed coat can be either wrinkled or smooth and of different colours including cream, buff, white, green, brown, dark and red (Ehlers *et al*., 1997).

2.3 Adaptation and Climatic Requirement

Black-eyed pea is a warm-weather, daylight plant and drought tolerant. Southern pea can withstand heat better than most other legumes (Singh *et al*., 2002). It is sensitive to frost in fall and spring and grow well primarily under humid condition. Cowpea is adapted to high temperatures (20–35°C) (Singh *et al*., 2002). It is receptive to enabling growth conditions, and adjusted to dry season and other abiotic factors. The plant interacts with soil bacteria (Rhizobium sp.) to fix atmospheric nitrogen in root nodule thereby enhancing soil fertility especially when used in rotation with cereal crops (Eloward and Hall, 1987; Sanginga *et al*., 2003). The crop grows well in a wide range of soil textures, from heavy clays, if well drained to sands. Light sandy loam soils are more suitable than heavy soils. It grows best in slightly acid to slightly alkaline soils (pH 5.5–8.3) (Hall *et al*., 2003). It has little tolerance to salinity but is somewhat tolerant to soils high in aluminum. Like most legumes, it does not withstand waterlogged or flooded conditions. Cowpea grows under a wide extreme of moisture conditions and moisture deficiency has an adverse effect on vegetative growth and seed formation. It is often grown in rainfed agriculture receiving at least 600 mm annual rainfall, or less if some minimal irrigation is available. Excessive vegetative growth at the expense of seed production may result in rich soils under the climate conditions of the forest zone, and the crop may be better utilized as a green manure or as leaf vegetables under those conditions (Singh *et al*., 2002). Cowpea, like most legumes, requires soil with adequate phosphorus and a good

balance of micronutrients (iron, sulfur and molybdenum) .Cowpea is sensitive to zinc deficiency (Singh *et al*., 2002).

2.4 Nutritional Composition

The diet of most people in developing countries including Ghana is based on cereal grains such as maize, sorghum, rice, and tubers such as cassava. They contain mostly starch and trace amounts of protein. Food legumes, as a result of their high protein content, constitute the characteristic protein needed to supplement staple foods. Cowpeas contain modestly low fat and a total protein composition that is 2-4 times higher than maize, rice and tuber produce. The seed protein content falls within 23 to 32% of seed dry weight (Nielson *et al*., 1993). In the same way, protein composition of twelve cultivars in North America and West Africa fall within 22-29 percent, with most genotypes having protein content values around 22 and 24 percent (Hall *et al*., 2003). On the other hand, it is lacking in methionine and cystine when contrasted with animal proteins. Southern pea seed is also a rich source of vitamins and minerals (Hall *et al*., 2003) and it has one of the largest measures of folic acid, a vital B vitamin that protects the defect of spinal tube in unborn children (http://www.cdc.gov/doc.do/id/ 0900f3ec8000d558).

Fat composition of 100 improved genotypes from IITA produced a fat composition ranging from 1.4 to 2.7 percent (Nielson *et al*., 1993), while fiber is around 6 percent (Hall *et al*., 2003). Apart from its low fiber and fat composition, the protein in blackeyed pea has been demonstrated to decrease low-density lipoprotein that cause coronary disorders (Phillip *et al*., 2003). Protein extracts from southern pea seeds have great emulsifying, solubility and foaming characteristics (Rangel *et al*., 2004), and could be a substitute for soy protein withdraws for persons (particularly babies) with soy protein sensitivities. Moreover, the grains contain micronutrient, for example, iron and zinc which are essential for healthy living (Boukar *et al*., 2010).

2.5 Economic Importance of Cowpea

Cowpea is of real significant value because it provides employment to a huge number of people generally needy individuals in less developed nations of the tropics (Timko *et al*., 2007). The cultivation of cowpea provide rural communities with food, animal fodder and income. The grain is generally saleable in the production centers; it gives an inexpensive and nutritious sustenance for moderately poor urban groups. The new leaves, juvenile pods and peas are utilized as vegetables, while a few snacks and fundamental feast, are made from the grain. All the plant parts that are utilized for food are nutritious, giving protein, vitamins and minerals. Selling of fresh produce and canned food from cowpea provide an opportunities for the rural and urban individuals for making money especially women (Timko *et al*., 2007). Farmers who can and store cowpea haulm, for resulting deal at the crest of the dry season have found to get as much as 25% of their yearly revenue by this source. As far as production is concerned, the spreading, determinate bushy cowpea gives ground cover, smother weeds and give some protection against soil disintegration (Hall *et al*., 2003).

2.6 Diseases of Cowpea

2.6.1 *Phytophthora* Stem Rot (*Phytophthora vignae)*

The influence of stem rot on cowpea cause die-back in patches and yellowing of plant leaf. At the point when expelled from the soil, a light brown patches might be seen totally supporting the base of the stem. In humid conditions, the upper part of the plant might be attacked specifically bringing about a shrinking and fall of the stem. The infection happens under wet and water logged conditions. The stem rot is a serious disease of cowpea and can destroy genotypes that are susceptible. (Schwartz *et al*., 2005). The infection causes dim dark sores on the surface of the lower stem by shrinking and inevitable collapse of the plant.

2.6.2 Wilt *(Fusarium oxysporum)*

This is a root infection of cowpea common after lengthy rain and water-logged conditions. It causes an interior decay and tanned discolouration of the vascular tissue inside the stem, trailed by the fall of the plant. Side effects vary from phytophthora stem rot in that there are no outside stem sores (Quinn, 2014).

2.6.3 Powdery Mildew

It is a cowpea defect which is pervasive under dry conditions or with late planted crops. Taking after a white fine film scattered over the surface of the foliage. At the point when plants are dampness distention it can bring about untimely leaf drop (Schwartz *et al*., 2005).

2.6.4 Tan Spot (*Curtobacterium flaccumfaciens)*

This is a cowpea infection that is wide and have sporadic yellow territories, beginning from the leaf edge and expanding inwards, trailed by a tan discolouration. The infection turn to be more serious and eventually traumatize crops. It can sometimes go undetected in crops developed in great conditions.

2.6.5 Anthracnose

Anthracnose incited by *Colletrotrichum* spp occur mainly in a wide variety of legumes where they cause significant yield losses (Masagwa *et al*.,2013). The disease is characterized by crowded black acervuli borne on well developed stomata. Symptoms usually appear in the early reproductive stages on stems, pods and petioles as irregular brown lesions which later turn black from presence of acervuli that produce minute black spines (setae) visible to the naked eye. The disease is characterized by necrosis of lamina veins, premature defoliation, pod blanking and shriveled seeds resulting in 16 to 26% yield reduction or total crop failure in severe instances (Enyiukwa *et al*., 2014).

2.6.6 *Cercospora* Leaf Spot

Cercospora leaf spot occur mainly on cowpea and on other grain legumes. The symptoms are prominent on the leaves. The affected leaves become sub circular to broadly irregular spots having pale tan to grey centre surrounded by dark brown or reddish margin. The spots coalesce to form round lesions which are brown and necrotic with dark and slightly depressed edges. The pods dry up and eventually damage the pods. Lesions are found on stems and cotyledons (CAB International 2007).

2.7 Pests of Cowpea

2.7.1 The Parasitic Weed (*Striga gesnerioides*)

Parasitic weed *S.gesnerioides* (Willd) Vatke is a commit root-parasitic blooming plant of the *Scrophulariaceae* family that basically pervade dicotyledonous species, including cowpea and other leguminous plants (Thalouran and Fer, 1993). The parasitic weed *Striga gesnerioides* likewise referred to in a few areas as "witch weeds", it is the most essential imperatives to cowpea cultivation in the dry savanna and causes extreme chlorosis, withering, impeding susceptible host, bringing about yield reduction (Omoigui *et al*., 2009). The seeds of these parasites can live in the soil for a long time (over 20 years) until a suitable genotype is planted. Under normal conditions, the seeds tumble to the soil, which pollute the soil in larger quantities. Because of small nature of the seed, they are effectively scattered by wind, water and animals. Under agricultural condition, seeds can be polluted with harvested produce and also soils can be contaminated through the use of farm implement such as plough and harrow. There are two kinds of parasitic weeds that affect cowpea, these *Striga gesnerioides* and *Alectra*. In any case, *Striga gesnerioides* has a more obliterating impact than *Alectra*. *Striga gesnerioides* is boundless in locality with low precipitation and poor soil fertility conditions that are normal all through the northern Ghana (Atokple, 1995).

2.7.2 Economic Importance of *S. gesnerioides* and Damage to Host

Striga and other weed parasites such as *Striga hermonthica* and *Alectra* are the leading biohazards to agricultural productivity in Africa (Sauerborn, 1991). *Striga gesnerioides* represents a critical danger to cowpea production especially in Northern Ghana. Cowpea yield reduces because of *Striga gesnerioides* might be up to 70% reliant upon the degree of harm and level of infestation (Aggrarwal and Ouedranogo, 1989; Alonge *et al*., 2005). On susceptible cultivars, yield losses could reach 100% when *S. gesnerioides* population was more than 10 plant for each host plant (Kamara *et al*., 2008). Omoigui *et al*., 2009 reported that yield reduction brought about by *Striga gesnerioides* in dry savannas of sub-Saharan Africa are evaluated in millions tons every year and the commonness of *Striga* pervaded soils is relentlessly expanding. This is ascribed to the a lot of seeds created by *Striga* plant. Each *Striga* plant can produce up to 90,000 seeds (Parker 1991). Also, acclimatization and inactive nature of *S. gesnerioides* allow the seeds to stay alive in the soil for quite a long time (20 years). *Striga* harm happens at different parts of cowpea plants (Alonge *et al*., 2004) influencing the physiological and biological processes of cowpea plants. Decrease leaf area, photosynthesis, inadequate blooming and podding, and reduced seed advancement have been published (Alonge *et al*., 2004). Such harm is frequently escalated by transpiration by the parasite when dry spell predominate. Once a field is invaded with *Striga* seeds the underground *Striga* seed stock will build up, which sets up a situation of potential yield loss in the future (Cardwell and Lane, 1995). Edaphic factors involve seriousness of *S. gesnerioides* in that its acuteness is higher in sandy soils than clayey soils. In any case, the rate of *S. gesnerioides* is controlled by the collaboration between the host and the parasites. (Cardwell and Lane 1995).

2.7.3 Geographical Distribution of *Striga gesnerioides*

The areas affected by *S. gesnerioides* comprise West and Southern Africa, India, Asia or Europe and USA (Mohamed *et al*., 2001). *S. herrmonthica* is confined to East and West

Africa and infests host similar to *S. asiatica*. In West Africa, *S. gesnerioides* was reported to occur in Benin, Burkina Faso, Mali, Nigeria, Niger, Ghana, Togo, and Cameroon with one race designated to each country (Cardwell and Lane 1995). These races were assigned as SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria and Niger), SG4 and SG4z (Benin), SG5 (Cameroon) and SG6 (Senegal) (Botanga and Timko 2005). The past studies have not examined parasite from Ghana leaving its phylogenetic position and damage range unknown. However, Asare *et al*., (2010) suggested that the Ghanaian form of *S. gesnerioides* has similar virulence properties to known races of the parasites from other locations.

2.7.4 Taxonomy of *Striga* species

There are roughly 3,000 plant species of parasitic weed grouped in 17 families (Kuiper *et al*., 1998). They can be parasites of cereals and legumes (Botanga and Timko, 2005). The genus *Striga* is predominantly African in origin and distribution and about 30 are endemic to Africa (Mohamed *et al*.,2001). The genus *Striga* belongs to the family *Scrophulariaceae* which comprises about 50 species (Botanga and Timko, 2005). They are also among the most specialized of all root-parasitic *Scrophulariaceae* (or *Srobanchaceae* depending on how the families are circumscribed). Most members of the *Scrophulariaceae* are holoparasitic (without chlorophyll and totally dependent on the host for organic carbon, water and nitrogen), some are hemiparasitic (with chlorophyll) (Matusova *et al*., 2005). They have chlorophyll that is masked with other pigments. As a result, plants are white, shades of purple, and red similar to *Orobanch*e species (Mohamed *et al*., 2001). In addition, plant of *S. gesnerioides* have leaves reduced to scales-feature common to all *Orobanche* species. *Striga* spp. belonging to *Orobanchaceae* are hemiparasites because of the aerial photosynthetic activity occurring after *Striga* emergence from soil (Matusova *et al*., 2005). These pathogens attack their hosts

underground and by the time the parasites emerges and is evident, the crop is damaged. Their destructive behaviour may be the source of the Latin name

"*Striga*" meaning "hag" or "witch". In this way hosts are "bewitched" because the farmer is unaware of the parasites until it comes up. There are different species of *Striga* of which *S. hermonthica* and *S. aspera* are parasites of cereals and form the largest among the agronomically important species, and the most destructive of all *Striga* species. *S. gesnerioides* is the only species attacking broadleaf host, which cause threats to dicotyledonous spp in particular cowpea (Berner and Williams, 1998). *S. gesnerioides* can also attack tobacco (*Nicotiana tabacum* L.), sweet potato (*Ipomea batatas* (L.) Lam) and other legumes

2.7.5 The Biology and Life Cycle of *Striga gesnerioides*

The life cycle of *Striga gesnerioides* constitute a series of growth phases that are linked to the developmental stages of the host plant. (Lane and Bailey, 1992; Matusova *et al*., 2005). There are biochemical signals that coordinate *Striga* life cycle to the hosts (Matusova *et al*., 2005). When the *Striga* seeds are formed, they need a post-harvest maturation period of six to seven months upon which *Striga* completes the physiological maturation process (Thalourarn and Fer, 1993). The seeds remain dormant if the temperature is below 25^0C or above 35^0C (Kuiper *et al*., 1996).

Temperatures ranging from 30 to 35 °C in a moist environment are ideal for germination. The seeds of *Striga* require an inhibition period of 10 to 21 days before they can germinate (Okonkwo 1991; Lane and Bailey, 1992). Host root exudates contain strigolactones, signaling molecules that promote *Striga* seed germination. Its seeds sprout when stimulated by the host's roots (Lane and Bailey, 1992; Matusova *et al*., 2005).

They must attach to the roots of suitable host soon after germination in order to survive. The radicle of *Striga* grows and a bell-like swell forms where the parasitic roots attach to

the roots of the host. After germination, a haustorium is shaped through separation of the reticular apex. A vascular association is consequently settled with the host, permitting the weed to obtain the water and supplements that are fundamental for its development (Dubé and Olivier, 2001). However, the *Striga* radicle cannot survive more than 7 days if the connection to the host is not achieved, because nutrients in seeds are very limited due to its small size (Berner and Williams, 1998). The *Striga* seeds are microscopic in size measuring 0.20mm to 0.35mm long, weighing 4 to 7µg (Dubé and Olivier, 2001). However, the nature of the seeds facilitate dissemination through water, wind and soil via animal vectors. The major means of dispersal, however is through human interaction, by means of machinery, tools and clothing (Mohamed *et al*., 2001). Due to this association with the crop plant *Striga* reduces the growth and markedly alters the architecture of crop plants.

Table 2.1 Different Species of *Striga* and their Host S*triga* Species Host Plant

Striga gesnerioides	Cowpea, Tobacco, Sweetpotato, *Tephrosiaspp, Indigofera tinctoria*
Striga hermonthica, Striga asiatica (clusters includes: *Striga hirsuta, Striga lutea, Striga elegans*	Sorghum, Millet, Sugar cane and Maize
Striga aspera	Maize, Rice and Sugar cane

2.7.6 The Sources of Resistance to *Striga gesnerioides*

In light of the differential resistance reaction of different cultivars, breeding lines and landraces, a minimum of seven particular races of cowpea-parasitic *S. gesnerioides* have been characterized inside the cowpea production areas of West Africa (Lane *et al*., 1996). Cowpea have different sources of resistance each combining the resistance to at least two races of *S. gesnerioides* of West Africa. As indicated by Botanga and Timko 2005, race development in cowpea *S. gesnerioides* was generally a consequence of hostdriven

selection in light of the fact that the parasite is autogamous, with flower anatomy that makes any possibility of out-crossing minimal. In cowpea, resistance depends on *Striga* strains and a combination of several mechanisms that influence the development of the parasite (Parker and Polniaszek, 1990; Muller *et al*., 1992; Lane *et al.*, 1996; Touré *et al*., 1997; Reiss and Bailey, 1998). The genetics of southern pea *Striga* resistance differs based on the biotype of the parasite and varieties, and is acquired predominantly as a single gene. (Singh and Emebeche, 1990; Atokple *et al*., 1993; Lane *et al*., 1993; Moore *et al*., 1995; Touré *et al*., 1997; Carsky *et al*., 2003). Notwithstanding, few studies identified that resistance is given by two independent dominant genes or recessive single genes (Dube, 2000). Therefore this study sought to confirm or verify the results of the previous studies.

2.7.7 Measures to Control *Striga gesnerioides*

It is difficult to manage witch weed due to the fact that the larger part of its life cycle happen subterranean, when it is not recognized before rise; it is past the point where it is possible to decrease crop infestation (www.wyoug.nsw.gov.au/environment/weeks).

The life span of *Striga* seeds in the soil and economic consequences of cultivation in Africa obstructs the effective weed control measures for *Striga* species (Lane *et al*., 1993). A few control techniques have been developed including enhanced cultural practices, chemical control methods and breeding for resistance genotypes (Berner *et al*., 1995). Chemical control techniques are costly for peasant farmers, whilst cultural practices offer essentially long term advantages. Germination stimulant of *Striga* seeds can be effective in controlling *Striga* by inducing suicidal germination (Berner and Williams 1998; Berner *et al*., 1997). However, such methods are expensive to smallholder farmers of Sub-Saharan Africa. Alternatively trap-crop can be used to reduce *Striga* seed stock in the soil. Among the effective trap crops, a variety of sorghum bicolor named Bagauda Farafara was found to be the highest germination stimulant of *S.gesnerioides*

(Berner and Williams 1998). Some studies recommend that, as a control measure postponing the sowing of black- eyed peas could diminish the level of *Striga* infestation (Lagoke *et al*., 1991). Toure *et al*. (1996) observed some varietal contrasts while postponing the sowing of cowpea brought about diminishing quantities of sprouted *S. gesnerioides*. However, as indicated by Parker (1991) the utilization of weed-resistant or tolerant genotypes is likely the most effective technique for small scale farmers to control *S. gesnerioides*. Alonge *et al*. (2004) demonstrated that *S. gesnerioides* infestation diminished the root nodulation, root and shoot dry weight of a considerable measure of cowpea particularly in the late planted trials.

2.7.8 Mechanism involved in the Resistance to *Striga gesnerioides*

The germination, haustorial incitement, connection to, and infiltration of the host circulation system are all basic phases in *Striga* life cycle (Botanga and Timko, 2005). A variety of molecules which vary in compound structure and particular action are generated by the host roots. After germination, the host-derived synthetic sign from the root, known as the haustorium initiation factor, is required for the separation of radical into the haustorium by which the *Striga* seedlings attach 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 association is built up between the host and parasite, the growth of the haustorium stops, the *Striga* seedling expands, becoming a dense mass of tissue called the tubercles. The haustorium permits the transfer of water and nutrients to the parasite. *Striga* penetration of host root tissue involves a mix intrusion and enzymes digestion (Godwa *et al*., 1999) Lane *et al*. (1993) observed that on the resistance line B301, the roots stimulate germination of *Striga* seeds and permit attachment, but hasutorial formation and growth are inhibited. *Striga* shows strain variety such that cultivars varies in their resistance from one location to another (Lane *et al*., 1994). Genomic research have demonstrated that three dominant non-allelic genes give

resistance to various *Striga* biotypes (Singh,1993). An alternate group of three dominant, nonoallelic genes has been recognized to be resistant to *Alectra* (Singh, 1993). Thorough examination has given confirmation to at least two unique processes of resistance to *Striga* parasitism in southern pea (Li *et al*., 2009; Li and Timko, 2009). One mechanism resembles the hypersensitive response (HR) observed in other plantplant pathogen interactions and suggested the presence of a specific R gene-mediated response mechanism. The second type of resistance response involves arrested development of the parasite tubercle following attachment and attempted penetration of the root cortex (Mohamed et al., 2010).

2.8 Breeding for Resistance to *Striga gesnerioides*

Evidence has shown that genetic enhancement of southern pea have taken place within national research facilities and universities in a couple of West African countries, India, Brazil, USA and International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Timko *et al*., 2007). The imbricate dispersion of the five parasite races has essential outcomes for breeding resistant cowpea. While most cowpea plants are prone to *Striga* parasitism, some native landraces and wild accessions have been discovered that are resistant to the parasite, and in many reports resistance is a dominant characteristic, acquired in a monogenic way (Aggarwal *et al*., 1984; Touré *et al*., 1997; Ouédraogo *et al*., 2001; Ouédraogo *et al*., 2002; Singh, 2005; Timko *et al*., 2007b). The use of *Striga* resistant/tolerant varieties is the most feasible and sustainable approach for mining the losses caused by this parasitic weed (De Vries, 2000; Badu-Appraku *et al*., 2005; Menkir *et al*., 2005). According to Parker (1991) the use of resistant varieties are probably the most appropriate way for subsistence farmers to control *S. gesnerioides*.

The most important source of resistance is the landrace B301, originally selected for its partial resistance to *Alectra vogelii* in Botswana (Parker and Riches, 1993). B301 fortunately shows high-level resistance to *A. vogelii* in West Africa (based on two

dominant genes) as well as to *S. gesnerioides* (based on a single dominant gene) (Singh *et al*., 1993; Atokple *et al*., 1995). The resistance, or virtual immunity of this line has been effective against all biotypes of the parasite in West Africa except that it occurs locally in southern Benin. Lane *et al*. (1996) describe the existence of five known parasite biotypes, varying in their virulence on different 'resistant' varieties of cowpea. Two other sources of resistance, Suvita-2 and IT82D-849, have different single dominant genes for resistance to the Mali biotype, and a different pattern of response to the five parasite biotypes (Atokple *et al.*, 1995). IITA (International Institute for Tropical Agriculture) has now developed lines with resistance to *Striga* and *Alectra*, as well as to various other pests and diseases. Among the developed lines include IT99K573-1-1, IT99K-573-2-1, IT82D-847 and IT81D-994. They were indeterminate, semierect, and photosensitive and high yielding (Guissai, 2010). Alternatively, cowpea genotypes could be assessed rapidly against the full range of virulence using *in vitro* tests. Information on parasite virulence is essential for determining the optimum development of resistance across West Africa. The virulence data can facilitate monitoring the changing distribution of *S. gesnerioides*. This become increasingly important with the greater development of *Striga* resistant germplam in West Africa (Timko *et al*., 2007).

2.9 Genetics of *Striga gesnerioides* Resistance in Cowpea

Around seven remarkable races of *S. gesnerioides* (assigned SG1-SG7) have been characterized (Lane *et al*., 1997a, Botanga and Timko, 2006). Many cowpea species are inclined to *Striga* infestation, despite the fact that some regional landraces appeare to be impervious to some *Striga* races (Timko *et al*., 2007) with resistance being given by single dominant gene (Aggarwal *et al*., 1984; Toure *et al*., 1997). Gene symbols *Rsg*1, *Rsg*2, *Rsg*3 and *Rsg*4 are proposed for resistance to *Striga generioides*. The genes have been shown to be independently assorted and non-allelic (Atokple *et al*., 1993). Initial inheritance studied demonstrated that resistance *S. gesnerioides* race-SG1, race-SG2,

race-SG3, and race-SG4 in some cowpea are monogenic (Touré *et al.*, 1997, Atokple *et al.*, 1993; Moore *et al*., 1995). Resistance to SG1 in the cultivar B301 and IT82D-849 might be presented by various alleles at the same locus as two class of resistance are expressed (Atokple *et al*., 1995). Studies conducted by Touré *et al*. (1997) confirmed that *S. hermonthica* and *S. asiatica* are controlled by a recessive gene. *Striga* resistance in maize is quantitatively inherited (Kim, 1994). Recently, Singh and Emechebe (1990b) and Singh *et al*. (1993) reported *Striga* and *Alectra* resistance in cowpea genotype B301 is influenced by a single dominant gene *Rsg* and duplicate dominant genes *Rav*1 and *Rav*2 respectively.

2.10 Mechanisms of Plants Resistance

2.10.1 Antibiosis

Antibiosis is the mechanism that describes the negative effects of a resistant plant on the biology of an insect which has colonized the plant (e.g. adverse effect on development, reproduction and survival). Both chemical and morphological plant defenses can induce antibiosis effects. The consequences of antibiosis resistance may vary from mild effect that influences fecundity, development time and body size through to acute direct effect resulting in death (Kogan and Omar, 1978). Antibiosis may be due to presence of toxic substances, absence of sufficient amount of essential nutrients and nutrients imbalance improve utilisation of nutrients.

2.10.2 Antixenosis

Host plant resistance is responsible for non-preference of the insects for shelter, oviposition and feeding. It denotes presence of morphological or chemical factors which alter insects or pest behaviour resulting in poor establishment of the insect or parasite. Antixenosis is the inability of a plant to serve as host to an insect herbivore. The basis of this resistance mechanisms can be morphological (eg. Leaf hairs, surface waxez, tissue

thickness) or chemical (eg repellants) or antifeedants. These plants would have reduced initial infestation and/or higher emigration rate of the insect than susceptible plants (Kogan and Omar 1978).

2.10.3 Tolerance

Ability to grow and yield despite pest attack. Tolerance is the ability of a plant to undergo stress (diseases, infected or physiologically challenged) but the extent of loss does not exceed the economic threshold level (an extent of loss which do not hamper the economic potential of the produce). It is generally attributed to plant vigour, regrowth of damage tissue, to produce additional branches compensation by growth of neighbouring plants.

2.11 Heritability

Heritability is a measure of the degree (0 to 100%) to which offspring resemble their parents for a specific traits. Heritability measures the strength of the relationship between performance (phenotype) and breeding value (genotype) of an individual. Breeding value is the sum of the additive effects of the alleles at the locus. Heritability tells the breeder how much confidence to place in the phenotype performance on an individual when choosing parents for the next generation (Provine, 2001). Heritability is one important component of the equation used to predict genetic progress from selection to improve a trait. Thus heritability denotes the proportion of phenotypic variance that is due to genotype. Heritability is classified as broad and narrow sense.

The broad sense heritability estimate heritability on the basis of all genetic effects (Wray and Visscher, 2008). It expresses total genetic variance as a percentage, and does not separate the components of genetic variance. Genetic variance include additive, dominance and epistatic effect. Generally broad sense heritability is a relatively poor predictor of potential genetic gain or breeding progress. Its usefulness depends on the particular population. Narrow sense heritability in contrast expresses the percentage of

genetic variance that is caused by additive gene action. Narrow sense heritability is always less than or equal to broad sense heritability because narrow sense includes only additive effects whereas broad sense heritability is all genetic effects. The usefulness of broad over narrow sense heritability depends on the generation and reproductive system of the particular population. In general, narrow sense heritability is more useful than broad sense heritability since only additive gene action can normally be transmitted to progeny (Wray and Visscher, 2008).

2.12 Conventional Breeding

Conventional plant breeding is the manipulation of plants attributes, structure and composition so that such plants become useful to mankind. New breeds of plants are engineered to adapt to specific weather conditions, improve taste or nutrition, adapt to pest and diseases better, to utilize water more efficiently. To traditionally breed a novel plant, two closely-related plants are 'sexually crossed' (Piepho and Mohring, 2007). The objective is to join the attractive characters from both plants and take out undesirable characters in a particular new and better plant strain. Nonetheless, the first filial generation gain a blend of traits from both parental plants and so both desirable and undesirable characters might be acquired. A breeder will need to re-examine all the offspring and select strains with the desirable characters whiles minimizing the selection of undesirable characters. He then crosses the selected offspring back to one of the first parent plants to attempt and exchange a greater amount of its desirable characters into the second filial generation. This procedure is termed "back-crossing" which normally takes many years until the offspring have all the desired attributes and none of the negative ones of the initial two parent plants.

2.12.1 Limitation to Conventional Breeding

Conventional breeding strategies have the impediment of a thousand of genes getting transferred in each cross, which might possibly be useful alongside the desired ones in

the target species. Another significant limitation in conventional breeding include the barriers for genes transfer through incompatibility. Although trait selection has habitually been fruitful to recognize genes with a substantial impact on the phenotype, on numerous occasions ideal alleles of little impact, specifically for multifactorial characters, have frequently remained hidden preventing them from being used for breeding purposes. (Morgante and Salamini, 2003). Thus, the masking impact of environment may diminish the efficiency of selection, bringing about the loss of great alleles during the selection process. As an account of these restrictions of conventional breeding, extra genetic advances in major crops for yield or for different characters for which extensive breeding has been done is becoming more and more complicated. In value, for some major crops the pace tested for genetic gain in yield in the twentieth century will be hard to be maintained if only existing conventional breeding advances are utilized (Araus *et al*., 2008).

2.13 Genetic Engineering

Since the beginning of farming, people have found a way to enhance plant characteristics, for example, hardiness, taste, versatility and beauty. Some years back, farmers just spared seeds from their best plants for replanting. Over time, plant breeders develop sophisticated techniques to advance specific traits. The latest, some might say greatest, technique is genetic engineering (GE), and advocates say it's only the next step in humanity's long history of development for enhancing crop plants. Genome Editing (GE) techniques have made it possible to insert genes from different sources to overcome issues of sexual incompatibility and gaps between species (Hammer and Teklu, 2008). This technique enable breeders and molecular geneticists to introgress the desired gene with specificity. It is nothing strange but a mere improvement in classical breeding technology. The core objective of advanced genome editing is engineer a living cell for specific beneficial activities, precisely and controllably. The result of GE innovation is a

transgenic or genetically modified (GM) organisms. GMOs have many favorable traits which comprise the ability to withstand biotic and abiotic stresses, adds nutritional quality to the products leads to increase in productivity (Hammer and Teklu, 2008).

2.13.1 Benefit of Genetic Engineering

As safety is concerned, the potential threats of genetically-engineered organisms (GEOs) should be evaluated and analyzed to identify ecological impact, and threats to traditional and other ago-practices like organic farming. Herbicide-tolerant and pestresistant transgenic breeds may reduce the use of environmentally dangerous chemicals to control insects. Herbicide-tolerant product may incite natural favorable circumstances by urging a move to conventional tillage method. Specifically, these crops may allow agriculturists to remove preemergent herbicides that are included into the soil and rely on upon post emergent herbicides, for instance, glyphosate. The move to post emergent control of weeds may propel no till and conservation tillage methods that can lessen soil erosion, water leakage and enhance the organic composition of soil. On the other hand, if genetically engineered products will enhanced yield, some propose that ecological advantages will be beneficial and natural habitats for development will be increase. Some genetic engineering of plants may give *in situ* remediation of contaminated soils, silt, surface waters, and aquifers. Transgenic plants can facilitate removal of poisonous metals from dirtied soils, water and sequester these into plant tissue available for harvest (Gleba *et* al., 1999; Zhu *et al*., 1999).

2.13.2 Limitation to Genetic Engineering

The arrival of GEOs highlights the general difficulty in forecasting when and how nonnative species brought into an ecosystem. Nonindigenous species have been brought into the United States intentionally and surprisingly and are not local (Pimentel *et al*., 2000). Direct non target effects on beneficial and wild species: Plants engineered to make pesticidal properties, for example, *Bacillus thuringiensis* (Bt) poison, may have both

incite and moderate acting consequences for individuals of non target species. One class of harmful substances from Bt basically targets Lepidoptera (butterflies and moths, for occurrence, the European corn borer), and another generally impacts scarab (Coleoptera) (Stotzky, 2000). GEOs may have control over species that rely on the parasites controlled for survival or reproduction. Ecological models recommend a more viable control of weeds by utilizing herbicide tolerant yields could induce be more relevant to peasant farmer (Watkinson, 2000).

2.14 Marker Assisted Selection

The utilization of DNA markers in plant breeding is called marker-assisted selection (MAS) and a segment of the new train of 'molecular breeding'. Marker Assisted Selection (MAS) is a strategy where molecular markers are utilized to choose genotypes that carry a trait of interest. The significance of this approach is that marker phenotypes can be characterized at the seedling phase, reducing plant maturation period in populace size (Yu *et al*., 2000).

Additionally, seeds screening disregard the potential for genotype by environment (G x E) interactions. Marker-assisted selection procedures are promising in light of the fact that the appraisal for *Striga* resistance in the field is intricate, costly and sometime unpredictable (Haussmann *et al*., 2000). Various genomic markers have been created for recognizing polymorphism in plants. The RFLPs (Restriction Fragment Length Polymorphisms) were the main comprehensively utilized genetic fingerprints (Tanksley *et al*., 1999).

RFLPs are to a great degree proficient in characterizing polymorphism; however intricate automating the method and the large amounts of DNA required has decreased the usage of this technique. The AFLPs (Amplified Fragment Length Polymorphism) were designed as a Polymerase Chain Reaction (PCR) based methodology for identifying

polymorphisms (Kochert, 1994), but modern genotyping uses the Simple Sequence Repeats (SSRs), particularly in maize. SSRs have multiple benefits including a simple automating process, more open SSR accessible, and cost-effective once the oligonucleotides are designed (McCouch *et al*., 1997). With the new database availability of genome sequences, SNPs (Single Nucleotide Polymorphisms) are turning out to be more utilized in both public and private genetic projects. MAS is preferred for characters controlled by major genes and by QTL (Quantitative Trait Loci). In the selection of gene of interest, a breeder first has to choose variable parental phenotypes and develop generations segregating for that phenotype. The parents are then screened for polymorphisms in their genome using bio-markers. Polymorphic markers are population-wide, and the data accumulated are used to map the genome. Screening of the population is done to identify phenotypes, and statistical software used to identify genomic markers from the linkage map are connected with the phenotype

(Ragot *et al*., 1995)

2.14.1 Advantages of Marker Assisted Selection

The widespread application of genomic markers in different fields of plant science, for instance germplasm assessment, genome mapping, map-based gene discovery, trait characterization has revealed that molecular techniques are effective and dependable approach in DNA engineering of agronomically desired phenotypes in crops (Xu, 2010; Jiang, 2013). However, marker assisted selection has significant advantages as follows (i) Marker assisted selection can permit a determination for a wide range of characteristics to be done at seedling stage and hence lessen the time required before the phenotype of an individual plant is known. For the characters that are expressed at later formative stages, undesirable genotypes can be quickly eliminated by marker-assisted selection MAS). (ii) The utilization of markers is not influenced by environment, in this way permitting the selection to be performed under any natural conditions (eg. Nursery and

off-season nurseries). This is highly useful for engineering of specific phenotypes that are expressed just when ideal ecological conditions are available. For lowheritability characters are usually influenced by the environment, MAS based on solid markers firmly connected to the quantitative trait loci (QTLs) for genes of interest can be more compelling and productive than phenotypic approach. (iii) Apply to codominance markers (eg SSR and SNP) can permit successful choice of latent alleles of desirable traits in the heterozygous mode. No selfing or test-crossing is expected to locate the genes influenced by latent alleles. (iv) For multifactorial traits and QTLs, genes in the same population can be recognized and simultaneously be selected in MAS, and hence MAS is especially adopted for gene pyramiding**.**

2.15 Genetic Markers

Genetic markers are DNA sequences with a precise locus on a chromosome that can be used to identify individuals. It is the variation (brought about by alteration in the DNA site) that can be characterized. Molecular markers employed in plant breeding and genetics falls under two groups: DNA Markers and Mendelian markers (Xu, 2010). Mendelian (Classical) markers contain morphological components, cytological markers and biochemical markers. DNA markers have advanced into various procedures in light of utilizing different polymorphism-identifying strategies (PCR, southern blotting, nucleic acid hybridization, PCR and DNA sequencing) (Collard *et al*., 2005), for instance, RFLP, AFLP, RAPD, SSR and SNP. These morphological markers for the most part reflect genetic variability which are effortlessly recognized and controlled. In this way, they are typically utilized as a part of development of linkage maps by established a few point tests. Some of these markers are connected with other agronomic attributes and hence can be utilized as alternate choice criteria as a part of functional breeding. Cell-based markers, chromosomal structures can be visualized by karyotype and bands (Xu, 2010). The banding arrangement, showed in colours, order, width, and position, display

the distinction in frequency of euchromatin and heterochromatin. For example, Q bands emerge from quinacrine hydrochloride, G bands are created by Giemsa stain, and R bands are reversed G bands (Collard *et al*., 2005). These chromosomal features are utilized not just for differentiation of normal and chromosome mutation analysis, they are further used as a part of physical mapping and linkage group detection. Protein or biochemical markers may likewise be divided into molecular markers however the last are usually synonymous with DNA markers. Isozymes are elective structures or auxiliary variations of a chemical that have distinctive molecular weights and electrophoretic portability however, have the same metabolic pathway. Isozymes represent the results of various alleles as opposed to various genes on the grounds that the distinction in electrophoretic mobility brought on by point mutation as an result of substitution in amino acids (Xu, 2010)

2.15.1 Simple Sequence Repeat (SSR) Markers

SSR, also termed as short tandem repeats (STRs) or microsatellites are PCR-based markers. They are short nucleotide motifs; random tandem repeats (2-6 bp/nucleotides long). Di-, tri-and tetra-nucleotide rehashes, e.g. (GT) n AAT) n and (GATA)n, are generally dispersed through the genomes of plants and other species. The duplicate number of these repeats differs among species and can lead to polymorphism in plants. Since the DNA sequences flanking microsatellite regions are normally conserved, primers-particular for these areas are intended for use in the PCR reaction (Song *et al*., 2010).

The distinguishing feature of microsatellite loci is that they exhibit high allelic variation, hence their use as molecular markers. The special sequences around SSR motifs give layouts to particular primers to increase the SSR alleles by means of PCR.

SSR loci are individually amplified by PCR utilizing sets of oligonucleotide precursors particular to one of the special sequences around the SSR region. The PCR-multiplied products can be isolated in high-resolution electrophoresis technique (e.g. AGE and PAGE) and the bands can be recorded by fluorescent marking or silver-staining. SSR markers are described by their hyper-variability, co-dominant nature, reproducibility, locus-specificity and mostly, random-genome wide. The benefits of SSR markers are that they can be rapidly be analysed using PCR and can simply be detected by AGE or PAGE

.

SSR markers can be multiplexed, have high throughput genotyping and can be robotized. SSR examination requires just little DNA concentration (~100 ng per individual) and low start-up expenses for manual assay protocols. In any case, SSR procedure requires nucleotide input for oligonucleotide design, laborious marker design protocols, capital intensive and expensive start-up for robotized discoveries. Beginning in the 1990s SSR markers have been widely utilized as a part of developing molecular linkage maps (Song et *al*., 2010), QTL mapping, marker-assisted determination and germplasm investigation in plants. In numerous species, a lot of breeder-friendly SSR markers have been designed and are accessible for researchers. For example, there are more than 35,000 SSR markers created and mapped onto every one of the 20 linkage bunches in soybean, and this data is accessible for the general public (Song *et al*., 2010).

2.15.2 Advantages of Simple Sequence Repeat Markers

In SSR markers low quantities of DNA template are required (10-100ng per reaction) and high in genomic abundance. Random distribution throughout the genome and high levels of polymorphism (alleles). SSR markers have band profiles that can be interpreted in terms of loci and alleles. SSR markers have co-dominance of alleles and allele sizes can be determined with accuracy of 1bp, allowing accurate comparison across different gels.

It is highly reproducible and different microsatellites may be multiplexed in PCR or on gel.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The development of F_1 and F_2 were done on the Research fields of CSIR-Crops Research Institute at Fumesua (01° 36W; 06 ° 43N) during the month of March to December, 2014 raining season. Fumesua falls within the Semi-deciduous forest zone with elevation of 186m above sea level and has bimodal precipitation. In the Semideciduous forest zone, the significant rains begin in March for major season and September for minor season. The soil at the site at Fumesua is Asuansi series; Ferric Acrisol (FAO/UNESCO, 1986). It has 16-20cm thick layer of sandy loam topsoil and a slope of 1-5%.

3.2 Planting Materials, Source and Attributes

The cowpea genotypes used for the experiment are provided in Table 3.1. Seeds of *S. gesnerioides*-resistant cowpea were obtained from CSIR-Plant Genetic Resource Research Institute, Bunso, Ghana, the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria and the CSIR-Savanna Agricultural Research Institute, Temale, Ghana. The susceptible varieties were obtained from CSIR-Crops Research Institute Fumesua, Ghana.

The resistant genotypes are mostly white seeded cowpea and obtained from IITA (Table 3.1). Their growth habit is indeterminate, semi-erect, photosensitive and high yielding. It has a long pods with bold seeds. It mature within 60 to 65 days. Songotra is a white seeded cowpea with black eye. The growth pattern is semi-erect and indeterminate. It matures within 65 to 70 days. However, the GH3684 is also semi-erect and indeterminate. The seeds are mostly red, the seeds size are small and mature within 75 to 80 days.

Table 3.1 Sources of Planting Materials and their Attributes

Genotype/Varieties	Source	Attribute
IT99K-573-2-1	IITA	Resistant
IT99K-573-1-1	IITA	Resistant
IT07K-303-1	IITA	Resistant
IT07K-291-69	IITA	Resistant
Songotra	SARI	Resistant
GH3684	PGRRI	Resistant
Asetenapa	CRI	Susceptible
Asomdwee	CRI	Susceptible
Hewale	CRI	Susceptible

Brief Description of the Susceptible Parents (Asomdwee, Hewale and Asetenapa):

These susceptible cowpea varieties "Asomdwee" and "Hewale" were released by CSIR-CRI in 2010 mainly for areas where *Striga* is not a problem. They are very high yielding varieties preferred in the Forest transition, Coastal and Savanna areas. Their growth habit is semi-erect and growth pattern is semi-determinate. They flower between 40 and 46 days with days to maturity ranging between 65 and 72 days. They have potential grain yield of about 2863kg/ha^{-1} to 3130kg/ha^{-1} under *Striga* free environment.

3.3 Crossing

The crossing block was designed using the North Carolina Design II. In this design, each member of a group of parents used as males was mated to each member of another group of parents used as females. Each male is crossed to a different set of females as shown in (Table 3.2). As such the females are nested within males. Each cross generate one family. Blocking used in this design to allow all mating involving a single group of males to a single group of females to the kept intact as a unit (Acquah, 2012).

Table 3.2 Crossing Block Layout

Males	Females		
	Asetenapa	Asomdwee	Hewale

IT99K-573-2-1	X	X	X
IT99K-573-1-1	X	X	X
IT07K-303-1	X	X	X
IT07K-291-69	X	X	X
Songotra	X	X	X
GH3684	X	X	X

3.4 Land preparation, Agronomic Practices and Development of F_1 Progenies

The land was prepared by slashing and ploughing followed by harrowing. As a weed control strategy, weed re-growth was allowed for a period of two weeks prior to sowing and were controlled by applying glyphosate (Roundup, Nova Agro (HK) Ltd, Hong Kong) at a rate of 300ml per 15L of water.

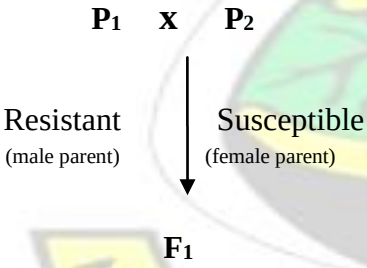
The field was demarcated into 5m long plots and the distance between each plots was 1.5m. There were 18, four- row plot in all with the first row containing the males and the remaining three rows containing the females. On each plot were four rows of cowpea sown at a spacing of 60cm between rows and 20cm within rows. In all, three seeds were sown per hill and later thinned to two plants per hill. The male parents were planted four days before the females. This was to ensure that adequate pollen was produced ahead of anthesis of the female parents although protandry mechanisms or flower types are observed in cowpea.

Field pests were controlled by applying preflowering insecticide Karate (Super 2.5EC, Dizengoff, Netherlands) at the rate of 15g/15L of water. Post flowering field pests were controlled using Cymethoate (Lambda super 2.5EC, Kumark company Ltd, China) 100-120ml at the rate of 1.5L/hectare.

In the late daytime on the day preceding fertilization, flower buds that were bound to open the next morning were identified and emasculated. Emasculation was performed by

opening the sepals encasing the keel, opening the keel and evacuating anthers. All operations were performed with tweezers sanitized in 70% ethanol between plants. Care was taken to ensure that anthers do not touch the stigma, which could bring about selffertilization since the stigma was open at that point. The emasculated blossoms were labeled for identification. In early hours between 6:00am to 9:00am in the morning, pollination was accomplished by taking open blossoms of the selected male (resistant parent) and brushing pollen from the burst anthers and style of the male onto the emasculated blossom. The male blossoms picked had enough pollen. A successful pollination normally takes places between the hours of 7 and 10 am. Pods were harvested three weeks after pollination.

The resistant parent was crossed to introduce traits/genes into susceptible parent. The P_1 was the donor or nonrecurrent parent (DP) where the source of desirable traits was coming from and P_2 was the recurrent parent (RP) where the desirable traits was transferred to. The F_1 (First filial generation) was the offspring which was referred to as heterozygous.



3.5 Backcrossing and Selfing of F_1 progenies

F_1 pods were dried and threshed and the seeds were put into envelopes. The F_1 seeds were then planted on the field at CSIR-Crops Research Institute along with parental varieties. Twenty seeds of F_1 were backcrossed to respective susceptible varieties (recurrent parent) to obtain Bc_1F_1 and ten seeds were also backcrossed to resistant genotypes (donor parent) to obtained Bc_1F_1. The remaining F_1 seeds were selfed to

obtained F_2 . Each set of Bc_1F_2 were harvested separately and put into envelopes and labelled. These seeds were then sent to the CSIR-Savanna Agricultural Research Institute at Bawku-Manga for further evaluation in pots.

F_1 x Recurrent Parent (Susceptible Variety) …….BC_1F_1

F_1 x Donor (Resistant Parent)…….BC_1F_1

3.6 Screening of Parental Lines, F_1 and F_2 Populations using Three SSR Markers Associated with *S. gesnerioides* Resistance

3.6.1 DNA Isolation

Total genomic DNA was isolated from the leaf tissues of cowpea plant using ZR plant/seed DNA MiniPrep ^{Tm} (Zymo Research Corporation, South Africa). After taking the leaf tissue from the plant was placed in a silica gel and brought to the Molecular Laboratory at CSIR-Crops Research Institute for extraction. Young cowpea leaves of about two to three weeks old were extracted from parental lines, F_1 and F_2. A 150mg of leaf sample was weighed with an electric scale into ZR Bashing Bead^{TM}. A total of 96 samples were crushed in liquid nitrogen and 750µl lysis buffer added. The ZR Bashing Bead^{TM} containing the sample together with the lysis buffer was centrifuged at 10,000xg for a minute. About 400µl of the supernatant was transferred into ZymoSpin^{TM} IV spin filter in a collection tube and centrifuged at 7,000rpm (-7,000 xg) for a minute. Following centrifugation, 1,200µl of plant/seed DNA binding buffer was added to the filtrate in the collection tube. However, to get high quality DNA, beta mercaptoethanol was added. Also 800µl of the mixture was transferred into a ZymoSpin^{TM} IIC column in a collection tube and centrifugation was done at 10,000xg for a minute. The flow through was discarded from the collection tube and Zymo-Spin^{TM} IIC column containing the DNA was rescued. The DNA pre-wash buffer was pre heated for 30 minutes at 37^{o}C. After heating, 200µl of pre-wash DNA buffer was added to the

Zymo-Spin™ IIC column and centrifuged at 10,000xg for a minute. In the ZymoSpin™IIC column, 500µl DNA wash buffer was added and centrifuged at 10,000 xg for

1 minute. The Zymo-Spin™IIC column was transferred to a clean 1.5ml tubes and 100µl DNA elution buffer was added to the column matrix. Centrifugation was done at 10,000xg for 30 seconds to elude the DNA. The eluted DNA was then transferred into Zymo-Spin™IV-HRC spin filter in a clean 1.5ml tubes and centrifuged at 8,000xg for a minute. Samples were assessed by electrophoresis using 0.8% (w/v) agarose gel to check the quality of DNA extracted. The concentration of the DNA was determined using Nanodrop (spectrophotometer 2000C, Inqaba biotec™, South Africa). Working solution of 10ng/µl was prepared for each sample.

3.6.2 Polymerase Chain Reaction (PCR) Analysis

Each PCR reaction contained "One Taq Quick-Load 2x Master Mix"(Biolab Inc, South Africa) (which include 20mM Tris-HCl, 1.8mM $MgCl_2$, 22mM NH_4Cl, 22mM KCl, 0.2 mM dNTPS and 25units/ml One Taq DNA Polymerase), Molecular Grade Distilled Water (MGDW), 1µl of each primer and 10ng/µl of genomic DNA sample to make a total volume of 10µl. The PCR amplifications were performed in an Eppendorf Master Cycler (Gene Amp, PCR System 9700, Germany). The thermal cycle used comprised an initial denaturation at 95°C for 1 min followed by 35 cycles of denaturation for 1 min at 94°C, annealing at 55 or 60°C for 1 min, extension at 72°C for 1 min and with final extension at 72°C for 10 min. The PCR products were resolved for 45 min at 120V on 1.5% (w/v) agarose gel in 1XTAE buffer using horizontal gel electrophoresis apparatus. The gel was stained with ethidium bromide and photograph documented with minibus camera (DNR-Bio imaging systems)+ high performance UV transiluminator (Upland) .

3.6.3 Simple Sequence Repeat Markers

Two SCAR markers (Sequence Characterized Amplified Region) and one microsatellite marker (simple sequence repeat) known to be associated with *S. gesnerioides* resistance in cowpea were used. The SCAR markers used were 61RM2 and C42-2B, and SSR marker used was SSR-1. Sequence of primers used to amplify the DNA are given in Table 3.3.

Table 3.3: Primer Sequence and their Annealing temperatures

Primer		Primer Sequence 5'-3'	Annealing Temperature
61RM2	Forward	5´-GATTTGTTTGGTTTCCTTAAG-3´	55 °C
61RM2	Reverse	5´-GGTTGATCTTGGAGGCATTTT-3´	
SSR-1	Forward	5´-CCTAAGCTTTTCTCCAACTCCA-3´	55 °C
SSR-1	Reverse	5´-CAAGAAGGAGGCGAAGACTG-3´	
C42-2B	Forward	5´-CAGTTCCCTAATGGACAACC-3´	60 °C
C42-2B	Reverse	5´-CAAGCTCATCATCATCTCGATG -3´	

(Asare *et al*., 2010)

3.6.4 Hybridity Test in F_1

After DNA extraction from the parental lines and the F_1 progenies, they were subjected to amplification to check for the presence or absence of the markers in the F_1 progeny. After amplification, three primers were present and polymorphic in the genotypes between IT99K-573-1-1/Hewale and GH3684/Asomdwee. These genotypes were then selected for further evaluation in the field where *S. gesnerioides* were very prevalent. In order to determine the genotype of the F_2 plants, progeny testing was performed on 93 representative samples from the individuals that were taken from the segregating populations from cross between IT99K-573-1-1 and Hewale. Between 2 and 3g of leaf

tissue were collected from the selected F_2 plants and immediately desiccated following the procedure described in section 3.6.1.

3.6.5 Scoring of bands from Agarose gel Electrophoresis

Scoring of bands on agarose gel was done with the minibus camera + high performance UV transiluminator connected to a computer. A 100-bp DNA ladder from invitrogen was used as a molecular-weight size marker for each gel alongside the DNA samples. Those that corresponded to the product size of the marker were scored present (1) and those below or above the molecular weight of the marker were score absent (0).

3.7 Sources of *S. gesnerioides* Seeds

The *Striga* seeds were obtained from farmers' field in the Mamprusi District in the Upper East Region. The *Striga* plants were harvested. The harvested plants were placed in sacks and transport to Savanna Agricultural Research Station in Manga-Bawku for drying. The plants were dried in a well ventilated covered area in the screen house for about a week. Stick was used to beat the *Striga* plants to released the seeds. The seeds were then collected and placed in envelopes to be used for pot evaluation..

3.8 Pot Culture Screening of Cowpea Genotypes against *Striga gesnerioides* Infestation

Pots experiments took place at Savanna Agricultural Research Institute at MangaBawku. The F_2 and the parental lines were planted on 9th June and harvested on September, 2015 in pots. The pot screening was used to test two F_2 populations of cowpea against *S. gesnerioides*. Based on the markers, two populations derived from the cross of IT99K-573-1-1/Hewale and cross of GH3684/Asomdwee were selected. Each population together with its parental lines were planted separately in pots arranged in Completely Randomised Design (CRD) with three replications. Plastic pots of 23cm diameter and depth of 20cm were filled with sandy soil and 1 teaspoon (5g) of *Striga* seeds inoculated into the soil. Inoculation was done by mixing thoroughly small part of the sand with the

Striga seeds before putting on top of the pot. Three seeds of cowpea were subsequently planted per pot and thinning was done two weeks after emergence to leaving two plant per pot. The pot was kept moist by watering as and when necessary. Pre-flowering insects were controlled using karate (Dizengoff, Netherlands) at the rate of 15g per hectare and against post flowering insects with 400g cymethoate (Kumark Company Ltd, Singapore) per hectare applied to control aphids, thrips, pod sucking bugs and *Maruca*.

3.9 Estimation of Yield Loss due to *Striga* Infestation

Eight F_3 cowpea genotypes were selected based on their responses to *S. gesnerioides* attack in pots. The F_3 genotypes were selected from the two populations that were evaluated earlier against *S. gesnerioides*. Two separate experiments were conducted in the screen house at Savanna Agricultural Research Institute at Manga-Bawku in pots during September to December 2015. The first experiment was designed to inoculate the pots with *Striga* seeds. This second experiment was designed with no inoculation of *Striga* seeds in pots (non infested). The experimental design was Complete Randomized Design with replications. The varieties were the treatments with 4 replications (two resistant, two susceptible, eight F_3 progenies from a cross between IT99K-573-1-1/ Hewale and GH3684/Asomdwee). The soil was sterilized to eliminate *Striga* seeds that may be in the soil. The soil was sterilized using steam sterilization method.

The results obtained on these parameters from *Striga* infested cowpea plants were compared with that from uninfested cowpea plants, as shown below:

$$\text{Percentage (\%) change in yield} = \left(\frac{INF - C}{C}\right) \times \frac{100}{1}$$

(Aggrarwal and Ouedraogo, 1989; Thalorouarn and Fer, 1993)

Where:

INF = Infested Cowpea Plants (Inoculated)

C =Uninfested Cowpea Plants (Uninoculated)

3.9.1 Description of Genotypes used for Yield Loss estimation

The description of genotypes used as treatment for the estimation of cowpea yield loss are provided in Table 3.4 below:

Table 3.4: Description of genotypes

Treatments (Genotypes)	Description
Asomdwee	Susceptible
Hewale	Susceptible
GH3684	Resistant
IT99K-573-1-1	Resistant
F_3 Progeny (s52)	GH3684/Asomdwee (susceptible)
F_3 Progeny (s37)	GH3684/Asomdwee (susceptible)
F_3 Progeny (r246)	GH3684/Asomdwee (resistant)
F_3 Progeny (r286)	GH3684/Asomdwee (resistant)
F_3 Progeny (s147)	IT99K-573-1-1/Hewale (susceptible)
F_3 Progeny (s272)	IT99K-573-1-1/Hewale (susceptible)
F_3 Progeny (r282)	IT99K-573-1-1/Hewale (resistant)
F_3 Progeny (r69)	IT99K-573-1-1/Hewale (resistant)

3.10 Data Collected from Pot Experiments

3.10.1 Days to *Striga* Emergence

Days taken to first *Striga* emergence was recorded in each pot. At five weeks after planting (WAP), emerged *S. gesnerioides* plants were observed in each pots and days to emergence in each pot was taken. The date the *Striga* shoot emerged was subtracted from the date it was inoculated, to get the actual days it took the *Striga* to emerged.

3.10.2 Days to 50% Flowering

The date of flowering was taken on each of the F_2 progenies to ascertain the number of days its took to flower compared to the parental lines used for crossing.

3.10.3 Plant Height

The cowpea plant height (cm) was measured as the distance from the soil surface to the tip of the shoot with long meter rule.

3.10.4 *Striga* Height

Striga height (cm) was measured on 15 randomly selected plants as the distance from the soil surface to the tip of the shoot with meter rule and the number of branches were counted. The height was measured two weeks after emergence and every week until the cowpea plant matured.

3.10.5 *Striga* Attachment Score

Destructive sampling was carried out at eight weeks after planting. The plant-soil mass was removed from every pot, submerged into a basin of water, and gently agitated to loosen soil mass. The roots were washed completely free of soil and analyzed for necrotic hypersensitive bruises, attachment of *S. gesnerioides* and tubercles. Plants that had connection, vigorous growth and appearance of *S. gesnerioides* were delegated as susceptible and those that seemed free from the parasites, with no attachment were regarded as resistant type.

3.10.6 *Striga* Biomass

After the destructive sampling, the *Striga* plants were washed and sun-dried for two to three days. After thorough drying the samples were weighed to determine their weight in grammes with electric scale.

3.11 Analysis of Data

All field data recorded were subjected to analysis of variance (ANOVA) using GenStat Version 12. Means were compared using Least Significant Difference (LSD) at 5% level of probability. Pearson's correlation coefficient was used to compute correlation between emerged *Striga*, number of pods per plant, grain and fodder yields.

The dendrogram was constructed using power marker software using the three polymorphic markers with UPGMA tree method. Power marker version 6.25 was used to construct the dendrogram.

3.11.1. Estimate of Heritability

Broad sense heritability (h^2_b) and narrow sense heritability (h^2n) were calculated using Wright (1968) Warner (1952) respectively.

(a) Broad sense heritability: $(h^2_{b)} = \{VF2 – [(VP1 + VP2 + 2VF1) /4]\}/ VF2$

(b) Narrow sense heritability: $(h^2n) = [VF2 – (VBC1 + VBC2) /2] / VF2$

Where:

VF_2 – Variance of second filial generation ($F_{2)}$

VP_1 – Variance of parent 1 (resistant parent)

VP_2 – Variance of parent 2 (susceptible parent)

VF_1 - Variance of first filial generation ($F_{1)}$

VBC_1 –Variance of backcross to susceptible parent (BC_1)

VBC_2 – Variance of backcross to resistant parent (BC_2)

3.11.2 Chi-Square analysis

In order to determine the gene action controlling the resistance to *S. gesnerioides* in cowpea a Chi-Square test was used to test the goodness-of-fit of observed ratios to the expected ratios in two different segregating F_2 populations.

$\chi_2 = \underline{(\lceil \text{Observed} – \text{Expected} \rfloor -0.5)^2}$

Expected (Yates, 1934)

Where:

Observed-the observed frequencies are those in the samples.

Expected- the expected frequencies are those computed.

The observed frequencies in each response category was compared to the expected frequencies that was computed.

3.11.3 Estimation of Harvest Index

After harvesting of pods, cowpea plant was cut with knife at the ground level, standardization of cutting height at ground level is also necessary to avoid bias in comparisons of genotypes of differing height. The fresh weight of haulm was taken with electric scale. The haulm was then placed in a electric oven for 24 hours at a temperature of 150^{o}C to dried to constant weight. The dried samples were removed and allow to cooled for 5 to 10 minutes, before taken the dried weight. The dried weight of the haulm plus the dehusked weight of cowpea pod were put together to get the total cowpea haulm. The economic (grain) yield was the cowpea seeds.

Harvest Index (HI) was calculated using Donald and Hamblin (1976) method as follow:

$$= \frac{\text{Economic (grain) yield}}{\text{Total above ground dry weight of cowpea haulm}}$$

3.12 Rainfall Trend during Evaluation

Evaluation of genotypes was affected by the amount of water that was distributed throughout the growing period. Low availability of water during critical stage had higher impact on yield. Low yield obtained from some of the cowpea genotypes were due to low rainfall in the month of October through December as shown in Table 3.5. Ideally, cowpea produced more pods per peduncles but due to low rainfall, high temperature and low relative humidity, the number of pods per peduncles were reduced.

Table 3.5: Rainfall Data during Evaluation (June to December 2015)

Months	Rainfall (mm)	Temperature (^{0}C)		Relative Humidity (%)	
		Max	Min	Max	Min
June	99.7	39.3	24.4	91	63
July	112.8	32.5	23.8	92	72
August	284.8	41.9	23.5	94	78
September	351.2	32.6	23.4	94	73
October	45.7	35.3	23.0	93	63
November	0.00	37.4	20.4	65	39
December	0.00	31.9	20.1	39	20

Source: Weather Station (SARI) Manga-Bawku

CHAPTER FOUR

4.0 RESULTS

4.1 Total number of Pods and Seeds obtained from crosses

The outcome of the cross between the resistant genotypes and susceptible varieties are shown in Table 4.1. In all, 18 set of F_1 progenies were obtained from the various crosses.

Table 4.1: Number of Pods and Seeds obtained from crosses for F_1 progenies

Entries	No. of Pods	No. of Seeds
IT99K-573-2-1 x Asomdwee	19	94
Songotra x Asomdwee	9	47
IT99K-573-2-1 x Hewale	13	53
Songotra x Hewale	8	86
Songotra x Asetenapa	7	28
IT99K-573-2-1 x Asetenapa	9	45
IT07K-291-69 x Asetenapa	5	29
IT99K-573-1-1 x Asetenapa	8	39
IT99K-573-1-1 x Asomdwe	7	45
IT07K-303-1 x Asomdwee	19	110
IT07K-303-1 x Asetenapa	10	35
IT07K-291-69 x Asomdwee	6	27
IT07K-291-69 x Hewale	12	72
IT99K-573-1-1 x Hewale	5	38
IT07K-303-1 x Hewale	7	38
GH3684 x Hewale	10	45
GH3684 x Asomdwee	9	39
GH3684 x Asetenapa	11	27

4.2 Gene Action controlling *S. gesnerioides* Resistance

Two F_2 populations were generated. The F_2 population emanated from the cross IT99K-573-1-1/Hewale (population I) was shown in Table 4.2. Calculated χ^2 value, that is less than corresponding tabular value indicates that, the observed value showed a goodness-of-fit to the genetic ratio expected. In population I (IT99K-573-1-1/Hewale), the F_2 generation segregated into 68 susceptible and 225 resistant filling into 3R:1S ratio (χ^2 = 0.41). This indicated that the *Striga* resistance was controlled by a single dominant gene (monogenic resistance).

Chi-square values were obtained as follows:

Table 4.2 The χ^2 value for the F_2 of the IT99K-573-1-1/Hewale Cross

Chi-Square	**Resistant**	**Susceptible**
Observed (obs)	68.00	225.00
Expected (Exp)	73.25	219.75
Obs - Exp	-5.25	5.25
(⌈Obs-Exp⌋-0.5)	4.75	4.75
(⌈Obs-Exp⌋-0.5)^2	22.56	22.56

$\chi^2 = \frac{22.56}{73.25} + \frac{22.56}{219.75}$

0.13 + 0.10

χ^2 = 0.41

χ^2 (5% 1 df) = 3.841

In population II (GH3684/Asomdwee), the F_2 generation segregated into 236 resistant and 60 susceptible individuals filling into 3R:1S ratio (χ^2 = 3.28) as shown in Table 4.3. Calculated χ^2 value, that is less than corresponding tabular value indicates that, the

observed value show a goodness-of-fit to the genetic ratio expected. This further supported the results obtained in population I above indicating that *Striga* resistance is controlled by a single dominant gene (monogenic resistance).

Table 4.3 The χ^2 value for the F_2 of the GH3684/Asomdwee Cross

	Susceptible	Resistant
Chi-Square		
Observed (Obs)	60	236
Expected (Exp)	74	222
Obs – Exp	-14	14
(⌈Obs - Exp⌋ -0.5)	13.5	13.5
(⌈Obs - Exp⌋ -0.5)^2	182.25	182.25

$$\chi^2 = \frac{182.25}{74} + \frac{182.25}{222}$$

$$2.46 + 0.82$$

$$\chi^2 = 3.28$$

χ^2(5% 1 df) = 3.841

4.3 Broad sense and Narrow sense Heritability estimates for *S. gesnerioides* Resistance and other Traits

To estimate both broad sense and narrow sense heritability for *S. gesnerioides* resistance and three other traits, cowpea variance components were calculated. The means, standard errors and variances of the parental lines, F_1, F_2, BC_1 and BC_2 are presented in (Table 4.4) BC_1 and BC_2 are backcrosses made to parent 1(resistant) and parent 2 (susceptible) respectively.

The mean value for the days to flowering ranged from 41 to 53 and days to maturity ranged from 60 to 68 with P_1 flowering and maturing earlier. P_1 had no *Striga* attached to the roots implying the ability to resist the parasite (resistance) while P_2 had a number of *Striga* attached to the roots indicating the ability to accommodate the parasite (susceptible)

The F_1 and F_2 flowered within 47 to 50 days and matured within 66 to 68 days. The BC_1 and BC_2 flowered within 47 to 48 days and matured within 65 to 68 days.

Table 4.4: Mean, Standard Errors and Variances of Days to Flowering, Days to Maturity, Plant Height, Susceptible and Resistant for the Two parents IT99K-573-1-1/Hewale and their Six Progenies

Generations	Days to Flowering		Days to Maturity		Plant Height (cm)		Susceptible		Resistant	
	Mean ± SE	S_2	Mean ± SE	S^2	Mean ± SE	S^2	Mean ± SE	S^2	Mean ± SE	S^2
P1	41.6 ± 0.96	13.83	60.67± 0.78	9.09	15 ± 0.71	7.50	0.00 ± 0.00	0.00	17.87 ± 1.10	17.98
P2	53.47 ± 0.75	8.41	66.93 ± 0.30	1.35	23.6 ± 1.37	35.46	22.27 ± 1.77	46.78	0.00 ± 0.00	0.00
F1	50.13 ± 0.74	8.27	68.8 ± 0.85	10.74	20.2 ± 1.38	28.6	2.4 ± 0.72	7.83	4.47 ± 0.89	11.98
F2	47.55 ± 1.04	21.73	66.85 ± 0.86	14.87	27.7 ± 1.26	31.91	7.7 ± 1.45	42.22	7.45 ± 1.53	46.68
BC1	48.6 ± 1.25	23.26	69.73 ± 0.82	10.12	25.47 ± 1.29	25.12	7.4 ± 1.16	20.26	3.87 ± 1.18	20.70
BC2	47.73 ± 0.54	4.35	65.33 ± 0.59	5.24	22.87 ± 1.41	29.70	4.8 ± 1.16	20.17	4.27 ± 1.14	19.35

*SE= Standard Error of Mean, S^2= Variance

Broad sense and narrow sense heritability estimates for the cross IT99K-573-11/Hewale are presented in Table 4.5. The broad sense heritability for the five characters measured expressed as percentages ranged from 27 to 78% while the narrow sense heritability ranged from 14 to 57%. Among the traits studied, plant height had the least broad and narrow sense heritability of 27 and 14%, respectively indicating the traits were largely influenced by the environment. *Striga* resistance had the highest broad and narrow sense heritability of 78 and 57%, respectively meaning the trait was less influenced by environment.

Table 4.5: Percentage Broad and Narrow Sense Heritability of the Cross IT99K573-1-1/Hewale Characters Heritability (%)

	Broad Sense	**Narrow Sense**
Days to Flowering	55	36
Days to Maturity	46	38
Plant Height (cm)	27	14
Susceptible	63	52
Resistant	78	57

Broad sense and narrow sense heritability for same traits were also estimated in the cross GH3684/Asomdwee. This was necessary since the sources of resistance (the resistant lines) may have different genotypes. The variance components were computed (Table 4.6) and heritability estimates.

Table 4.6: Mean, Standard Errors and Variances of Days to Flowering, Days to Maturity, Plant Height, Susceptible and Resistant for the Two parents GH3684/Asomdwee and their Six Progenies

Generations	Days to Flowering		Days to Maturity		Plant Height (cm)		Susceptible		Resistant	
	Mean ± SE	S_2	Mean ± SE	S^2	Mean ± SE	S^2	Mean ± SE	S^2	Mean ± SE	S^2
P1	55.07 ±1.09	17.78	70.4 ±0.53	4.26	41.53 ±1.52	34.41	0 .00±0.00	0.00	18.67 ±0.99	14.95
P2	50.6 ± 0.71	7.54	69.07 ± 0.71	7.50	24.4 ± 0.89	12.11	18.87 ± 1.08	17.55	0.00 ± 0.00	0.00
F1	51.47 ± 1.37	37.98	70.47 ± 0.89	12.12	39.13 ± 1.64	40.12	8.67 ± 1.90	53.95	11.73 ± 1.81	49.21
F2	56.4 ± 1.25	31.09	73.45 ± 0.99	19.42	42.45 ± 1.95	75.94	11.55 ± 1.75	61.10	11.6 ± 1.65	54.67
BC1	51.6 ± 0.77	8.83	69.33± 0.82	10.10	38.87 ± 1.89	53.41	7.4 ± 1.36	27.54	10.6 ± 1.48	32.69
BC2	60.13 ± 1.06	16.98	77.53 ± 1.08	17.41	38.4 ± 1.34	27.26	10.4 ± 1.85	51,54	13.0 ± 1.73	44.86

*SE= Standard Error of Mean, S^2= Variance

The mean, standard error, variance of the six generations are presented in Table 4.6. The mean values for the days to flowering for the six generations ranged from 51 to 60, days to maturity from 69 to 77 and plant height from 24.4 to 41.53. P_2, F_1 and BC_1 flowered earlier than the P_1, F_2 and BC_2. The P_1 was resistant (had no *Stiga* attached to roots) while P_2 was susceptible (had some amount of *Striga* attached to the roots). BC_2 flowered within 60 days and matured in 77 days.

Broad and narrow sense heritability for the cross GH3684/Asomdwee expressed as percentages are presented in (Table 4.7). The percentage of the broad sense heritability of the four trait studied ranged from 48 to 58% and narrow sense ranged from 29 to 47%. Among the traits measured, days to flowering had the highest broad sense heritability (58%) followed by plant height (57%). On the other hand, plant height had a higher value on narrow sense heritability (47%) and days to maturity, and resistant both had a lower value of narrow sense heritability of 29%.

Table 4.7: Percentage Broad and Narrow sense Heritability of the cross GH3684/ Asomdwee Characters Heritability (%)

	Broad Sense	Narrow Sense
Days to Flowering	58	35
Days to Maturity	54	29
Plant Height (cm)	57	47
Susceptible	48	35
Resistant	49	29

4.4 Marker Assisted Selection of *S. gesnerioides* Resistant in F_2 Individuals using SCAR and SSR markers

In order to introgress *S. gesnerioides* resistance into susceptible genotypes the presence or absence of markers associated with the resistance traits was first confirmed in the parental lines (Figures 4.1 and 4.2). The markers used were SSR-1 and C42-2B.

M	P_1	P_2	P_3	P_4	C

M	P_1	P_1	P_2	P_2	P_3	P_4	C

M= Marker, Molecular weight of marker = 50bp, P_1= Susceptible variety (Asomdwee), P_2= Susceptible variety (Hewale), P_3= Resistant genotype (IT99K-573-1-1), P_4= Resistant genotype (GH3684) and C= Control (-ve).

Figure 4.1: Presence of the SSR-1 marker in parental line. P_3 and P_4 are the resistant parents while P_1 and P_2 are susceptible parents

Figure 4.2: Presence of the C42-2B marker in parental line. P_3 and P_4 are the resistant parents while P_1 and P_2 are susceptible parents

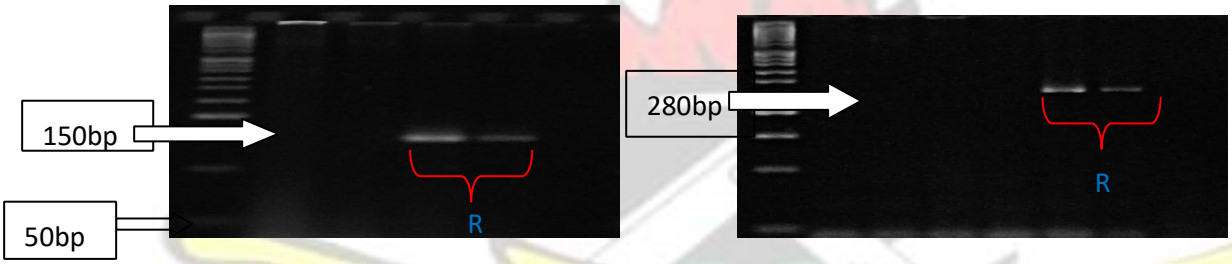


Figure 4.1 shows SSR-1 markers with amplification of 150bp PCR product in the resistant genotype and completely absent in the susceptible genotypes. Figure 4.2 showing C42-2B marker with amplification of 280bp PCR product in the resistant genotype.

4.4.1 Marker – Traits Association in F_2 Population

A total of 93 F_2 progenies from a cross IT99K-573-1-1/Hewale were screened using three markers associated with resistance to *S. gesnerioides*. The three markers have previously been shown to be associated with *S. gesnerioides* resistance (Ouedraogo *et al*., 2012). Thus, the presence of the three markers in a genotype was an indication that, the genotype had the *Striga* resistant allele(s). The presence of a marker was scored 1, meaning resistant and absence of a marker was scored 0, meaning susceptible. Figures 4.3, 4.4 and 4.5 show the results of the markers (SSR-1, C42-3B and 61RM2) on agarose gel stained with ethidium bromide. The product size of the three markers, SSR-1, C42-2B and 61RM2 were 150bp, 280bp and 400bp, respectively. All bands that corresponded to the product size of the markers were scored present. Those below or above the marker weight were scored as absent.

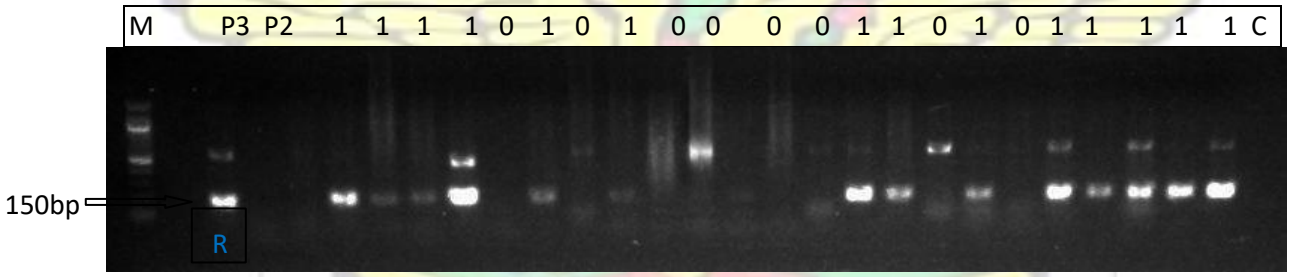


Figure 4.3: SSR-1 marker scored on ethidium bromide stained agarose gel (1.5%). Marker amplified a band only on resistant genotypes. Arrow pointing to band of interest. M= Marker, P_3=resistant genotype (IT99K-573-1-1), P_2= susceptible variety (Hewale), C= Control. The 0 and 1 represent susceptible and resistant genotypes of F_2. Molecular weight of marker =100bp

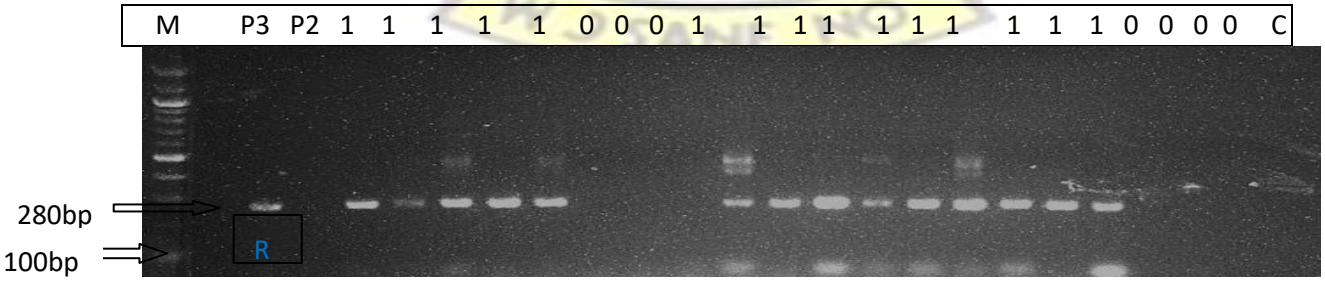


Figure 4.4: C42-2B marker scored on ethidium bromide stained agarose gel (1.5%). Marker amplified a band only on resistant genotypes. Arrow pointing to band of interest. M=Marker, P_3=resistant genotype (IT99K-573-1-1), P_2= susceptible variety (Hewale), C= Control. T he 0 and 1 represent susceptible and resistant genotypes of F_2.

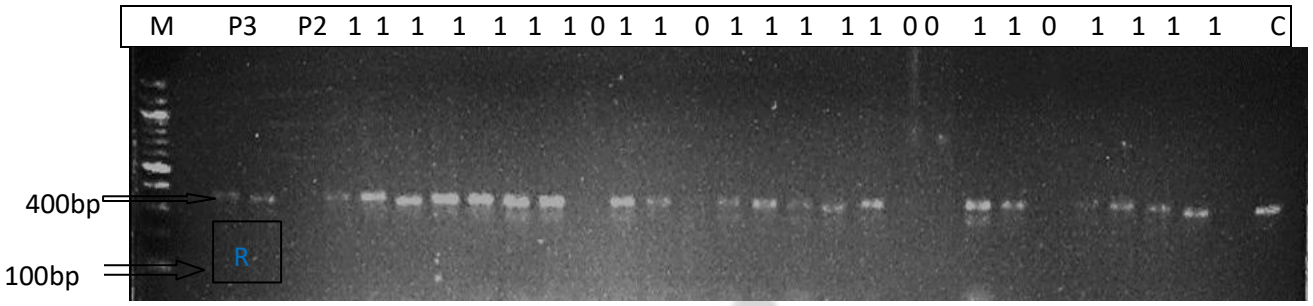


Figure 4.5: 61RM2 marker scored on ethidium bromide stained agarose gel (1.5%). Marker amplified a band only on resistant genotypes. Arrow pointing to band of interest. M=Marker, P 3=resistant genotype (IT99K-573-1-1), P_2= susceptible variety (Hewale), C= Control. The 0 and 1 represent susceptible and resistant genotypes of F_2.

Note: The same genotypes were tested on the three markers.

4.4.2 Polymorphism in the F_2 progenies as Revealed by the Three Markers

The level of polymorphism as shown by the three markers are presented in Table 4.8.

The number of individuals having the SSR-1, C42-2B and 61RM2 marker alleles were 61, 58 and 68, respectively.

Table 4.8: The polymorphism in the F_2 progenies as revealed by the three markers

Marker	Sample Size	Number of individuals with the marker	Number of individuals without the marker
SSR-1	93	61	32
C42-2B	93	58	35
61RM2	93	68	25

4.4.3 Cluster analysis and Identification of F_2 Individuals with Markers

To identify F_2 individuals having all three markers or any two or one or none of the markers, a cluster analysis was performed. It was expected that those that have similar

genotypes with respect to the presence or absence of a given marker combination would be clustered together.

The dendrogram (Figure 4.6) showed the combined data of the three polymorphic markers which delineated the 93 F_2 progenies into five major clusters I, II, III, IV and V. The five major clusters had the following genotypes below:

Cluster	Markers	Number of Individuals
1. Cluster I	SSR-1,C42-2B and 61RM2	26
2. Cluster II	C42-2B and 61RM2	16
3. Cluster III	SSR-1 and C42-2B	11
4. Cluster IV	SSR-1 and 61RM2	31
5. Cluster V	No marker present	9

The 14 sub-clusters were also identified but were not relevant since the data were not adequate for grouping at a higher resolution.

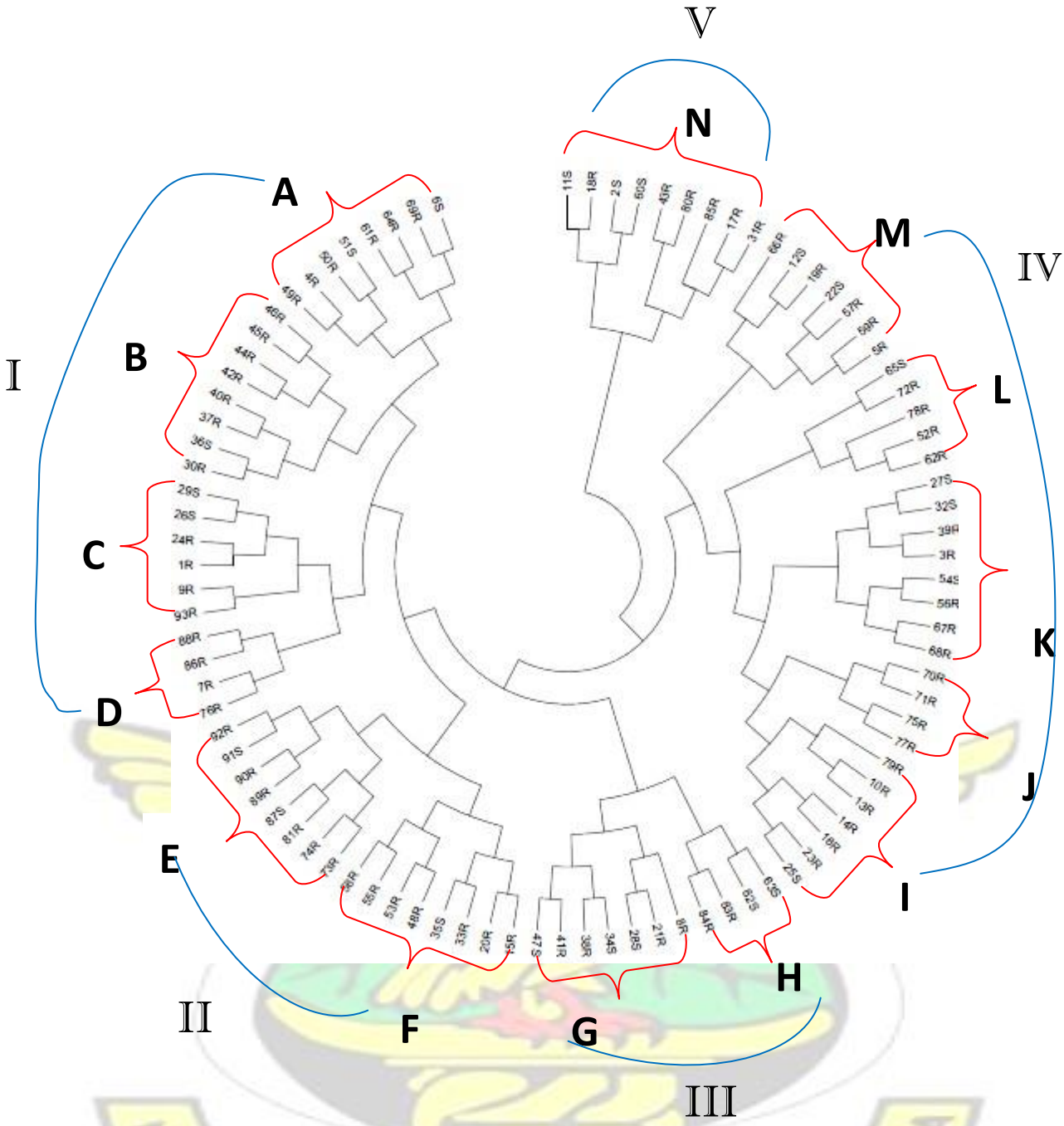


Figure 4.6: A dendrogram of 93 F_2 progenies constructed from power marker using three polymorphic markers with UPGMA tree method.

4.4.4 Allele frequency, Gene diversity and Polymorphic Information Content (PIC)

The alleles frequencies for the three markers within the F_2 population are given in Table 4.9. Allele frequencies yielded by the three markers ranged from 0.62 to 0.73 with the mean of 0.67. The allele number was two based on scoring for present and absent. The heterozygosity was zero because the markers were dominant markers. Dominant markers are

only able to tell if an allele is present or not and detect polymorphic bands in homozygotes. Gene diversity was low ranging from 0.39 to 0.47 with the mean of 0.44. The gene diversity is based on the strength of association between the genetic marker and phenotype. Based on the genetic diversity, each locus for allelic polymorphism information content (PIC) was calculated and the values ranged from 0.32 to 0.36 with the mean of 0.34. However, a marker with more allelic frequency had lower alleles under PIC as found in 61RM2 and a maker with lower allele frequency had a larger PIC as also found with C42-2B. In this case, the higher major allele frequencies (MAF), the lesser the PIC and vice versa.

Table 4.9: Allele frequency, gene diversity and polymorphic information content (PIC) of the markers used in the study

Markers	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
SSR1	0.6559	2.0	0.4514	0.00	0.3495
C42-2B	0.6237	2.0	0.4694	0.00	0.3592
61RM2	0.7312	2.0	0.3931	0.00	0.3158
Mean	**0.6703**	**2.0**	**0.4380**	**0.00**	**0.3415**

*PIC= Polymorphic Information Content

4.5 Estimation of Cowpea Yield Loss due to *S. gesnerioides* Infestation

4.5.1 The Effect of *S. gesnerioides* Infestation on Yield Components

The analysis of variance revealed that differences among the genotypes were highly significant (P≤0.05). *Striga* infestation was highly significant (P≤0.05) among the genotypes and significantly reduced the yields of all the susceptible genotypes. The details of *Striga* emergence and degree of parasite infestation as influenced by genotypes are presented in Table 4.10. The mean days to *Striga* emergence ranged from 35 to 48 days. The results showed that two susceptible parents and four of the F_3 progenies

supported *Striga* emergence (See Appendix 1-4) .On susceptible cultivars, emergence was observed 28 days after planting. The two resistant parents and four F_3 progenies were completely devoid of *Striga* shoots emphasizing that these lines are resistant genotypes. In terms of *Striga* shoots per plant the susceptible had 27 *Striga* shoots (emerged seedlings) while F_3 progenies had *Striga* shoots ranging from 2 to 15 per plant, indicating that some of the F_3 progenies were moderately resistant or tolerant. Some *Striga* plants attached to the host failed to emerge and therefore, total number of parasites was difficult to estimate from the number of emerged *Striga*. *Striga* height was significantly ($P \leq 0.05$) different among genotypes (Table 4.10). *Striga* height was higher on genotypes that supported higher number of *Striga* compared with those that had fewer *Striga* emergence. This implies that the susceptible genotypes were effective hosts for the parasite's growth while resistant genotypes were ineffective hosts. *Striga* plant with high biomass values contributed water and nutrients for the parasite's growth and development rather than the hosts. Thereby, reducing the efficiency of the cowpea plants.

Table 4.10: Mean days to *Striga* Emergence, *Striga* shoot/plant, *Striga* Height and *Striga* Biomass of Twelve Cowpea Genotypes

Genotype	Days to *Striga* emergence	*Striga* shoots/plant	*Striga* height (cm)	*Striga* biomass (g)
Asomdwee	42.75a	27.75a	12.50a	7.57a
Hewale	42.25a	27.00a	11.00a	7.60a
GH3684	0.00b	0.00b	0.00b	0.00b
IT99K-573-1-1	0.00b	0.00b	0.00b	0.00b
F_3 (s52)	35.25a	15.50a	13.25a	5.65a
F_3 (s37)	35.25a	15.25a	13.75a	0.92a
F_3 (r246)	0.00b	0.00b	0.00b	0.00b
F_3 (r286)	0.00b	0.00b	0.00b	0.00b
F_3 (s147)	41.50a	2.50a	7.75a	1.93a
F_3 (s272)	47.75a	11.50a	11.25a	4.83a

F_3 (r282)	0.00b	0.00b	0.00b	0.00b
F_3 (r69)	0.00b	0.00b	0.00b	0.00b
Mean	20.7	8.29	5.79	2.38
CV(%)	10.27	8.566	3.224	1.164

Means followed by the same letter in each column are not significant ly different (P≤ 0.05) .

Significant differences (P≤0.05) were observed among the genotypes for number of pods per plant, grain yield (kg/ha^{-1}) and fodder yield (kg/ha^{-1}). Number of pods per plant was significantly different (P≤0.05) among the genotypes (Table 4.11). The susceptible genotypes recorded fewer pods per plant compared with the resistant or F_3 progenies.(See Appendix 7 ,12, 13, 16, 21.and 22). The percentage yield loss for the number of pods per plant for the susceptible genotypes was -45.12 to -49.53%. Meaning that most of the assimilates for plant growth were exported to the parasite rather than the host. There was significant difference (P≤0.05) among the genotypes for grain yield. The susceptible genotype had grain yield between 100 and 212kg/ha^{-1} while resistant genotype had between 1045 and 1062kg/ha^{-1} and F_3 progenies moderately resistant or tolerant ranged from 316 to 1050kg/ha^{-1}. Yield losses were statistically highly significant (P≤0.05) for the susceptible genotypes than the resistant and F_3 progenies. The grain yield loss from susceptible varieties ranged from -78.22 to -87.17%. In effect *S. gesnerioides* had negative impact on cowpea growth and yields.

Fodder yield also showed significant difference (P≤0.05) among the genotypes. The susceptible varieties suffered fodder yield loss (-70.59 to -73.03%). The pod length, 100 seed weight and number of seeds per pod had significant differences (P≤0.05) among the genotypes (Table 4.12). The *S. gesnerioides* had significant effect on the pod length, 100 seed weight, as well as the number of seeds per pod on susceptible genotypes. The susceptible genotypes had reduced pod length ranging from 7.40 to 8.40cm while resistant genotypes had 13 to 14cm and F_3 progenies had 10-13cm. The infestation of *Striga* led to

the reduction of pod length -31.88 to -37.17%, 100 seed weight -31.39 to 36.30% and number of seeds per pod to -32.29 to -37.15%. (See Appendix 8, 10, 11, 17, 19 and 20). Therefore, *Striga* drastically reduced a number of yield components.

Table 4.11: Mean Pods per plant, Grain yield (kg ha^{-1}) and Fodder yield (kg ha^{-1}) Cowpea response to Infection by *S. gesnerioides*

Genotypes	Pods/plant			Grain yield (kg ha^{-1})			Fodder yield (kg ha^{-1})		
	Infested	Not infested	Loss (%)	Infested	Not infested	Loss (%)	Infested	Not Infested	Loss (%)
Asomdwee	3.25 d	6.00 a	-45.12 a	212.5 c	979 b	-78.22 a	254.2 c	892 b	-70.59 a
Hewale	2.25 d	4.75 a	-49.53 a	100.0 c	913 b	-87.17 a	275.0 c	1488 a	-73.03 a
GH3684	5.50 b	5.25 a	-4.17 b	1045.8 a	1258 a	-16.13 c	1470.8 a	1483 a	-27.58 b
IT99K-573-1-1	6.25 b	6.50 a	-16.91 b	1062.5 a	1017 a	+5.32	1041.7 b	1183 a	-32.93 b
F_3 (s52)	4.25 cd	6.60 a	-29.60 a	425.0 c	925 b	-48.83 b	458.3 c	900b	-69.04 a
F_3 (s37)	6.75a	7.00 a	-16.17 b	966.7 a	1104 a	-11.53 c	1208 b	1521 a	-45.95 a
F_3 (r246)	7.50 a	6.00 a	-18.75 b	1050.0 a	1004 b	+9.41 c	1841.7 a	1733 a	-17.10 b
F_3 (r286)	7.00 a	6.25 a	-26.34 ab	1016.7 a	1058 a	-1.08 c	1091.7 b	1008 b	-43.17 b
F_3 (s147)	5.50 b	6.75 a	-29.11 a	870.8 bc	1113 a	-20.88 c	787.5 bc	1029ab	-23.41 b
F_3 (s272)	4.25 cd	7.25a	-38.84 a	316.7 c	879 b	-62.99 ab	475.0 c	1008 b	-44.41 ab
F_3 (r282)	6.00 b	6.50 a	-13.84 b	950.0 a	1021 a	-13.99 c	1137.5 b	1404 a	-26.74 b
F_3 (r69)	6.75 a	7.00 a	-3.57 b	1016.7 a	1021 a	-10.60 c	1094.7 b	1250 a	-35.94 b
Mean	5.44	6.31	-5.94				928	1242	-42.49
753					1024				-28.06
CV(%)	15.7	20.9	-296.07	17.4	17.1	91.42	44.9	36.9	-55.08

Means followed by the same letter(s) in each vertical column are not significantly different (P≤ 0.05)

Table 4.12: Mean Pod length, 100 Seed weight and Number of seeds per pod of Twelve Cowpea Genotypes

Genotypes	Pod Length (cm)			100 Seed Weight (g)			Number of seeds/pod		
	Infested	Not infested	Loss (%)	Infested	Not infested	Loss (%)	Infested	Not infested	Loss (%)
Asomdwee	8.40 d	12.40 b	-31.88 a	9.38d	14.40 a	-31.39 a	6.50 b	10.00 a	-32.29 a
Hewale	7.40 d	11.90 b	-37.17a	6.97e	11.10b	-36.30a	5.50 c	8.75 b	-37.15 a
GH3684	13.9a	15.47 a	-7.78 b	11.80 c	13.22 a	-9.69 b	11.00 a	12.00a	-20.17 a
IT99K-573-1-1	14.87 a	13.27 a	+5.29b	15.15a	14.92 a	+4.10 b	8.00 b	7.75 b	-18.68 a
F_3 (s52)	10.35 c	10.80 b	+0.71b	9.72 d	11.20 b	-10.84 b	8.50 b	10.75 a	-18.37a
F_3 (s37)	11.60 b	11.05 b	+7.33b	11.05cd	13.22 ab	-16.22 ab	9.25 ab	10.25a	-7.53 b
F_3 (r246)	12.50 b	11.22 b	+5.29b	11.67c	10.92 b	+8.71b	9.25 ab	9.75 ab	-3.61b
F_3 (r286)	11.07c	12.72 b	-11.55ab	12.25bc	11.65 b	+5.58 b	10.50 a	11.75a	-13.05ab
F_3 (s147)	11.30bc	12.20 b	-5.71 b	12.20c	12.17 b	-6.20 b	8.00 b	9.75 ab	-16.92 a
F_3(s272)	10.75 c	11.27 b	-3.01b	9.22de	12.50 b	-26.17 a	6.25 bc	8.50 b	-25.16 a
F_3 (r282)	13.05 b	13.15 a	-0.64 b	14.62 a	15.52a	-19.95 a	6.25 bc	7.75 b	-17.71a
F_3 (r69)	13.85ab	13.05ab	+7.87 b	14.82a	15.82 a	-4.82 b	7.50 b	7.25 b	-7.29 b
Mean	11.59	12.38	-5.94	11.57	13.06	-11.9	8.04	9.52	-18.16
CV(%)	11.1	14.7	-296.07	12.9	16.0	141.3	20.0	19.6	-103.63

Means followed by the same letter(s) in each vertical column are not significantly different (P≤0.05).

There was significant difference (P≤0.05) among the genotypes for days to flowering, days to maturity and plant height (Table 4.13). Flowering and maturity were delayed in the susceptible genotypes in the infested compared with the noninfested. The plant height also showed similar trend. The susceptible genotypes in the infested had lower height compared to non-infested. The susceptible genotypes showed stunted growth with poor flowering or no flowering.

Table 4.13: Mean days to Flowering, Days to Maturity and Plant Height of Twelve Cowpea Genotypes

Genotype	Days to flowering		Days to maturity		Plant height (cm)	
	Infested	**Not infested**	**Infested**	**Not infested**	**Infested**	**Not Infested**
Asomdwee	57.50a	51.25ab	81.50 a	66.25 b	19.8c	31.50 a
Hewale	52.00 bc	45.00 cd	66.25 b	58.50 d	18.38c	23.75c
GH3684	53.00 b	52.50 a	64.25 b	66.75 ab	31.75a	31.12 a
IT99K-573-1-1	46.75 d	44.25d	63.50 bc	60.00c	24.38b	27.75 b
F_3 (s52)	52.25 b	47.75b	65.00 b	63.75 bc	23.62b	26.75bc
F_3 (s37)	51.75c	46.50 c	66.75 b	62.00 c	23.12 b	24.12 c
F_3 (r246)	54.00 b	52.25a	67.25 b	69.50 a	29.88ab	32.38 a
F_3 (r286)	51.50 c	49.75 b	64.75 b	61.00 c	23.50 b	24.12 c
F_3 (s147)	53.75 b	48.25b	60.75c	60.00 c	24.50 b	32.12 a
F_3(s272)	55.25ab	47.50 bc	67.00 b	58.75 d	16.12d	18.62 d
F_3 (r282)	48.00 d	48.25b	61.00c	59.75 cd	18.38 c	18.88 d
F_3 (r69)	50.00cd	47.00 c	62.50 c	60.00 c	26.38 b	23.75 c
Mean	52.15	48.35	65.88	62.19	23.32	26.24
CV(%)	3.0	3.4	5.0	3.1	9.4	7.1

Means followed by the same letter(s) in each column are not significantly different (P≤0.05).

Table 4.14: Correlation Coefficients between Parameters measured and its components caused by *S. gesnerioides*

	***Days to Striga* emergence**	**Fodder yield kg/ha**	**Grain yield kg/ha**	**No. of pods/plant**	**No. of seed /pod**	**Pod Length (cm)**	***Striga* height (cm)**	***Striga* shoot/plant**	**100 seed weight (kg)**
Fodder yield kg/ha	-0.5639**								
Grain yield kg/ha	-0.7237**	0.6483**							
No. of pods/plant	-0.6257**	0.6337**	0.8428						
No. of seed /pod	-0.336*	0.2969*	0.4687	0.4537					
Pod Length (cm)	-0.6402**	0.5268**	0.7425**	0.608**	0.4492**				
Striga height (cm)	0.8729**	-0.5502**	-0.6898**	-0.5834**	-0.2871*	-0.645**			
Striga shoot/plant	0.6492**	-0.5616**	-0.7619**	-0.6975**	-0.3781**	-0.712**	0.7942**		
100 seed weight(kg)	-0.7342**	0.4263**	0.7325**	0.6593**	0.2185ns	0.6894**	-0.7119**	-0.7209**	
Striga biomass (g)	0.749**	-0.6846**	-0.9247**	-0.7975**	-0.4749**	-0.7804**	0.7174**	0.8089**	-0.7444**

*Significant at 5% level of probability, **Significant at 1% level of probability

The correlation coefficients among most of the parameters were negative and highly significant (Table 4.14). There were significant correlation (r=0.56) found among percentage yield reduction and percentage reduction in various yield components. However, there was no significant correlation (r= 0.061) found between the non infested and its yield components (Table 4.15).In this case, the susceptible, resistant and F_3 progenies put on their best performance under *Striga*-free environment.

Table 4.15: Correlation Coefficient between Yield Component of Uninfected Trial

	Fodder yield kg/ha	Grain yield kg/ha	No. of seeds/pod	Pod length(cm)	No. of Pods/plant
Grain yield kg/ha	0.4097**				
No of seeds/pod	-0.0013 ns	0.0617 ns			
Pod length	0.2465 ns	0.4876 ns	0.0836 ns		
Pods/plant	0.0493 ns	0.3364*	-0.2908*	-0.0953 ns	
100 seed weight	-0.0901 ns	0.1379 ns	-0.4314**	0.2908*	0.3211*

*Significant at 5% level of probability, **Significant at 1% level of probability and ns= not significant

There was no significant difference (P≤0.05) among genotypes in terms of harvest index (Table 4.16). Under non infested condition, susceptible genotypes obtained a harvest index ranging from 0.36 to 0.48, resistant genotypes 0.37 to 0.43 and F_3 progenies 0.33 to 0.47. However, under infested condition, there was significant difference (P≤0.05) among the genotypes. The susceptible genotypes obtained a harvest index of 0.19 to 0.39, resistant 0.32 to 0.39, and F_3 progenies from 0.29 to 0.41.

Table 4.16 Harvest Index of the Twelve Cowpea Genotypes Harvest Index

Genotypes	Infested with *Striga*	Non-infested
Asomdwee	0.39a	0.48a
Hewale	0.19b	0.36b
GH3684	0.32ab	0.43a
IT99K-573-1-1	0.39a	0.37b
F_3 (s52)	0.32a	0.47a
F_3 (s37)	0.41a	0.39a
F_3 (r246)	0.32ab	0.33b
F_3 (r286)	0.37a	0.38ab
F_3 (s147)	0.42a	0.45a
F_3 (s272)	0.29 b	0.42a
F_3 (r282)	0.35a	0.38ab
F_3 (r69)	0.42a	0.46a
Mean	0.35	0.41
CV(%)	25.80	18.70

Means followed by the same letter(s) in each column are not significantly different (P= 0.05)
.

4.6 Visual Observation of *S. gesnerioides*

The phenotypic observation of *S. gesnerioides* on cowpea plants

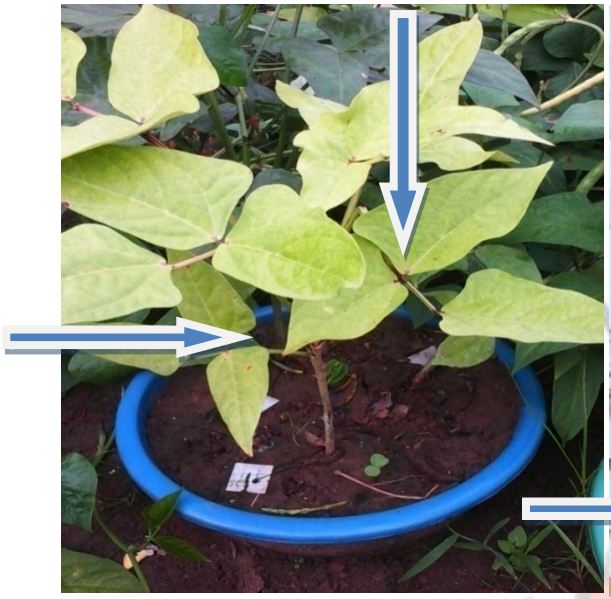


Plate 4.1: F_2 progeny of a cross IT99K-573-1-1/Hewale. A susceptible genotype showing necrotic symptoms with no emergence of *Striga* shoot.

Plate 4.2: After washing *Striga* shoots attached to the roots. Arrow pointing to *Striga* shoots in black

Plate 4.3: Susceptible genotypes showing heavy infestation of *Striga* shoots.

Plate 4.4: Arrows pointing to *Striga* shoot in benches attached to susceptible genotype

Plate 4.5: F_2 progeny of a cross IT99K-573-1-1/Hewale. Arrow pointing to resistant genotype without *Striga* attached to the roots.

Plate 4.6: F_2 progeny of a cross GH3684/Asomdwee showing emergence of *Striga* shoot

Plate 4.7:F_2 progeny of a cross GH3684/Asomdwee showing resistant as well as podding

CHAPTER FIVE

5.0 DISCUSSION

5.1 *S. gesnerioides* Resistance is Controlled by a Single Dominant Gene Action

The segregation analysis of the F_2 progenies of the two populations revealed that resistance to *S. gesnerioides* is conferred by single dominant gene action and demonstrated as monogenic resistance. The chi-square values obtained from these findings ranged from 0.41 to 3.28 which are less than the corresponding tabulated chisquare value of 3.841 (P=0.05) indicating that the observed ratios showed a goodness – of- fit to Mendelian ratio of 3:1 for a single locus. The inheritance of resistance to the race SG5 of *Striga* in Ghana is monogenic with segregating pattern of 3R:1S. This finding also confirmed initial inheritance studies by Atokple *et al*. (1993) and Moore *et al*. (1995) 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. These genes could be exploited in breeding for *S. gesnerioides* race resistance in cowpea, and effort should be made to develop *Striga*-resistant cowpea varieties that meet end user preferences.

5.2 Heritability Estimates for *S. geserioides* Resistance

Broad sense and narrow sense heritability estimates for *Striga* resistance in population 1 (IT99K-573-1-1/Hewale) were 0.78 (78%) and 0.57 (57%), respectively. These values are relatively high, indicating that *Striga* resistance in population 1 (IT99K-573-11/Hewale) is less influenced by the environment. It also suggests that the trait is governed by additive genes, and selection for improvement would be effective. In this case, selecting genotypes that are completely resistant to the parasite would be helpful for breeding subsequent generation. On the other hand, broad sense and narrow sense heritability estimates in population 2 (GH3684/Asomdwee) were 0.49 (49%) and 0.29

(29%), respectively. These estimates are relatively low, suggesting that the resistance in population 2 (GH3684/Asomdwee) is influenced by environment. Selection for this traits in population 2 (GH3684/Asomdwee) will therefore not be effective. Traits such as days to flowering, days to maturity and plant height had differences in their genotype and phenotype. The higher values obtained in broad sense heritability for some of the traits do not indicate that the environment had less impact on genotype because broad sense heritability comprises both fixable (additive) and nonfixable (dominance and epistatic) variance and therefore, selecting based on these characters may not be useful.

In the present study the estimated broad sense heritability for days to flowering was 55%. Other studies testing on different genotypes and limited environment reported relatively higher heritability values for days to flowering at 86% (Ishiyaku *et al*.,2005) and (Omoigui *et al*., 2006). Ishiyaku *et al.* (2005) suggested that days to flowering is a quantitative traits in *V. unguiculata* and its inheritance is controlled by seven genes and therefore such high value does not reflect the true nature of that trait under *Striga* infestation. However, lower values for some of the traits measured in broad sense revealed that the characters were highly influenced by the environment and therefore, genetic improvement through selection would be difficult due to masking effects of the environment on the genotypic effects. The present study estimated moderately higher broad sense heritability value of 54% for days to maturity in cowpea under *Striga* infestation (Table 4.4). Omoigui *et al.* (2006) reported high broad sense heritability of 79% for days to maturity. In this case, the present findings are not in agreement with Omoigui *et al.* (2006) because of environmental influence on the days to maturity. The environmental variance had effect on maturity.

5.3 Marker Assisted Selection of *S. gesnerioides* Resistant in F_2 Individuals using SCAR and SSR Markers

The study showed that the three markers had discriminating power to distinguish between the resistant and susceptible genotypes. These markers recognized genotypes with magnification of bands in resistant genotypes. As indicated by Omoigui *et al.* (2009), marker C42-2B distinguished resistant genotypes with a distinct band while susceptible one had no bands. On the other hand, "Asomdwee "and "Hewale" had no bands of the markers used, indicating they are susceptible. This confirmed the phenotypic data where the susceptible genotypes had a number of *Striga* shoots while the resistant genotypes were completely devoid of *Striga* shoots. The allele frequency or the gene frequency is the relative frequency of an allele (variant of a gene) at a particular locus in a fraction of all chromosomes in the population that carry that alleles

(Moghaddam *et al*., 2009). In this study, the allele frequency for marker SSR-1 was 65% suggesting that the resistant alleles associated with the marker SSR-1 is highly repeatable within the population. This also means that the population had high breeding values. The 61RM2 also had 73% of the alleles frequency suggesting that such a marker can be very useful in discriminating resistant alleles from susceptible alleles within the population. This suggests that markers could be used to improve upon a variety to facilitate long-term gains from selection, and reduce genetic vulnerability to parasite epidemics. Li *et al.* (2001) demonstrated that microsatellite markers were conserved among *Vigna* species. Hence microsatellite markers could provide a simple approach to assaying the introduction of such genetic material. The polymorphic information content (PIC) is often used to measure the informativeness of a gene related to expected heterozygosity and is calculated from allele frequency (Norman *et al*., 2012). The PIC value of SSR-1 markers in the present study was not very high and ranged from 0.31 to 0.35. The PIC values of the SSR-1 markers can be compared to results reported by Li *et al*. (2001) with PIC ranging from 0.02 to 0.73. The PIC obtained by Asare *et al*. (2010) varied from 0.07 to

0.66 with an average of 0.38. The PIC values recorded in the current study compared favourably with results obtained by Asare *et al* (2010) because of the same markers were used for this study. The amount of PIC is a function of detected alleles and distribution of their frequency (Moghaddam *et al*., 2009). Markers with high allelic frequency had lower PIC value as in the case of 61RM2. The genetic diversity refers to a measure of variance of alleles in a population that enable individual to survive in a given environment. It must be noted that the allelic frequency, PIC and gene diversity reported in this study are in relation to the marker alleles used in the study.

The result of the cluster analysis based on molecular markers revealed individuals that possessed all three markers associated with resistance to *Striga*. The molecular data was consistent with the morphological data. Cluster I, showed those individuals that had all the three markers present and also showed the resistant phenotypes under field conditions. Clusters II, III and IV indicate individuals with either two of the markers present and resistant under field condition. Cluster V indicate individuals that did not have any of the three markers and were susceptible under field conditions. However, for some individuals, there were lack of consistency between the marker and the phenotype. For those individuals, marker showed resistance but under field condition they were susceptible. Some of them also showed resistance while the marker showed susceptible. This indicated that there might be epistatic interactions among the genes or the marker may have segregated away from the genes conferring the resistance. As indicated by Li *et al*. (2009) gene markers connected with resistance have been characterized, and a few many SCARs (sequence-characterized amplified regions) have been designed for use in marker-based breeding programs.

5.4: Estimation of Cowpea Yield Loss due to *Striga* Infestation

The result revealed significant difference ($P \leq 0.05$) among the genotypes for all tested characters. The results obtained from this study revealed grain yield losses (78.22 to 87.17%) on susceptible genotypes compared with resistant genotypes with a yield loss (5.32 to 16.13%). This indicated that *Striga* had greater influence on yield. Alonge *et al*. (2005) pointed out that on susceptible cowpea, *Striga* infestation induced grain yield losses by 78.9-86.2%. The susceptible varieties "Asomdwee" and "Hewale" had a number of *Striga* shoots emerging while the resistant genotypes (GH3684 and IT99K573-1-1) and some of the F_3 progenies were completely *Striga*-free. Godwa *et al*. (1999) pointed out that the response to *Striga* varied among genotypes suggesting that differences exist in the ability of these plants to recognize the pest and to activate defense response mechanisms. Botanga and Timko (2005) reported that incompatibility appeared to be the result of the failure of the parasite to establish proper vascular connection (xylem-xylem linkage) with the host. In contrast, the resistant genotypes were ineffective host. The higher infestation rates of the susceptible genotype were due to soil sterilization. The sterilization of the soil may have affected both micro and macro nutrients and thereby making the soil low in fertility. When compared with unsterilized soil the number of pods obtained were higher than sterilized soil. The soil was sandy and there was also low rainfall during the time of the experiment. Ideally the sterilization help to eliminate any *Striga* seeds in the soil before inoculation to avoid bias. No *Striga* emerged from the uninfected pot and this indicated that soil sterilization was perfect. The resistant genotypes have relatively good growth and probably less export of assimilate to the parasite would have ensured adequate biomass accumulation and grain development. Similar reports were published by Hibbered *et al*., (1996) and Alonge *et al*. (2005). According to Hibberdd *et al*. (1996), final biomass accumulation by cowpea infested with *S. gesnerioides* was significantly lower than that by uninfected plants. The higher number

of *Striga* shoot and low yield observed among the susceptible genotypes in this study corroborate the earlier report of Singh (2002) who reported that *Striga* infestation in cowpea causes severe damage in areas with sandy soils, low fertility and low rainfall. According to Kamara *et al* (2008), the parasite activity is higher with increasing sandy soil with poor soil productivity and low precipitation. The study revealed a reduction in cowpea characters measured on susceptible genotype. For this situation the affected plants were less able to produce adequate dry matter per pod. Analysis has demonstrated that affected plants recorded lower biomass as a consequence of parasite-host competition for water, carbon, as well as photosynthetic activity in the leaves of affected plants (Pres,1995). The pervaded plants have hindered development, chlorosis, senescence, defoliation, decreased size of flesh leaves, poor blooming and poor pod development. This could be due to nitrogen deficiency and poor adsorption of essential nutrients for growth. The lessening of blackeyed pea haulm will prompt critical deficiencies since cowpea hay is essential feed for livestock during dry seasons. In this study, genotypes that upheld higher number of *Striga* additionally recorded lower number of units per plant, 100 seed weight, grain yield and fodder yield. This suggests that reduced photosynthesis could have resulted in lower number of pods per plant and translocation of photosynthate to the sink. The decrease in grain yield could also be due to the reduction in root nodulation and root growth by the parasite. The number of pods per plant, 100 seed weight, grains and fodder yields was significantly influenced by developed *Striga* shoot showing that *Striga* infestation could be ascribed to competition between the host and parasites. The study revealed that number of pods per plant were highly negatively correlated with emerged *Striga* shoot.

5.5 *Striga gesnerioides* and its Effect on Harvest Index

Harvest index is the ratio of reproductive yield to total plant biomass and has been taken as a measure of efficiency in partitioning assimilated photosynthates to harvestable

products. The result for the harvest index showed that *Striga gesnerioides* had effect on assimilates partitioning. *S. gesnerioides* has taken greater part of the assimilate thereby reducing the harvestable products in the susceptible genotypes. Since *S. gesnerioides* is a parasite and depend completely on the host for nutrients and water, less photosynthates were exported to the host for grain yield and dry matter production. High harvest index and early maturity reflects the two adaptations for plants to withstand stress from the environment. Enhanced harvest index shows partitioning of the limited assimilated photosynthates under the stress conditions into harvestable products. It has emerged that applying adequate dry matter from the season's harvest can act as a mechanism to enhance yield. However, it is more difficult estimating crop's economic yield as source constrained or sink restricted due to the fact that amid improvement and development of the sink, the relationship amongst source and sink unavoidably change. On the other hand, in the presence of any severe stress such as moisture, diseases or insect or pest that may alter plant growth, the source efficiency may be yield limiting factor.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

The study on the gene action controlling *Striga* resistance in cowpea shows that *Striga* resistance is control by a single dominant gene. The F_2 generations segregated in the ratios (3:1) respectively. The result of the inheritance study indicated the environment had great impact on days to flowering, days to maturity, plant height, *Striga* attachment and non attachment. The high values for narrow sense heritability for *Striga* attachment and non attachment indicated that such characters are governed by additive genes and selection would be effective.

Molecular markers were used to identify F_2 individual that have all three markers associated with *Striga* resistance. The marker data were consistent with the phenotypic data. This facilitate the selection process. Markers are useful tools in detecting the gene of interest. Therefore molecular marker could be an auxiliary selection means for breeding new cultivars or lines. The use of MAS makes easier the selection of plant traits and reduces the time needed to develop new varieties.

This study made use of *Striga* resistant lines IT99K-573-1-1 and GH3684 to improved upon farmer preferred varieties Asomdwee and Hewale. Therefore such genotypes are very good breeding lines which could be used for subsequent generations. The result of the yield loss indicated that susceptible varieties are effective hosts for the parasite and if farmers cultivate such varieties then they stand the chance of losing everything.

The result estimated grain yield loss of about 78.22 to 87.17%.

6.2 Recommendations

At present very limited sources of *Striga* resistant varieties are available therefore there is the need to develope new *Striga* resistant cowpea varieties that meet end-user preference. It is recommended that the F_2 progenies will be screened and those identified as completely resistant will be further evaluated. Promising lines will be screened with more *Striga* resistant markers to determine their level of genetic status.

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APPENDICES

Appendix 1: Days to *Striga* emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	342.73	114.24	1.23	
Rep.*Units* stratum					
Trt	11	19665.06	1787.73	19.18	<.001
Residual	33	3076.02	93.21		
Total	47	23083.81			

Appendix 2: *Striga* height (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	28.917	9.639	2.22	
Rep.*Units* stratum					
Trt	11	1495.917	135.992	31.36	<.001
Residual	33	143.083	4.336		
Total	47	1667.917			

Appendix 3: *Striga* shoots per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	39.50	13.17	0.36	
Rep.*Units* stratum					
Trt	11	5105.17	464.11	12.69	<.001
Residual	33	1207.00	36.58		

Total	47	6351.67			

ndix 4: Striga biomass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.7042	0.2347	0.36	
Rep.*Units* stratum					
Trt	11	428.2992	38.9363	59.50	<.001
Residual	33	21.5958	0.6544		
Total	47	450.5992			

Appendix 5: Days to Flowering Uninfected

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2.896	0.965	0.35	
Rep.*Units* stratum					
Trt	11	308.729	28.066	10.14	<.001
Residual	33	91.354	2.768		
Total	47	402.979			

Appendix 6: Days to Maturity Uninfected

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	12.729	4.243	1.12	
Rep.*Units* stratum					
Trt	11	561.562	51.051	13.48	<.001
Residual	33	125.021	3.789		
Total 47	699.312				

Appendix 7: Number of pod per Plant Uninfected

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep stratum	3	11.229	3.743	2.15	
Rep.*Units* stratum					
Trt	11	23.562	2.142	1.23	0.308
Residual	33	57.521	1.743		
Total 47 92.312					

Appendix 8: Number of seeds per pod Uninfected

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2.729	0.910	0.26	
Rep.*Units* stratum					
Trt	11	106.229	9.657	2.77	0.012
Residual	33	115.021	3.485		
Total	47	223.979			

Appendix 9: Plant Height (cm) Uninfected

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	9.849	3.283	0.93	
Rep.*Units* stratum					
Trt	11	1039.682	94.517	26.90	<.001
Residual	33	115.964	3.514		
Total 47 1165.495					

Appendix 10: Pod Length Uninfected

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	13.477	4.492	1.36	
Rep.*Units* stratum					
Trt	11	74.497	6.772	2.05	0.055
Residual	33	109.130	3.307		
Total 47 197.105					

Appendix 11: 100 Seed Weight Uninfected

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	23.901	7.967	1.83	
Rep.*Units* stratum					
Trt	11	135.976	12.361	2.84	0.010
Residual	33	143.802	4.358		
Total	47	303.678			

Appendix 12: Grain yield (kg/ha) Uninfected

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	261204.	87068.	2.84	
Rep.*Units* stratum					
Trt	11	464200.	42200.	1.38	0.229
Residual	33	1010228.	30613.		
Total	47	1735632.			

Appendix 13: Fodder yield (kg/ha) Uninfected

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2154507.	718169.	3.41	
Rep.*Units* stratum					
Trt	11	3446596.	313327.	1.49	0.182
Residual	33	6941615.	210352.		
Total	47	12542717.			

Appendix 14: Days to Flowering Infested

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	11.562	3.854	1.63	
Rep.*Units* stratum					
Trt	11	386.229	35.112	14.82	<.001
Residual	33	78.188	2.369		
Total	47	475.979			

Appendix 15: Days to Maturity Infested

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	33.42	11.14	1.03	
Rep.*Units* stratum					
Trt	11	1279.75	116.34	10.72	<.001
Residual	33	358.08	10.85		
Total	47	1671.25			

Appendix 16: Number of pods per plant Infested

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.2292	0.0764	0.10	
Rep.*Units* stratum					
Trt	11	115.5625	10.5057	14.43	<.001

Residual	33	24.0208	0.7279		
Total 47	139.8125				

Appendix 17: Number of Seed per Pod Infested

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4.250	1.417	0.55	
Rep.*Units* stratum					
Trt	11	133.917	12.174	4.69	<.001
Residual	33	85.750	2.598		
Total 47	223.917				

8: Plant Heig ıt Infested

Appendix 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	19.391	6.464	1.35	
Rep.*Units* stratum					
Trt	11	954.307	86.755	18.06	<.001
Residual	33	158.547	4.804		
Total 47	1132.245				

Appendix 19: Pod Length Infested

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	3.113	1.038	0.63	
Rep.*Units* stratum					
Trt	11	219.012	19.910	12.04	<.001
Residual	33	54.552	1.653		
Total	47	276.677			

Appendix 20: 100 seed Weight Infested

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	10.946	3.649	1.64	

Rep.*Units* stratum					
Trt	11	275.092	25.008	11.24	<.001
Residual	33	73.417	2.225		
Total	47	359.455			

Appendix 21: Grain yield (kg/ha) Infested

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	45694.	15231.	0.89	
Rep.*Units* stratum					
Trt	11	6094581.	554053.	32.46	<.001
Residual	33	563190.	17066.		
Total	47	6703465.			

Appendix 22: Fodder yield (kg/ha) Infested

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	160340.	53447.	0.31	
Rep.*Units* stratum					
Trt	11	10583127.	962102.	5.55	<.001
Residual	33	5723154.	173429.		
Total	47	16466622.			

Appendix 23: Scoring of genotypes with three markers

Sample ID	**Genotyping**			**Phenotyping**
	SSR-1	**C4-2B**	**61RM2**	
1	1	1	1	R
2	0	0	0	S
3	1	0	1	R
4	1	1	1	R
5	0	0	1	R
6	1	1	1	S

7	1	1	1	R
8	1	1	0	R
9	1	1	1	R
10	1	0	1	R
11	0	0	0	S
12	0	0	1	S
13	1	0	1	R
14	1	0	1	R
15	0	1	1	R
16	1	0	1	R
17	0	1	0	R
18	0	0	0	R
19	0	0	1	R
20	0	1	1	R
21	1	1	0	R
22	0	0	1	S
23	1	0	1	R
24	1	1	1	R
25	1	0	1	S
26	1	1	1	S
27	1	0	1	S
28	1	1	0	S
29	1	1	1	S
30	1	1	1	R
31	0	1	0	R
32	1	0	1	S
33	0	1	1	R
34	1	1	0	S

35	0	1	1	S
36	1	1	1	S
37	1	1	1	R
38	1	1	0	R
39	1	0	1	R
40	1	1	1	R
41	1	1	0	R
42	1	1	1	R
43	0	1	0	R
44	1	1	1	R
45	1	1	1	R
46	1	1	1	R
47	1	1	0	S
48	0	1	1	R
49	1	1	1	R
50	1	1	1	R
51	1	1	1	S
52	1	0	0	R
53	0	1	1	R
54	1	0	1	S
55	0	1	1	R
56	1	0	1	R
57	0	0	1	R
58	0	1	1	R
59	0	0	1	R
60	0	0	0	S
61	1	1	1	R
62	1	0	0	R
63	1	1	0	S
64	1	1	1	R
65	1	0	0	S
66	0	0	1	R
67	1	0	1	R
68	1	0	1	R
69	1	1	1	R
70	1	0	1	R
71	1	0	1	R
72	1	0	0	R

73	0	1	1	R
74	0	1	1	R
75	1	0	1	R
76	1	1	1	R
77	1	0	1	R
78	1	0	0	R
79	1	0	1	R
80	0	1	0	R
81	0	1	1	R
82	1	1	0	S
83	1	1	0	R
84	1	1	0	R
85	0	1	0	R
86	1	1	1	R
87	0	1	1	S
88	1	1	1	R
89	0	1	1	R
90	0	1	1	R
91	0	1	1	S
92	0	1	1	R
93	1	1	1	R

PCR COMPONENTS X1/µL

NFSW (Nuclease free sterile water)	1
One taq 2x master mix	6
Primer-F	0.5
R	0.5
DNA	2

2x Master Mix with Standard Buffer Contains

1.8mM $mgcl_2$, 0.2mM dNTPs, 25units/ml one Taq DNA polymerase, 20mM TrisHCL (pH8.9), 22mM NH_4CL, 22Mmkcl and tracking dyes.

PCR CONDITIONS

Initial pre-denaturation 95^{o}C for 5 minutes.

Followed by 35 cycles of denaturation at 95^{o}C for 1 minutes

Annealing 55^{o}C for 1 minute

Extension 72^{o}C for 2 minutes

Final extension 72^{o}C for 10 minutes