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GENETIC MARKERS ASSOCIATED WITH *STRIGA GESNERIOIDES* RESISTANCE AND SEED SIZES IN COWPEA [*VIGNA UNGUICULATA* (L.) WALP.] INBRED LINES

FRANK ESSEM

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BY

FRANK ESSEM

Thesis submitted to the Department of Molecular Biology and Biotechnology, School of Biological Sciences of the College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Molecular Biology and Biotechnology

NOVEMBER 2017

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature ……………………………. Date …………………….

Name: Frank Essem

Supervisor's Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature …............................. Date …………………

Name: Dr. Aaron Tettey Asare

Co-Supervisor's Signature …………………………....... Date …………………

Name: Michael P. Timko, PhD

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ABSTRACT

Production of cowpea is severely hampered by different races of the parasitic weed, *Striga gesnerioides*. Cultivation of *Striga*-resistant cowpea is the most reliable protocol to effectively combat the parasite. The current study reported the identification of genetic markers associated with multi-race-*Striga* resistance and seed size across the genome of cowpea RIL populations. The landrace GH3684 from Ghana was tested for resistance against all known races of *Striga gesnerioides* in West Africa. Pot experiments were used to test for resistance in each line against *Striga* populations collected from Northern Ghana (GH) and Nigeria (SG3). Seed size was measured in field trials. SSR and SNP markers were identified and used for phylogenetic analysis and genetic mapping. A genetic linkage map was constructed with QTL IciMapping. Segregation of SSR-1 marker with known association with the *Striga* resistance gene *rsg3* was 100 % consistent with the cowpea phenotypes in SG3. This study indicated that the SG3 resistance gene is located at 12.60 cM away from that of the GH race. On the whole, 70 % of the inbred lines of cowpea were resistant to *Striga* in Ghana and the lines designated UCC-11, UCC-24, UCC-32, UCC-122, UCC-221, UCC-241, UCC-328 (best RIL candidates for release) and GH3684 were immune to all 7 known races of *Striga* in West Africa. SARC-LO2 had resistance to four races of *Striga* (SG2, SG4z, SG5 and SG6). The low genetic diversity and polymorphism information content suggest close genetic relatedness within the RIL population. One and five SNP marker(s) were found to be associated with cowpea seed size and *Striga* resistance respectively.

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KEY WORDS

Deoxyribonucleic Acid

Host Plant Resistance

Polymerase Chain Reaction

Recombinant Inbred Lines

Single Marker Analysis

Linkage Map

QTL Mapping

Multi-race *Striga* Resistance

Simple Sequence Repeat

Striga gesnerioides

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DEDICATION

To my family

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LIST OF ACRONYMS

%	Percentage
Cm	Centimetres
µL	Microlitre
mM	Millimolar
LSD	Least Significant Difference
RILs	Recombinant Inbred Line
IITA	International Institute of Tropical Agriculture
SARI	Savvana Agriculture Research Institute
PGRRI	Plant Genetic Resource Research Institute
CTAB	Cetyl Trimethylammonium Bromide
DNA	Deoxyribonucleic Acid
T.E	Tris EDTA
PCR	Polymerase Chain Reaction
TAE	Tris Acetate EDTA
SSR	Simple Sequence Repeat
Bp	Base Pairs
QTL	Quantile Trait Locus
LOD	Logarithm of Odds
cM	Centimorgan
UCC	University of Cape Coast
UVA	University of Virginia
T_A	Annealing Temperature
LG	Linkage Group
MGDw	Molecular Grade Distilled Water

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PAGE	Polyacrylamide Gel Electrophoresis
TBE	Tris Borate EDTA
SMA	Single Marker Analysis
UPGMA	Unweighted Paired group method with Arithmetic mean
ANOVA	Analysis of variance
PIC	Polymorphism Information Content
MAS	Marker Assisted Selection
RAPD	Random Amplified Poymorphic DNAs

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CHAPTER ONE

INTRODUCTION

This chapter is about the general introduction of the current work; background to the study, statement of the problem, justification, objectives and the associated hypothesis.

Cowpea (*Vigna unguiculata* (L). Walp) is a dicotyledonous leguminous food crop. It is also commonly referred to as crowder pea or lubia. Cowpea is known to have originated from West Africa close to six thousand years ago and widely cultivated in different parts of the world including Latin America, Southeast Asia and in the southern United States of America (Girija & Dhanavel, 2009). Cowpea is usually cultivated by small scale farmers and sometimes intercropped with maize or sorghum. Diversity of cowpeas exist in West Africa particularly, Nigeria, Southern Niger, Ghana, northern part of Togo, part of Burkina Faso, northern Benin and the North-Western part of Cameroon (Ng & Marechal, 1985). Cowpea is an important food crop for both man and animals (Davis et al., 1991). It improves soil fertility and serves as income for farmers and traders. The fibers are used for making fishing lines and have also been considered as a source of pulp to make good quality papers (Summerfield, Huxley & Steel 1985).

Cowpea production is suitable for subsistence farming systems in which low inputs are involved due to its ability to thrive on relatively poor soil (Pasquet, 2000; Pronaf, 2003). It has high level of adaptation due to its inherent ability to withstand drought, tolerate shade, and fix atmospheric nitrogen (Singh, 1997). Despite the huge potential of cowpea to ensuring food security and good soil nutrient turn over, several factors are known to affect its

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production. The low productivity of cowpea is particularly due to intense biotic pressure by insects and other pest.

Cowpea is attacked by a host of pathogens, most prominent is the parasitic flowering plant *Striga gesnerioides*, whose parasitism causes severe chlorosis, wilting and stunting of susceptible hosts, leading to yield losses estimated to be around millions of tonnes annually (Aggarwal & Ouedraogo, 1989; Muleba et al., 1997; Singh & Emechebe, 1997). Studies conducted in West Africa by Lane et al. (1996) revealed that there are five different races of the *Striga gesnerioides* designated as SG1 through SG5. In addition, Botanga and Timko (2006) identified SG4z and SG6 bringing the known races to seven. Various control measures, including cultural practices, chemical control, biological control and host plant resistance have been suggested (Dube & Olivier, 2001; Boukar, Kong, Singh, Murdock & Ohm 2004). No single method, however, seems to be fully adequate in the control of this parasite. One practice, host plant resistance appears to effectively and economically control the parasite in that, it is affordable to resource-poor farmers (Omoigui et al., 2007). The only challenge with this control method is that different cowpea varieties react differently to different races of the parasitic plant at different locations in West Africa (Lane et al., 1993; Singh, 2004). This fact was established when 2 cultivars (58-57 and Suvita-2) were found to be completely resistant to *S. gesnerioides* in Burkina Faso (IITA 1982, 1975) but proved to be heavily susceptible to *Striga* in Niger and Nigeria when regional trials were conducted on these cultivars, suggesting strain variation in *S. gesnerioides* (Aggarwal, 1985). Well-adapted high-

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yielding cultivars resistant to all races of *S. gesnerioides* are under development but not widely available (Singh, 2000).

One of the important desirable traits of cowpea in West Africa is large seed size (Drabo, Redden, Smithson, & Aggarwal, 1984; Langyintuo et al., 2003; Tchiagam et al., 2011; Egbadzor et al., 2014). However, much breeding objectives have not been directly focused on seed size compared with such traits as biotic and abiotic stress tolerance (Orawu et al., 2013). Highly specific seed market classes for cowpea and other grain legumes exist because grain is most commonly cooked and consumed whole. Size, shape, colour, and texture are critical features of these market classes and breeders target development of cultivars for market acceptance. Resistance to biotic and abiotic stresses that are absent from elite breeding material are often introgressed through crosses to landraces or wild relatives. When crosses are made between parents with different grain quality characteristics, recovery of progeny with acceptable or enhanced grain quality is problematic. Several cycles of backcrossing help recover elite characteristics including seed size. However, this process can be cumbersome and inefficient due to possible linkage drag and the polygenic nature of the trait. Thus genetic markers for grain quality traits can help in pyramiding genes needed for specific market classes. Allelic variation dictating the inheritance of seed size can be tagged and used to assist the selection of large seeded lines.

The aim of cowpea breeding and genetic improvement programmes around the world is to put together desirable agronomic traits with resistances to the major diseases, insect pests or parasites that afflict cowpea in agro-ecologically adapted cultivars (Timko et al., 2007; Timko & Singh, 2008).

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Approximately, ten years is required to breed a superior improved line using traditional selection and hybridization strategies. The overall efficiency and effectiveness of cowpea improvement programmes can be facilitated by the knowledge of the genetic diversity available within local and regional germplasm collections (Hall, Cisse, Thiaw, Elaward, Ehlers & Ismail, 2003; Hegde & Mishra, 2009). At present, analysis of Simple Sequence Repeat (SSRs) has proven to be useful since these sequences, besides being abundant and distributed throughout eukaryotic genomes, are highly polymorphic, inherited co-dominantly and reproducible, with simple screening requirements (Dib et al., 1996). Until recently, SSRs have been considered as the marker system of choice for the majority of applications. However, recent advances in sequencing and genotyping technologies now permit generation of large sets of single nucleotide polymorphism (SNP) markers from relatively under studied crop species such as faba bean at an acceptable level of cost. As a consequence, SNPs have become more widely used due to high abundance and capacity to be multiplex-formatted for high-throughput genotyping. In addition, SNP discovery from transcribed regions of the genome provides the basis to establish a direct link between sequence polymorphism and putative functional variation.

The cowpea breeding programme in the Department of Molecular Biology and Biotechnology, University of Cape Coast has the aim to introgress *Striga*-resistance quantitative trait locus (QTL) into local susceptible lines as well as improve their seed sizes. This has led to the development of new cowpea recombinant inbred lines, which warrant adequate genetic analysis to elucidate potential genotypes for optimum

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utilization of the crop. Besides, there is a need to validate multi-*Striga* race resistance status and seed size of the advanced cowpea recombinant inbred lines using simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers for subsequent selection and release of novel varieties.

Statement of the Problem

The most important challenge to cowpea cultivation in the major production regions of Northern Ghana and sub-Saharan Africa as a whole, is infestation of variable races of *Striga gesnerioides* in drought prone areas under poor soil conditions. The variable races of *Striga* warrant multi-resistance gene in cowpea to widely control the parasite across the value chain and trade routes in sub-Saharan Africa. The stress imposed on cowpea by *Striga* causes yield loss of between 80 % - 100 % (Asare et al., 2010). Besides, consumer preference for specific seed sizes has also become an important selective trait in breeding.

Obviously, the lack of improved *Striga* resistant cowpeas for farmers to cultivate, most especially, those at Ghana's hub of production (Upper East, Upper West and the Northern regions) largely contributes to the continual importation of cowpea from neighboring countries including Togo, Benin, Nigeria and Burkina Faso. The need for cultivation of improved *Striga*-resistant cowpeas in the affected regions in Ghana to meet consumer preference is critical towards sustainable production and food security. Though advanced recombinant inbred lines (RILs) of cowpea have been developed in the Department of Molecular Biology and Biotechnology, University of Cape Coast, it has not been fully explored for improved multi-*Striga*-resistance trait to mitigate the infestation of the parasitic weed. Besides,

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only few SSR markers exist and no SNP markers have been identified for genetic analysis to select multi-*Striga*-race resistant genotypes of cowpeas with improved seed sizes among inbred lines of the crop.

Justification

Variations in RILs of cowpea may be associated with useful multi-race-*Striga*-resistant and seed size traits. The use of molecular markers together with phenotypic information could be a more reliable protocol to identify and select desirable traits in crop improvement. Indeed, morphological and agronomic data coupled with molecular markers may facilitate reliable selection of multi-race-*Striga*-resistant cowpea genotypes with improved seed size. SSR and SNP genotyping are promising platforms for providing plant breeders with the simplest, most useful and cost-effective services, which can be employed to analyze the advanced RILs of cowpea which have been developed in the Department of Molecular Biology and Biotechnology, University of Cape Coast. This will enhance identification of multi-race-*Striga*- resistance and seed size QTLs across the genome of the inbred lines of cowpea.

Main Objective

The main objective of this study was to assess multi-race-*Striga*-resistance and seed size traits as well as genetic variability across the genome of recombinant inbred lines of cowpea.

Specific Objectives

The specific objectives were to determine:

1. SSR markers associated with *Striga* resistance across the genome of cowpea inbred lines and GH3684

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2. Multi-*Striga*-race resistance status of some cowpea inbred lines and GH3684
3. SNP markers associated with *Striga*-resistance and seed size among cowpea inbred lines.
4. Genetic relatedness among the cowpea inbred lines

Hypotheses

1. Specific SSR markers are linked to *Striga*-resistance across the genome of cowpea inbred lines.
2. Differential multi-*Striga*-race resistant traits exist among some cowpea genotypes
3. Specific SNP markers are associated with *Striga*-resistance and seed size
4. Genetic variations exist among cowpea inbred lines

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CHAPTER TWO

LITERATURE REVIEW

This chapter provides an overview of previous researches on cowpea and the application of molecular markers for the improvement of the crop. It introduces the framework for the case study that comprises the main focus of the research described in this thesis.

Origin and Domestication

The origin of cultivated cowpea has been discussed through centuries and scientists have still not come into consensus as to where exactly cowpea originated from. Cowpea has been mentioned since antiquity by Dioscoride. It has also been described by Linne from a cultivated species of Antilles as *Dolichos unguiculata*, then, *Vigna sinensis* and later became *Vigna unguiculata* (Faris, 1963; Pasquet & Baudoin, 1997). Timko and Singh (2008) suggested that both Asia and Africa could be independent centers of origin based on early observations of the crop. Cytological and morphological studies of the crop, suggest that Nigeria is the center of domestication of cowpea in West Africa (Faris, 1963). Some other studies confirmed that *V. unguiculata* originated from West Africa where some wild relatives are found at the edge of the forest (Pernes, 1984). However, Coulibaly et al., (2002) proposed that cultivated cowpea was domesticated in north eastern Africa based on DNA marker analysis. This buttresses the fact that scientists have not been able to come to a compromise as to where exactly cowpea was domesticated.

V. unguiculata has 22 chromosomes (2n= 2x= 22). The genus *Vigna* is pantropical and highly variable (Timko & Singh, 2008). This genus contains,

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in addition to cowpea, other members like mungbean (*V. radiata*), and the bambara groundnut (*V. subterranea*). The genus was initially divided into several subgenera based upon morphological characteristics, extent of genetic hybridization or reproductive isolation, and geographic distribution of species (Marechal, Mascherpa & Stinier, 1978).

In contrast to many other important world crops, relatively little is known about the domestication history, worldwide dispersal and distribution of genetic variation of cowpea (Huynh et al., 2013). The location of cowpea domestication in Africa is still uncertain. Different centers of origin and diversity have been proposed (Ba et al., 2004). Evidence were provided based on molecular markers that early domestication occurred in northeastern Africa (Coulibaly et al., 2002). In addition, Steele (1976), suggested that cowpea could have been domesticated together with sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum typhoides*) in the third millennium B.C. in Africa. Some speculations support that cowpea may have followed the same route out of Africa as sorghum, moving first from eastern Africa to the Arabian Peninsula and then onto the Asian subcontinent (Faris, 1965; Pant et al., 1982) and to East Asia. Tosti and Negri (2002) were of the view that cowpea may have also moved to Europe from the Middle East because the crop was known in southern Europe during Roman times. Therefore, it is plausible that cowpea first moved from western Africa to the New World with African people during the slave-trade period (Huynh et al., 2013), but little or no documentation exists to support the extent of this movement.

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Botany

Cowpea is a self-pollinated crop with a little rate of outcrossing attributed to insect activities (Rachie & Roberts, 1974). The floral structure of cowpea is characterized by a symmetric flower with a style and a short beak (stigma) (Marechal et al., 1978). One flower contains ten stamens and each stamen carries one anther sac which contains pollen grains for pollination and fertilization. Flower opening occurs after pollination and fertilization, which reduces chances for out-crossings due to foreign pollen (Marechal et al., 1978). The plant has large flower buds that facilitate emasculation during the process of artificial crossing.

The cowpea is a herbaceous, warm-season annual crop which requires temperature of at least 18°C throughout all stages of its development and requires an optimal growing temperature of about 28°C (Craufurd et al., 1997).

Importance and Uses

Cowpea is one of the most economically important indigenous African grain legume with enriched proteins as source of food for both human and animal nourishment and a major crop in regional trade within West and Central Africa (Langyintuo et al., 2003; Asare et al., 2013). The relatively high protein content of cowpea makes it an essential supplement to the diet of many Africans (Bressani, 1985; Asare et al., 2013) who consume high carbohydrate but low in protein cereals, root and tuber crops (Omoigui et al., 2007; Asare et al., 2013). These proteins have good functional properties including solubility, emulsifying and foaming activities (Rangel et al, 2004) and could be a substitute for soy protein isolates for persons (especially

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infants) with soy protein allergies. Cowpea crop can be used at all stages of its growth. In the area of agriculture, it is widely used as a nitrogen-fixing crop and as a green manure crop, a cover crop to inhibit weeds or for erosion control (Davis et al., 1991). Cowpea can also be used as a feed for livestock. The fibers are used for making fishing lines and have also been considered as a source of pulp to make good quality papers (Summerfield & Roberts, 1985). In terms of food, cowpea seed is highly nutritious. It is made up of 24% protein, 63.6% carbohydrate, 6.3% fiber among other important nutrients (Oelka et al., 1991). The tender green leaves are used as an important food source in Africa and are prepared as a pot herb like spinach (Davis et al., 1991). Green cowpea seeds are boiled as a fresh vegetable or may be canned or frozen. Dry beans may also be boiled or canned. Examples of food prepared in Ghana using cowpea are as follows; cowpea stew, cowpea cake, Yikpono (cowpea biscuits), Apapransa, cowpea pie, Tsintsin (cowpea sticks) etc.

Fat contents of 100 advanced breeding lines of cowpea from IITA showed a range from 1.4 to 2.7% (Nielson, Brandt & Singh, 1993), while fiber content is about 6% (Bressani, 1985). Besides its low in fat and high in fiber, the proteins in grain legume which includes cowpea have been shown to reduce low-density lipoproteins which are implicated in heart disease (Phillips et al, 2003).

Another important area under the uses and importance of the cowpea crop is employment creation. Employment opportunities are created through cowpea production, processing and sales. In 2008, Nagai described typical market channels for cowpea grain observed in Benin, Togo, Burkina Faso and Ghana. He said rural assemblers buy cowpea surpluses from farmers and sell

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them to small or large urban wholesalers or through commission agents; retailers buy from wholesalers or commission agents or sometimes at harvest time directly from farmers and sell to consumers. The price of cowpea grain is influenced by quality, time of selling, transport and storage costs, market tolls and taxes, and sales location (Langyintuo, Ntoukam, Murdock, Lowenberg-DeBoar & Miller, 2003). Prices decline during and soon after the harvest season (October through January in Niger, Benin, Nigeria and Ghana) and increases in the following months when grains are scarce.

The share of different varieties sold in markets varies from one country to the other and even from one market to the other within the same country. Cowpea varieties have different attributes ranging from grain size, grain texture through to eye color (Langyintuo, Ntoukam, Murdock, Lowenberg-DeBoar & Miller, 2003). These variations affect the type of foods prepared and storage conditions (Langyintuo, Ntoukam, Murdock, Lowenberg-DeBoar & Miller, 2003). Consumers pay a premium for larger grains in most markets of Cameroon and Northern Ghana (Langyintuo, Ntoukam, Murdock, Lowenberg-DeBoar & Miller, 2003), most markets of Southern Ghana (Mishili, 2005), and all the markets of Senegal (Faye, 2005).

In West and Central Africa, the drier Sahelian countries that produce a larger amount of cowpeas tend to be net exporters, while more humid coastal countries tend to be net importers (Langyintuo et al., 2003). Nevertheless, Nigeria is an important exception serving as the largest producer and the largest exporters of cowpea in the region. Langyintuo et al., (2003) identified two largely independent cowpea trade zones. The first is the “Nigerian cowpea grainshed,” composed of Niger, Burkina Faso, Mali, Cameroon, Chad, Benin

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(net exporters listed in descending order by the average annual amounts exported during the 1990s) and Nigeria, Ghana, Cote d'Ivoire, Togo and Gabon (net importers listed in descending order except for Gabon, for which data is unavailable. The second is the "Senegalese cowpea grainshed," composed of Senegal (a net exporter during the 1990s) and Mauritania, Gambia and Guinea Bissau (net importers). Per capita consumption of cowpeas estimated by Langyintuo, Ntoukam, Murdock, Lowenberg-DeBoar & Miller, (2003) showed enormous differences among countries; in Nigeria, it was estimated that people consumed 18kg of cowpea per capital per year during the 1990s; while per capital annual consumption was estimated at 9 kg to 16 kg in Benin, Ghana and Togo; and between 1.5 to 2.5 kg in Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Mali, Mauritania, Niger and Senegal.

Cowpea Production Systems

In West Africa, cowpea cultivation is principally based on small scale subsistence farming systems in the lowland dry Savanna and Sahelian regions. Traditionally, cowpea is grown as an intercrop or in relay-cropping with cereals such as sorghum [*Sorghum bicolor* (L.) Moench] or pearl millet (*Pennisetum glaucum*) (Ajeigbe, & Singh, 2006). It is less frequently cultivated as a sole crop but intercropped with maize (*Zea mays* L.), cassava (*Manihot esculenta* Crantz) or cotton (*Gossypium* sp.) (Langyintuo et al., 2003). However, due to the economic importance of the crop and the demands, cowpea cultivation is moving towards monocropping system. In Europe, both fodder and grain type varieties are grown mostly as a pure crop. Among the developed countries, only the United States is a large scale

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producer and exporter of cowpea (Imrie, 2000). The production of cowpea in USA is highly mechanized and purely on commercial basis.

The long term drought in the Sahelian zone of West Africa has caused many farmers to shift more of their crop production to cowpea because of it drought tolerance (Duivenboo-den, Abdoussalam & Mohamed, 2002). In the light of this, Timko et al. (2007) indicated that rapidly growing populations with high per-capital cowpea consumption in West and Central African regions have fueled demand for cowpea grain which is expected to continue, thus attracting more farmers to boost productivity

World Cowpea Production

Subsistence farmers in the semi-arid and sub-humid regions of Africa are the mojor producers and consumers of cowpeas. Nigeria is the world's leading cowpea producing country with 850, 000 t per annum, hence Africa is the leading cowpea producer in the world (Figure 1). Outside Africa, the major production areas are Asia, Central and South America with Brazil as the world's second leading producer of cowpea seed of 600,000 t annually (Guazzelli, 1989).

Cowpea is cultivated in the tropics and sub-tropics covering 65 countries in Africa, Asia and Oceania, the Middle East, Southern Europe, and Central and Southern America (Singh, Chamblis & Sharma, 1997). According to available information from FAO, it is estimated that globally cowpea cultivation covers about 14.5 million hectares of land with an annual production of over 4.5 million tons (Singh et al., 2002). World cowpea cultivation was estimated at 3, 319, 375 MT and 75 % of that production is from Africa (FAOSTAT, 2000). West Africa is the predominant cowpea

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producing zone, mainly in the dry savanna and semi-arid agro-ecological zones. The principal cowpea producing countries are Nigeria, Niger, Senegal, Ghana, Mali, and Burkina Faso (FAOSTAT, 2000): among these countries, Nigeria and Niger are the leading producers in the sub-regions respectively, with the rest of Africa contributing 68 % of global production.

Current estimates of cowpea production vary widely according to sources, the statistics are probably conservative. Recent estimates indicate that over 12.5 million tons of cowpea grains are produced worldwide, with Africa's arid sahel region accounting for 64 % of the total cowpea seed production (Fery, 2002; Timko & Singh, 2008) (Figure 1). The majority of the cowpea production in Africa takes place on low-input, subsistence farms in West and Central sub-Saharan Africa (Alene, Abdoulaye, Rusike, Manyong, & Walker, 1993).

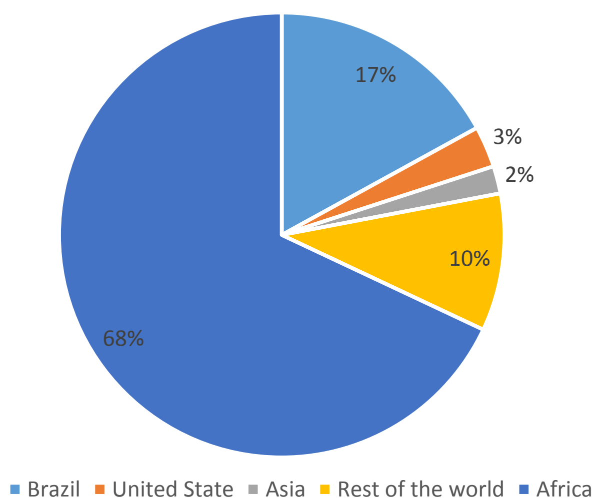

Figure 1: Cowpea production throughout the world (dry grains)

Source: Gomez 2004

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Cowpea Production in Ghana

Cowpea is the Second most important legume in Ghana after groundnut; an average of 143, 000 MT is produced annually on about 156, 000 ha making Ghana the fifth highest producer of cowpea in Africa, with a projection increase rate for the period between 2010 and 2020 to be 11.1 % for cowpea, International Crops Research Institute in the Semi-Arid Tropics, ICRISAT (2012, December, 16) retrieved August 2016 from http//www.icrisat.orf/tropicallegumesII. In Ghana, cowpea is produced predominantly in the Sudan Savanna agro-ecological zone as a main food crop by subsistence farmers.

The major limitations to cowpea production in Ghana are biotic and abiotic factors such as insect pest (aphids, flower thrips, maruca, pod sucking bugs, bruchid), diseases caused by bacteria, fungi, and viruses; *Striga* and *Alectra* infestations which can cause yield loss ranging from 15 to 100 % depending on the level and severity of infestation, susceptibility or resistant status of the variety; besides, drought and low soil fertility, lack of input such as fertilizers, insecticides and improved seeds, poor cultural practices and lack of appropriate machinery for expanding planting area are other constraints (IITA, 2010). Hence, Ghana produces less cowpea than it consumes as the population increases and as such the country imports cowpea from neighboring countries like Burkina Faso, Niger and Nigeria to supplement local production.

Ghana has the necessary agro-ecological conditions for cowpea production: a warm, humid climate with mean annual rainfall estimated at 83 to 220 cm; relative humidity between 50 % and 80 % with mean temperatures

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ranging from 21 °C near the coast to 31 °C in the extreme north. Annual potential open water evaporation has been estimated as ranging between 1350 mm in the south to about 2000 mm in the north (FAO, 1999). There are six agro-ecological zones defined on the basis of climate, natural vegetation and soil types (Table 1). Rainfall distribution is bimodal in the forest, transitional and coastal zones, giving rise to a major and a minor growing season. In the remaining two agro-ecological zones, the unimodal rainfall distribution gives rise to only one growing season.

Challenges to Cowpea Production

Among the constraints in crop production in tropical and subtropical regions of the world are the activities of insect pests on economic crops in the field and storage. One major food crop that has been so much plagued and damaged by insect pests is the legume, cowpea (*Vigna unguiculata* (L.) Walp). It is now known that a spectrum of insect pests decimate the crop at all stages of growth (Jackai et al, 1988). These insects which include the cowpea aphid, *Aphis craccivora*, Koch., flower bud thrips, *Megalurothrips sjostedti* Trymb., the legume pod borer, *Maruca vitrata* Fab, and a complex of pod sucking bugs among which are *Clavigralla tomentosicollis*, *Anoplocnemis curvipes* Fab., *Aspavia armigera* Fab. and *Nezera viridula* L, have been reported to cause low yield in cowpea in Africa (Olatunde et al., 1991). Man has, therefore, been deprived of the maximum benefit from cowpea cultivation as cheap source of protein (IITA, 1982; Alabi et al., 2003), fibre (Rachie, 1985) and fodder for livestock (Job et al., 1983).

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Table 1: *Characteristics of agro-ecological zones in Ghana*

Zone	Rainfall (mm/yr)	Portion of total area (%)	Length of growing season (days)	Dominant land use systems	Main food crops
Rain forest	2 200	3	Major season: 150-160 Minor season: 100	Forest, plantations	Roots, plantain
Deciduous forest	1 500	3	Major season: 150-160 Minor season: 90	Forest, plantations	Roots, plantain
Transition zone	1 300	28		Annual food and cash crops	Maize, roots,plantain
Guinea savannah	1 100	63	180-200	Annual food and cash crops, livestock	Sorghum, maize
Sudan savannah	1 000	1	150-160	Annual food and cash livestock	Millet, sorghum, cowpea
Coastal savannah	800	2	Major season: 100-110 Minor season: 50	Annual food crops	Roots, maize

Source: SRID, Ghana (2001)

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Drought

Drought is one of the most important constraints threatening the food security of the world (Barters & Nelson, 1994). Cowpea production in Ghana is hampered by recurrent drought especially in the three Northern Regions which is the centre of production. The rainfall patterns have been irregular and below normal throughout the semi-arid zones of West Africa including Ghana. In the Sudan and Sahelian semi-arid regions, the frequency and intensity of drought have increased over the last 30 years (Hall et al., 2003) due to climatic changes and human activities (Wittig et al., 2007). Estimates on yield reduction due to terminal drought range from 21-30% between stressed and non-stressed conditions (Chiulele, 2010). However, yield losses in plant production depend on geographical region and length of cropping season (Sabaghpour et al., 2006). Drought spells in farmers' field has resulted in reduction of yields of available cowpea genotypes. Most of these cowpeas are susceptible to drought. Drought can strike at anytime, anywhere. Plants are most prone to damage due to limited water during flowering and pod setting stages (Bahar & Yildirim, 2010). Therefore, it is desirable to improve these adapted genotypes for tolerance to drought in order to obtain high and stable yields.

Colletotrichum disease of cowpea

Colletotrichum sp. induces two major diseases in cowpea (anthracnose and brown blotch) in the humid areas. These diseases are induced by two different species of the genus *Colletotrichum*. Emechebe & Florini (1997) had suggested that the cowpea anthracnose pathogen be regarded as a species that is distinct from *Colletotrichum lindemuthianum*, the *Phaseolus* bean

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anthracnose pathogen. Latunde-Dada et al., (1999) have provided strong evidence in favour of considering the cowpea anthracnose pathogen as a form of *Colletotrichum destructivum* O'Gara and this has been accepted and adopted (Allen & Lenne, 1998). In Savanna agro-ecologies, cowpea brown blotch disease is induced by *Colletotrichum capsici* (Allen & Lenne, 1998; Emechebe & Shoyinka, 1985). However, *Colletotrichum truncatum* (Schew) is regarded as the causal agent of brown blotch of cowpea in humid areas (Adebitan, 1984). Symptoms of the disease includes purplish brown discolouration on pods, which may also extend to petioles, leaf veins and peduncles. Pod infection often leads to maldevelopment and distortion of pods (Allen et al., 1998). The diseases have been found to be seed borne (Emechebe & McDonald, 1979). Yield loss associated with this infection has been estimated as ranging between 46 % and 74 % depending on the susceptibility of the cowpea used for the evaluation (Alabi, 1994). Currently, due to susceptibility of cowpea germplasm *Colletotrichum* diseases stand as one of the most destructive diseases of cowpea in the humid areas. Adebitan, Fawole, & Hartman (1996) has reported greater reduction of brown blotch in monocropped cowpea as against intercrop. Moreover, it was shown that wide spacing of cowpea resulted in lower incidence and severity of brown blotch compared to the closer planted crop, both monocrop and intercrop in Ibadan the humid forest of Southern Nigeria. Anthracnose incidence and severity were lower in the intercrop relative to the sole crop while reductions in both inter and intra-row spacing resulted in an increase in the incidence and severity of anthracnose (Adebitan & Ikotun, 1996). Employing these control

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measures means only few cowpea plants will be needed on an acre as spacing will become a necessity to reducing the disease infection.

Cowpea leaf smut disease

Protomycopsis phaseoli (Ramak and Subram) is the causal agent of the cowpea leaf smut disease in Nigeria (Adejumo et al., 2000). It was first reported in Nigeria in 1975 (IITA, 1975) and later by Williams and Allen (1976). This pathogen formed dark ash-grey to sooty-black lesions of 3 - 10 mm in diameter, while young lesions had yellow haloes. False smut occurs mostly in humid and fertile soil causing yield losses of between 23 and 48% (Allen, 1979; Singh and Allen, 1979; Adejumo & Ikotun, 2003). In 1999 cropping season, about 65% of the 71-cowpea lines evaluated had leaf smut infection (Ajibade & Amusa, 2001). The potential of *Bacillus sp*., *Aspergillus fumigatus, Fasarium oxysporum, Trichoderma harzianum, Trichoderm koningii* and *Trichoderma* sp. and yeast as biological control agents of *P. phaseoli* has been reported (Adejumo et al., 1999). Destruction of leaf debris before crop emergence, long period of rotation and no tillage cropping are suggested to prevent the onset and spread of leaf smut disease of cowpea.

Web blight and related diseases

Thanatephorus cucumeris (Frank) Donk and its anamorphic state, *Rhizoctonia solani* Kuhn, are soil-borne and ubiquitous in nature as causal agents (Emechebe & McDonald, 1979) for two distinctly different diseases in cowpea, thus web blight and a root rot-seedling disease complex in South-western Nigeria. The root and seedling phase results in root rot and in damping-off or seedling blight, the latter being due to collar or foot rot. Web blight is induced by aerial types, usually belonging to AG-1, while the strains

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that induce root rots or seedling diseases are strongly soil-borne, in contrast to the aerial strain, which has only a transient association with the soil. The 2 phases of the disease complex have been reported to be seed-transmitted (Emechebe & McDonald, 1979). These diseases are often severe under localized, waterlogged conditions in the humid forest of South-western Nigeria. Web blight pathogens infect leaves and many other young stem tissues. Initial symptoms are small circular brown spot which enlarge and often show concentric banding and become surrounded with irregular shaped water soaked areas. Under humid conditions the lesions develop rapidly and coalesces leading to extensive blighting and defoliation (Allen & Lenne, 1998). Out of 71 cowpea lines evaluated in 1999 and 2000, in Ibadan, 39% was found susceptible to web blight disease. The two diseases have been regarded as major important diseases in the forest belt of West Africa (Emechebe & McDonald, 1979). Similarly, web blight has been described as a destructive disease of cowpea in Latin America and in hot humid regions of India (Lin & Rios, 1985; Verma & Mishra, 1989).

Sclerotium rot

The fungus *Sclerotium rolfsii* infects the cowpea stems at the base of the plant, producing a fan of silking mycelium and large round sclerotia which are initially white and gradually darken. The infected plants usually wilt and die leading to 100 % grain loss of the cowpea plant involved (Adejumo & Ikotun, 2003). Though sclerotium rot is often severe on infected crops, it is more localized in endemic areas and generally does not constitute major constraints to cowpea production.

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Charcoal rot (damping off)

Damping off caused by *Macrophomna phaseolina* (Tassi) Goid. *Rhizotonia* (Taub) Butler is one of the most destructive diseases of cowpea in the tropics and subtropics (Chidamboram & Mathur, 1975; Dhingra & Sinclair, 1977; Reuveni et al., 1983). Besides charcoal rot, the pathogens also induce diseases such as dry root rot, wilt, leaf blight and ashy stem blight (Abdon et al., 1980; Singh et al., 1990). Seed, soil and plant residue are the sources of primary inoculum (Reuveni et al., 1983, Short et al., 1980). The epidemic outbreak and yield losses due to charcoal rot of cowpea have been observed in many bean growing areas in Nigeria (Singh et al., 1990). Screening for resistance to *M. phaseolina* has been advocated and is being adopted in the humid forest of Southern Nigeria.

Cowpea parasitic nematodes

About 51 species in 23 genera of parasitic nematodes have been associated with cowpea plants (Caveness & Ogunfowora, 1985), while Florini (1997) reported about nine species of parasitic nematode on cowpea. The most important of the species of *Meloidogyne* pathogenic in cowpea is *Meloidogyne incognita* (Sarmah & Sinha, 1995; Khan et al., 1996; Adegbite et al., 2005). The rootknot nematodes, *M. incognita*, *Meloidogyne javanica* and *Meloidogyne areneria* were first reported in Nigeria on cowpea in 1958 and documented in 1960 (Anonymous, 1961). However, *M. incognita* and *M. javanica* have been found to be predominant in the southern forest zone of Nigeria (Olowe, 1976). It has been shown that those root knot nematodes are responsible for yield reduction in cowpea. Caveness (1979) and Ogunfowora (1976) reported yield losses of 20 and 59%, respectively due to infestation by

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M. incognita. Cowpea grain yield loss of 69% caused by root knot nematodes was reported by Babatola & Omotade (1991). Severe root knot nematode infestation has been observed to lead to crop failure in cowpea (Olowe, 1981; Adegbite et al., 2005).

Pest of cowpea

The parasitic weed Striga gesnerioides

Parasitic weeds are serious problems in many agricultural systems in the tropics. *Striga* also known as "witchweed" is an angiospermic, hemi-parasite belonging to the family orobachaceae (formerly scrophulariaceae). There are about 30 species or more of *Striga* described, but only 5 are presently of economic importance in Africa. These include, *Striga hermonthica* (Del) Benth, *Striga asiatica* L. Kuntze, *Striga gesnerioides* (Wild) Vatke, *Striga aspera* Benth, and *Striga forbesii* Benth. All these, except *Striga gesnerioides* are parasitic to African cereal crops and these include maize, rice, millet and sorghum. *Striga gesnerioides* is a parasite of cowpea and other wild legumes (Berner et al, 1997). *Striga gesnerioides* has greater impact on human welfare than any other parasitic angiosperms because their hosts are subsistence crops in areas marginal for agriculture (Singh, 2000). As a parasite, *Striga* is entirely dependent on its host. Exudates from roots of the crop plants stimulate germination of *Striga* seed. The sprouting seeds attach themselves to the root of the host crop and draw nutrients for their own growth. Farmers recognize two types of *Striga* damage (underground and above ground) with greater damage being caused by the underground *striga. Striga* undergoes considerable development underground at the expense of its host, by the time *Striga* emerges at the soil surface they may have devastated

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the crop. In this way hosts are "bewitched" because the farmers are unaware of the parasite until it comes up. *Striga* parasitic weeds are considered to be one of the major biological constraints to food production in Sub-Saharan Africa, probably a more serious agricultural problem than insects, birds or plant diseases (Singh, 2000). *Striga gesneriodes* attacks cowpea in West and Central Africa, particularly in the Sahel and Sudan savanna zones (Musselman & Parker, 1982; Ramaiah et al., 1983). It is also found in coastal savanna along the Atlantic Ocean as well as on sandy or shallow gravelly soils in the Guinea Savannas in Benin, Ghana, Togo and Sierra Leone (RENACO, 1990). In northern Nigeria, *Striga* may cause cowpea yield losses varying from few $Kgha^{-1}$ to total crop failure (Obilana, 1987). In Ghana, the witchweed can cause yield losses of between 30% and 100% (Asare et al., 2010) and in Sub-Sahara Africa alone the disease costs an estimated US$ 1 billion, affecting the livelihood of 100 million people. According to Emechebe & Leleji (1988) this had also led to abandonment of *Striga* infested fields to cowpea production. *Striga* tolerates a relatively wide range of climatic and soil conditions. It grows in areas with annual rainfall ranging from 25 to 150cm per year with increased severity of infestation in areas with low rainfall (Musselman & Ayensu, 1984), poor soil fertility and continuous cropping of the host crop.

Geographical distribution and races of Striga gesnerioides

Striga species fall into two main groups, based on their host preference (Pieterse 1985; Mohamed et al., 2001). The first group contains *Striga hermonthica, S. aspera*, and *S. asiatica*. This group parasitizes primarily members of the Poaceae family, including important food and forage grains (corn, sorghum, rice, and millet). The second group contains *S. gesnerioides,*

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the most widely distributed of the witchweeds. Isolates of *S. gesnerioides* from various locations are distinguishable by size, stem succulence, internode distance, and flower color (Musselman 1980; Musselman & Parker, 1981; Mohamed, 1984; Mohamed et al., 2001). However, the morphological differences observed among isolates are not sufficient to justify their classification as different species or subspecies at this time (Mohamed et al., 2001). There is considerable variation in host specificity among races of *S. gesnerioides*, and different host species vary in their susceptibility to different isolates of the parasite (Lane et al., 1996).

Host-plant resistance breeding was thought to have made a major stride in 1981, when 2 cultivars (58-57 and Suvita-2) were found to be completely resistant to *S. gesnerioides* in Burkina Faso (IITA, 1982, 1983). However, regional trials revealed that these cultivars were susceptible to *Striga* in Niger and Nigeria, suggesting strain variation in *S. gesnerioides* (Aggarwal, 1985). Subsequently, in the early 1990s, another cowpea cultivar, B301, thought to be resistant to *S. gesnerioides* throughout West Africa, was found to be heavily parasitized when grown in field plots in Zakpota, located in the southern part of the Republic of Benin (Lane et al., 1993). Subsequent evaluation of differential host-resistance responses, using a variety of different cowpea cultivars and breeding lines, led Lane et al. (1996) to propose that 5 distinct races of *S. gesnerioides* exist in West and Central Africa. Botanga and Timko (2006) recent studies on phenetic relationships among different races of *Striga gesnerioides* from West Africa revealed additional two races (SG4z and SG6) of the witch weed. In view of this, the number of known races now stand at seven. To date, no cowpea variety or cultivar has been identified that is

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naturally resistant to all races of *S. gesnerioides*. However, work by breeders at the International Institute of Tropical Agriculture (IITA) in Nigeria has generated a number of breeding lines that appear to be resistant at all locations tested in West and Central Africa, including Zakpota (Carsky et al., 2003).

The areas affected by *S. gesnerioides* comprise West and Southern Africa, India, Asia or Europe and USA (Mohamed et al., 2001). 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 & 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 & 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. One or more species of the parasite are found in the crop lands and or grasslands of almost all African countries below the Sahara (Gressel et al., 2004). The graphical representation of the distribution of the parasitic weed *Striga gesnerioides* is indicated in Figure 2.

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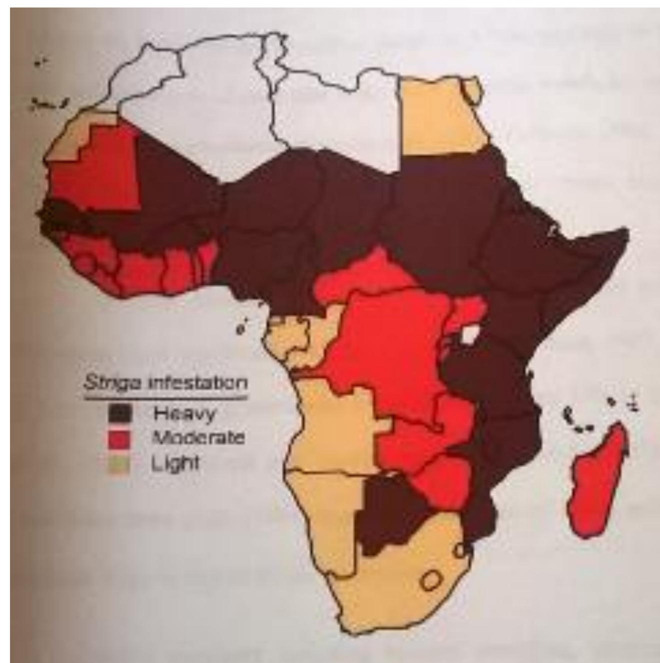

Figure 2: Striga infestation in Africa; is most severe in the most food insecure areas

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 & 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 & 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,

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plant of *S. gesnerioides* have leaves reduced to scales-feature common to all *Orobanche* species. *Striga* spp. belonging to 13 *Orobanchaceae* are hemiparasites because of the aerial photosynthetic activity occurring after *Striga* emergence from soil (Matusova et al., 2005). These parasitic weeds 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 & Williams, 1998). *S. gesnerioides* can also attack tobacco (*Nicotiana tabacum* L.), sweet potato (*Ipomoea batatas* (L.) Lam) and other legumes.

The biology and life cycle of Striga gesnerioides

The life cycle of *Striga gesnerioides* constitutes a series of growth phases that are linked to the developmental stages of the host plant. (Lane & 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 (Thalouran & 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

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Striga require an inhibition period of 10 to 21 days before they can germinate (Okonkwo, 1991; Lane & 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 & 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 & Williams, 1998). The *Striga* seeds are microscopic in size measuring 0.20 mm to 0.35 mm long, weighing 4 to 7 µg (Dubé & 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. Figure 4 is the diagrammatic representation of *Striga gesnerioides* life cycle,

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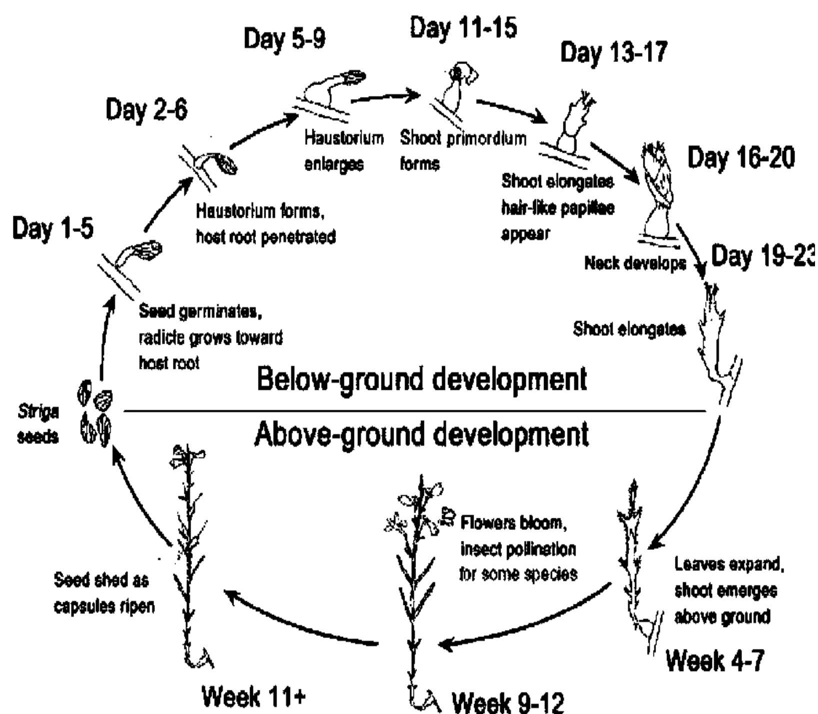

Figure 3: Diagrammatic representation of *Striga gesnerioides* life cycle
Retrieved from (https://nwezejustus.wordpress.com/2015/07/28/witchweed-striga-asiatica-a-destructive-crop-plant-parasitic-weeds/ (01-04-2017)

The sources of resistance to Striga gesnerioides

In light of the differential resistance reaction of different cultivars of cowpeas, 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 has different sources of resistance each combining the resistance to at least two races of *S. gesnerioides* in West Africa. Botanga and Timko (2005), indicated that race development in cowpea *S. gesnerioides* was generally a consequence of host-driven 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 & Polniaszek, 1990;

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Muller et al., 1992; Lane et al., 1996; Touré et al., 1997; Reiss & 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 & 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).

Measures to control Striga gesnerioides

It is difficult to manage the 'witch' weed, *Striga* due to the fact that the larger part of its life cycle occurs underground. Seed germination and development of *Striga* is not recognized until seedling emergence from the soil. In this respect, it precedes the point where it is possible to decrease crop infestation (www.wyoug.nsw.gov.au/environment/weeks). 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, & 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 & Williams, 1998).

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Some studies recommend that, postponing the sowing of black- eyed peas could diminish the level of *Striga* infestation (Lagoke et al., 1991). Toure et al. (1997) also observed that postponing the sowing of cowpea brought about diminishing quantities of sprouted *S. gesnerioides*. However, as indicated by Parker (1990) 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.

Breeding for resistance to Striga gesnerioides

The theoretical advantages of using genetic markers and the potential value of genetic marker linkage maps and direct selection in plant breeding were first reported about eighty years ago (Crouch & Ortiz 2004). However, it was not until the advent of DNA marker technology in the 1980s that a large enough number of environmentally insensitive genetic markers generated to adequately follow the inheritance of important agronomic traits. DNA marker technology has dramatically enhanced the efficiency of plant breeding and genetic engineering (Joshi et al., 2011). A number of breeding companies have in the past two decades started using markers to increase the effectiveness in breeding and to significantly shorten the development time of varieties. Therefore plant geneticists consider molecular marker assisted selection a useful additional tool in plant breeding programs to make selection more efficient (Bueren et al., 2010; Joshi et al., 2011).

Evidence has shown that genetic enhancement of cowpea have taken place within national research facilities and universities in a couple of West

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African countries, India, Brazil, USA and International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Timko et al., 2007). The imbricate dispersion of *Striga* 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 or 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 (1990) the use of resistant varieties are probably the most appropriate way for subsistence farmers to control *S. gesnerioides*.

Mechanisms of Plants Resistance

Antibiosis

Antibiosis is the mechanism that describes the negative effects of a resistant plant on the biology of an insect which had colonized it (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 & Omar, 1978). Antibiosis may be due to presence of toxic substances, absence of sufficient amount of essential nutrients and nutrients imbalance.

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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 wax, 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 & Omar 1978).

Tolerance

Tolerance is the ability of a plant to undergo stress (disease, 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 damaged tissue, to produce additional branches to compensate for the loss.

Genetics of *Striga gesnerioides* Resistance in Cowpea

Seven remarkable races of *S. gesnerioides* (assigned SG1-SG6) have been characterized (Lane et al., 1996, Botanga & Timko, 2006). Many cowpea species are inclined to *Striga* infestation, however, some regional landraces appear 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

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inheritance studied demonstrated that resistance to *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 classes 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. (1997) reported that *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.

Seed Size

Cowpea seed characteristics are of high importance to both the farmers and the consumers in Africa and the world at large. The overall shape, seed coat colour and the seed sizes of cowpea are three of the many characteristics that are looked out for by farmers to establish successful commercial production of the crop. Among these three characteristics, the cowpea seed size is perhaps the most important trait considered by both farmers (subsistence and commercial) and consumers. In Ghana, the successful commercial production of the crop is largely dependent on the cowpea seed size ranging from medium to large seed sizes. Seed size has several agronomically important impacts. Large seeded cowpea have enhanced emergence when planted deep (up to 5 cm), tend to emerge earlier, and produce larger plants during early development (Lush & Wien, 1980).

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Seed size is a very stable component of grain yield with high heritability for many crop plants including wheat (Giura & Saulescu, 1996), soybean (Cober et al., 1997), cowpea (Drabo et al., 1984), and mung bean (Fery, 1980). Several genes are known to control the inheritance of seed size in cowpea. Drabo et al. (1984) proposed that at least eight loci contribute to the quantitative inheritance of seed size and Fatokun et al. (1992) identified two major, unlinked genomic regions, one of which is orthologous to a seed size QTL in mung bean. The orthology of this locus was later confirmed by its identification and association to seed size in soybean (Maughan et al., 1996). The introgression of novel traits from diverse collections typically compromises seed size among progeny. Because of the importance of grain size in market appeal, recovery of adequate grain size is an important objective following elite exotic crosses.

Inheritance of cowpea seed sizes

Seed size is a very important component of grain yield and is controlled by several genes (quantitative trait loci) (Xian – Jun *et al.*, 2007). Seed formation starts with floral induction which is controlled by a number of factors including the plant and its age, environmental conditions, dry matter accumulation among others. The final seed size is believed to be controlled by genes (Li *et al.*, 2008). These genes restrict the period of cell proliferation thereby determining the maximum size the seed can reach. A number of genes controlling seed size have been reported by different authors and for different crops. A point to note is that, some authors use seed size and weight synonymously (Drabo *et al.,* 1984). Arabidopsis is probably the plant in which the mechanism of seed size inheritance is best understood. However, even in

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the Arabidopsis where three genes (Auxin response factor2, Apetala2 and Da1) have been identified to control the trait, the subject is not completely understood (Adamski *et al.*, 2009). Two loci with two alleles each have been reported to control seed size in chickpea with additive and dominant epistasis (Upadhyaya *et al.*, 2011). Hossain *et al.* (2010) reported dominance of small seed over large in chickpea. Differences in seed size inheritance might exist for different crops. In cowpea, Drabo *et al.* (1984) reported eight genes to control seed size while Lopez *et al.* (2003) reported five. However, there are reports of six, ten and other numbers of genes to control the trait by other authors (Aryeetey and Laing, 1973; Lopes *et al.*, 2003). Selection of parents may be a contributing factor to the varying reports. Also, variability in General and Specific Combining Ability (GCA and SCA) for seed size was reported by Tchiagram *et al.* (2011) in cowpea. Apart from the genotypes contributing to varying estimates of genetic parameters, the environment also has effect. The use of molecular techniques will probably help in better understanding of the genetics of seed size as the use of molecular approach would limit the environmental influence on the estimates.

Cowpea Breeding and Molecular Markers

The development of cowpea industry relies heavily on the improvement of existing cultivars and breeding of new varieties. Traditional selection mainly depends on the phenotypic variation. However, morphological markers are easily influenced by the environment, and some of them have epistatic effects (Meglic & Staub, 1996). Simultaneously, conventional breeding program requires selection on many generations of the material, leading to the reduction of reliability and efficiency (Tan et al.,

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2012). DNA molecular markers are genetic markers based on individual nucleotide sequence variation, which are the direct reflection of genetic polymorphisms at the DNA level. Compared with morphological markers, cytological markers and biochemical markers, DNA molecular markers have some unique advantages; its multi-locus nature as well as high reproducibility, simplicity and low cost make it particularly attractive for analyzing a large number of samples with narrow genetic variation (Tantssawat, 2010). The technology mainly consists of Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Single Sequence Repeat (SSR), Random Amplified Polymorphic DNA (RAPD), (Single Nucleotide Polymorphisms (SNP), and so on. They are widely used in genetic diversity research, variety identification, phylogenetic analysis, gene mapping and resource classification, etc. (Zhou, 2005). Currently, the two most robust marker systems of choice normally employed in cowpea breeding are the simple sequence repeat (SSR) and the single nucleotide polymorphism (SNP) markers.

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

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(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, shown 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 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 a result of substitution in amino acids (Xu, 2010).

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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; (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 unique 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 sequences flanking the SSR region (Van Zijll de Jong, Guthridge, Spangenberg & Forster, (2011). The PCR-multiplied products can be isolated in high-resolution involving agarose gel electrophoresis (AGE) or polyacrylamide gel electrophoresis (PAGE)) and the bands can be visualized by fluorescent marking or silver-staining. SSR markers are described by their hyper-variability, co-dominant nature, reproducibility, locus-specificity and mostly, random-genomic distribution (Deng et al., 2016). The benefits of SSR markers are that they can be rapidly 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 low DNA quantity (~100 ng per individual) and low start-up expenses for manual assay protocols. In any case,

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SSR procedure requires nucleotide input for oligonucleotide design, capita-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).

Single Nucleotide Polymorphism (SNP) markers

SNPs were first discovered in human genome but proved to be universal as well as the most abundant forms of genetic variation among individuals of the same species (Ghosh, Malhotra, Lalitha, Guha-Mukherjee, & Chauhan, 2002). Although SNPs are less polymorphic than SSR markers because of their biallelic nature, they easily compensate this drawback by being abundant, ubiquitous, and amenable to high- and ultra-high-throughput automation.

SNP markers have become extremely popular in plant molecular genetics due to their genome-wide abundance and amenability for high-to ultra-high-throughput detection platforms. Unlike earlier marker systems, SNPs made it possible to create saturated, if not, supersaturated genetic maps, thereby enabling genome-wide tracking, fine mapping of target regions, rapid association of markers with a trait, and accelerated cloning of gene/QTL of interest. Currently, SNP markers have been applied on a number of plants which includes legumes such as soybean. In order to improve the effectiveness

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of marker assisted selection (MAS) and clone soybean aphid resistance gene, *Rag1*, fine mapping was done to accurately position the gene, which was previously mapped to a 12 cM interval (Kim, Bellendir, & Hudson, 2010). The authors mapped the gene between two SNP markers that corresponded to a physical distance of 115 kb and identified several candidate genes. Similarly, another aphid resistance gene, *Rag2*, originally mapped to a 10 cM interval, was fine mapped to a 54 kb interval using SNP markers that were developed by resequencing of target intervals and sequence-tagged sites (Kim et al., 2010). In another study that used a similar approach, the authors identified SNP markers tightly linked to a QTL conferring resistance to southern root-knot nematode by developing these SNP markers from the bacterial artificial chromosome (BAC) ends and SSR-containing genomic DNA clones (Ha, Hussey, & Boerma, 2007). In all of these examples the main idea behind the identification of closely linked SNP markers was to enhance the efficiency and cost effectiveness through MAS and increase the resolution within the target locus. Another crop that has seen tremendous development since the discovery of SNPs is cowpea. Cowpea diversity studies involving the use of SNP markers have been reported (Egbadzor et al., 2014). Knowledge about the diversity in the crop will facilitate the selection of those accessions with improved characteristics. Cowpea genomes have also been mapped using SNP markers allowing scientist to pinpoint the exact location of beneficial traits through gene studies (Nacira, Aili, Leo, Man-Wah, & Hon-Ming, 2017).

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CHAPTER THREE

MATERIALS AND METHODS

This chapter gives a detailed description of all the experiments performed in this study. It begins with the description of the research area and conditions and experimental procedures, data collection and analysis.

The Study Area

The research was conducted in the Timko's laboratory located at the Gilmer hall, University of Virginia, Charlottesville, Virginia State, United State of America. The conditions of the *Striga* licensed laboratory where cowpeas were screened for multi-*Striga*-race resistance are indicated in Table 2.

Table 2: *Growth Room Conditions*

Parameter	Condition
Temperature	31 ^{o}C
Relative Humidity	28%
Photoperiod	10 hrs of light and 14 hrs of darkness
Light Intensity	71.333 $\mu mols^{-1}m^{-2}$

Source of Plant Materials and Attribute

All the 115 advanced Recombinant Inbred Lines and three parental genotypes as well as GH3684 were obtained from the Department of Molecular Biology and Biotechnology, University of Cape Coast, Cape Coast, Ghana. The cowpea seeds were advance breeding lines made up of two populations. Population one was derived from the cross between IT97K-499-35 and SARC-LO2 at the F_8 generation. Population two was derived from a cross between IT97K-499-35 and Apagbaala at the F_9 generation. Population one is made up of 84 RILs while population two consist of 31 RILs.

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The *Striga gesnerioides* seeds from Ghana were obtained from farmers' field at Mamprusi in the Upper East Region, and *Striga* races 1, 2, 3, 4, 4z, 5 and 6 were obtained from Timko laboratory, University of Virginia, Virginia, U.S.A. The cowpea germplasm used in this study are presented in Tables 3, 4, and 5.

Table 3: *Source of cowpea parental genotypes and the local check (GH3684)*

Genotypes	Source	*Striga*-Trait
IT97K-499-35	UCC	Resistant
Apagbaala	UCC	Susceptible
SARC-LO2	UCC	Susceptible
GH3684	UCC	Resistant

Table 4: *List of F_8 Recombinant Inbred Lines of Cowpea derived from SARC-LO2 x IT97K-499-35*

RIL	Source	RIL	Source	RIL	Source
UCC-01	UCC	UCC-24	UCC	UCC-77	UCC
UCC-03	UCC	UCC-25	UCC	UCC-78	UCC
UCC-04	UCC	UCC-30	UCC	UCC-84	UCC
UCC-05	UCC	UCC-32	UCC	UCC-86	UCC
UCC-07	UCC	UCC-33	UCC	UCC-99	UCC
UCC-08	UCC	UCC-35	UCC	UCC-106	UCC
UCC-10	UCC	UCC-37	UCC	UCC-113	UCC

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Table 4 continued

RIL	Source	RIL	Source	RIL	Source
UCC-11	UCC	UCC-38	UCC	UCC-122	UCC
UCC-12	UCC	UCC-47	UCC	UCC-145	UCC
UCC-16	UCC	UCC-56	UCC	UCC-148	UCC
UCC-17	UCC	UCC-60	UCC	UCC-149	UCC
UCC-20	UCC	UCC-64	UCC	UCC-151	UCC
UCC-23	UCC	UCC-73	UCC	UCC-153	UCC
UCC-154	UCC	UCC-223	UCC	UCC-292	UCC
UCC-159	UCC	UCC-226	UCC	UCC-318	UCC
UCC-173	UCC	UCC-227	UCC	UCC-321	UCC
UCC-177	UCC	UCC-231	UCC	UCC-328	UCC
UCC-178	UCC	UCC-232	UCC	UCC-329	UCC
UCC-189	UCC	UCC-236	UCC	UCC-333	UCC
UCC-191	UCC	UCC-238	UCC	UCC-337	UCC
UCC-194	UCC	UCC-239	UCC	UCC-357	UCC
UCC-199	UCC	UCC-241	UCC	UCC-361	UCC
UCC-200	UCC	UCC-242	UCC	UCC-216	UCC
UCC-204	UCC	UCC-243	UCC	UCC-220	UCC
UCC-206	UCC	UCC-244	UCC	UCC-221	UCC
UCC-209	UCC	UCC-247	UCC	UCC-275	UCC
UCC-211	UCC	UCC-253	UCC	UCC-288	UCC
UCC-212	UCC	UCC-274	UCC	UCC-290	UCC

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Table 5: *List of F_9 Recombinant Inbred Lines of Cowpea derived from Apagbaala x IT97K-499-35*

RIL	SOURCE	RIL	SOURCE	RIL	SOURCE
UCC-365	UCC	UCC-454	UCC	UCC-487	UCC
UCC-366	UCC	UCC-457	UCC	UCC-489	UCC
UCC-377	UCC	UCC-460	UCC	UCC-490	UCC
UCC-390	UCC	UCC-464	UCC	UCC-497	UCC
UCC-396	UCC	UCC-466	UCC	UCC-498	UCC
UCC-419	UCC	UCC-471	UCC	UCC-505	UCC
UCC-421	UCC	UCC-473	UCC	UCC-513	UCC
UCC-428	UCC	UCC-478	UCC	UCC-514	UCC
UCC-445	UCC	UCC-482	UCC	UCC-523	UCC
UCC-446	UCC	UCC-484	UCC		
UCC-448	UCC	UCC-486	UCC		

Screening of Parental Lines and RILs Using *Striga*-Resistant Specific SSR Markers

DNA isolation

The 3 parental lines of cowpeas together with GH3684 and 115 RILs were germinated in conical pots for 7 days. Young leaves of each of them were harvested and genomic DNA was extracted using modified CTAB protocol (Doyle and Doyle, 1987). The CTAB buffer was sterilized using 33µm Syringe Filter and it was pre-warmed to 60 ^{o}C using the Precision Water Bath 181. Approximately, 150 mg of young leaf tissue was added to 2 ml Eppendorf tubes with a metal bead and immediately placed in liquid nitrogen. Using the TissueLyser II, the tissue was pulverized. Β-mercaptoethanol (1.6 µl

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/1 ml buffer) was added to the CTAB buffer in the HAMILTON fume hood and 700µl of CTAB buffer was added and incubated at 60 °C in the water bath for 60 minutes, inverting the tubes at 15 minutes intervals throughout the incubation period. Two hundred microlitres (200 µl) of 5 M potassium acetate was added to each sample and incubated on ice for 20 minutes. Seven hundred microlitres (700 µl) of 24:1 chloroform isoamyl alcohol was then added to each sample in the fume hood and it was gently mixed and allowed to sit at room temperature for 5 minutes. The samples were then centrifuged for 10 minutes at 10000 rpm using the Eppendorf Centrifuge 5415D (manufactured by Sigma-Andrich, U.S.A). After the centrifuging, the supernatant was transferred to the new 1.5 µl tubes and 500 µl of cold isopropanol was added in the fume hood. The samples were then incubated at -20 °C for 30 minutes. It was then centrifuged again at 10,000 rpm for 10 minutes. The supernatant was carefully discarded not to disturb the pellet. Hundred microlitres (100 µl) of cold 70 % ethanol was then added to each sample and centrifuged at 10,000 rpm for 5 minutes. The ethanol was discarded and the pellets allowed to air dry until all ethanol evaporated. Finally, the DNA was resuspended in 200 µl 1× TE buffer. The DNA concentration was determined using the NANODROP 2000 Spectrophotometer (ThermoFisher Scientific, USA) (Appendix A). Working solutions of 50 ng/µl for each sample were prepared for downstream application.

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Polymerase Chain Reaction (PCR) analysis

Each PCR reaction mixture contained 2 µl of 1x *Taq* buffer, 0.5 µl of 200 µM dNTPs, 0.5µl of 1 unit *Taq* polymerase, 1µl each of 1 µM forward and reverse primers, 1 µl of 50 ng genomic DNA and 14 µl Molecular Grade Distilled water (MGDw) to make up a 20 µl total volume. The PCR amplifications were performed in BIO RAD T100^{TM} Thermal Cycler with conditions consisting of denaturation at 94 ^{o}C for 2 min, followed by 35 cycles of denaturation for 30 seconds, annealing at 55 ^{o}C for 30 seconds, extension at 72 ^{o}C for 1 min and ended with final extension at the same temperature for 5 min. The PCR products were resolved for 2 hrs at 140 V on 2 % (w/v) Agarose gel in 1× TAE buffer using a horizontal gel electrophoresis apparatus (Model 81-2325; Class II; Galileo Bioscience). The gels were stained with ethidium bromide, visualized on a CHROMATO-VUE using a Transilluminator (Model TL-33) and photo-documented using EOS REBEL T2i Utility. The size of the DNA bands in base pairs were determined using the 1 kb DNA standard ladder.

Simple Sequence Repeat markers

Four microsatellite and one SCAR markers associated with various races of *Striga gesnerioides* resistance were used. Sequences of primers used to amplify the DNA are listed in the Table 6.

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Table 6: *SSR Primers used to amplify markers associated with Striga gesnerioides resistance*

Primer		Primer Sequence (5'-3')	Annealing Temperature	*Striga* Race Specificity
SSR-1	Forward	cctaagcttttctccaactcca	55 °C	Embedded in RSG3-301 resistance gene
SSR-1	Reverse	caagaaggaggcgaagactg		
C42-2B	Forward	cagttccctaatggacaacc	60 °C	Linked to SG5
C42-2B	Reverse	caagctcatcatcatctcgatg		
LRR9	Forward	gttccataacatgctctgac	55 °C	Unknown
LRR9	Reverse	gctttctcaactctcatctctc		
LRR11	Forward	ggtagctcctctgttgattcag	55 °C	Unknown
LRR11	Reverse	catatgtccaaccattgccacag		
CLM1320	Forward	cacaacttgcaacaacatgc	55 °C	Unknown
CLM1320	Reverse	Tacgtggatctggtctttcc		

Gowda, and Timko (unpublished)

Hybridity Test in F_8 and F_9

After the extraction of DNA from the parental lines of cowpea, GH3684 and the RILs, they were subjected to PCR amplification to ascertain the presence or absence of the markers in the F_8 and F_9 progenies. After amplification, five markers were present and polymorphic [out of 17 markers tested (Appendix B)] in the genotypes between SARC-L02/IT97K-499-35 (Population one) and Apagbaala/IT97K-499-34 (Population two). These RILs were further tested in pots containing *Striga gesnerioides* seeds from Ghana designated as Ghana race (GH).

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Scoring of Bands From Agarose Gel Electrophoresis

The scoring of bands from agarose gel was done using the EOS REBEL T2i Utility. A 1000-bp DNA ladder from Invitrogen was used as a molecular weight size marker for each gel alongside the DNA samples from the RILs and the parental lines. Those that corresponded to the product size of the marker were scored present (+) and those below or above the molecular weight of the marker or no visible DNA band were scored absent (-).

Pot Culture Screening of Cowpea RILs, Parental Genotypes And The Local Landrace Against *Striga Gesnerioides* (Ghana race) and Race Three (SG3) Infestation

Pot experiments took place at the *Striga* Research Laboratory within the Timko Laboratory in U.S.A.

A fine sand was obtained by sieving a sandy-loam soil using a 250 micron sieve. This ensured that each sand particle was approximately the same size as *Striga* seed. The *Striga* inoculum was prepared using a ratio of 500 g of fine sand to 1 g of *Striga* seeds. A small and equal volume of *Striga* seeds and sand were mixed thoroughly and the procedure was repeated until all of the *Striga* seeds were mixed with an equal amount of sand. A volume of sand equal to the amount of mixture was added and mixed thoroughly. It was repeated until all the fine sand was used up. By mixing small amounts and gradually increasing the volume of the mixture, a homogenous mix was achieved. Each pot (diameter; 5.08 cm and length; 17.78 cm) was filled with sandy-loam soil to about 75 % full and 15 g of the inoculum was applied per pot. This gave an estimate of 1000 viable *Striga* seeds per pot. The pots were watered gently to precondition the *Striga* seeds for ten days.

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To ensure that each pot contained viable cowpea seeds, the seeds were pre germinated in Petri dishes after seeds were surface sterilized with 10 % bleach. The pre-germinated cowpea RILs and the parental genotypes (Apagbaala, SARC-LO2 and IT97K-499-35) including a local land race, GH3684 genotype were then transplanted to separate pots (3 seeds per pot) containing the *Striga* seeds arranged in Completely Randomised Design with four replications. Soil was then added to the pots until they were filled and then watered subsequently. Two weeks after the emergence of the cowpea seedlings, thinning out was done leaving two plants per pot. The pots were kept moist by watering as and when it became necessary.

Data collected from pot experiment

Plant height

The cowpea plant height (cm) was measured at the 6th week by taking the distance from the soil surface to the tip of the cowpea shoot using meter rule.

Striga height

The *Striga* height (cm) was measured three weeks after emergence as the distance from the soil surface to the tip of the shoot using a meter rule. *Striga* heights were scored nil for all RILs that showed attachment below the soil but did not emerge.

Striga attachment score

Destructive sampling was carried out ten weeks after planting. The plant-soil mass was removed from each pot, immersed into a bucket of water, and gently agitated to loosen the soil mass. The roots were washed thoroughly free of soil and examined using hand lens for presence of necrotic hypersensitive lesions, attachment of *Striga gesnerioides* and tubercles. Plants that supported

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attachment, healthy development and emergence of *Striga gesnerioides* were classified as susceptible and those that were free from infection, without any attachment were categorized as candidate resistant genotypes. Total number of *Striga* were counted (both emerged and submerged) by observation using hand lens.

Determination of the Distance Between the Genes Controlling *Striga* resistance in GH Race and SG3

Data from all the polymorphic markers were used to construct a genetic linkage map using QTL IciMapping (Meng, Li, Zhang & Wang, 2015) version 4.1. Five SSR markers were used to screen 115 RILs. Phenotypic data obtained from pot infestation of the population samples with GH race and SG3 were added to the genotypic data. Linkage grouping was performed at an LOD (logarithm of odds) score of 3.0 as a significant threshold. Recombination frequencies were converted into map distances in centi-Morgans (cM) using the Kosambi (1944) mapping function. 'Group' command was used to identify linkage groups and 'Order' command was used to establish the most-likely order within each linkage group, while the orders were confirmed by permuting all adjacent markers by the 'Ripple' function.

Multi-race *Striga* Resistance Status Assessment

Sixteen cowpea RILs with early maturing and medium to large seed sizes were tested against all known *Striga* races to ascertain their multi-race *Striga* resistance status. Pot inoculation, preconditioning of *Striga* seeds, and planting of the cowpea seeds were done following previously described procedure. The multi-*Striga*-race resistance test was also conducted at the Timko Laboratory, University of Virginia (UVA), Virginia, U.S.A.

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Data collected from the multi–Striga-race resistance status testing

Plant height, number of flowers, days to *Striga* emergence, *Striga* height, number of *Striga* and *Striga* attachment were scored and analyzed following the previous procedure. The different races of *Striga gesnerioides* and the selected RILs used in this study is presented in Table 7 and 8 respectively.

Table 7: *Races of Striga gesnerioides seeds used and their Sources*

***Striga* Race**	**Geographic Location**	**Source**
SG1	Burkina Faso	Timko Lab, UVa
SG2	Mali	Timko Lab, UVa
SG3	Nigeria and Niger	Timko Lab, UVa
SG4	Benin	Timko Lab, UVa
SG4z	Benin	Timko Lab, UVa
SG5	Cameroon	Timko Lab, UVa
SG6	Senegal	Timko Lab, UVa

Table 8: *Sixteen selected RILs, parental genotypes and the local land race*

RILs			**Parental genotypes**	**Local landrace**
UCC-11	UCC-221	UCC-466	IT97K-499-35	GH3684
UCC-24	UCC-241	UCC-473	SARC-LO2	
UCC-32	UCC-328	UCC-478	APAGBAALA	
UCC-86	UCC-366	UCC-513		
UCC-122	UCC-377			
UCC-153	UCC-445			

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Detection of Single Nucleotide Polymorphic (SNP) Markers Associated with Cowpea *Striga*-resistance and Seed Size

Phenotype data

Seed size was determined as weight per 100 seed, seed thickness, seed length, and seed width. The seed weight was measured with electronic weighing balance. The seed length, width and thickness were measured using electronic digital calipers (Appendix C). The F_8 progenies of cowpea which were used are presented in Table 4.

Genotype data

One hundred and sixty six (166) PCR based SNP markers (Appendix D) fairly distributed across the genome of the crop were tested for polymorphisms between the parents (IT97K-499-35 and SARC-LO2). Thirty-four of the SNP markers found to be polymorphic were used to analyze the genotypes of cowpea inbred lines. The linkage map locations were constructed based on the cowpea consensus map by Munoz et al. (2017).

The SNP marker information are stated in Table 9.

Polymerase Chain Reaction (PCR) analysis

Each PCR reaction mixture contained 2 µl of 1x PCR buffer, 0.5 µl of 200 µM dNTPs, 0.5 µl of 1 unit Taq polymerase, 1 µl of 1 µM of each primer, 1 µl of 50 ng genomic DNA, 0 µl to 1 µl of 1.5 -2.5 mM $MgCl_2$ and 12 µl to 13 µl (depending on the Magnesium chloride concentration) Molecular Grade Distilled water (MGDw) to make up a 20 µl total reaction volume. The PCR amplifications were performed in BIO RAD T100™ Thermal Cycler comprising of an initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation for 30 seconds, annealing at 45 °C to 60 °C for 30 seconds,

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Table 9: *Polymorphic SNP markers used to genotype RILs*

Marker Name	T_A (°C)	LG	Map Distance (cM)	Frangment Size (bp)
2_08868	55	1	49.56	163
2_16911	45	1	72.3	220
2_17191	55	2	75.04	174
2_14148	50	3	15.4	194
2_14205	55	3	125.6	167
2_49906	55	4	11.25	190
2_21262	55	5	25.41	151
2_02661	45	5	41.61	198
1_0018	55	6	30.95	143
2_02471	55	7	34.4	264
2_03317	55	8	0	144
2_19666	50	8	50.49	146
2_05151	55	8	61.42	197
2_19243	55	9	2.01	146
2_26050	55	9	15.61	179
2_00054	55	9	34.08	168
2_08679	55	9	51.16	177

*T_A = Annealing temperature; LG = Linkage group; cM = Centimorgan
Locus of each marker is based on the cowpea consensus map (Muñoz-Amatriaín et al., 2017).

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Table 9: Continued

Marker Name	T_A(°C)	LG	Map distance (cM)	Frangment Size (bp)
2_03702	60	9	57.02	162
2_24219	55	9	58.88	166
2_54689	55	9	60.3	97
2_21105	55	9	63.77	169
2_14455	55	9	65.44	170
2_16636	55	10	0.63	126
2_23117	50	10	15.14	200
2_05766	55	10	21.62	162
2_13064	55	11	1.41	165
2_21345	55	11	2.98	107
2_27951	55	11	20.14	173
2_08233	55	11	35.16	213
2_23898	55	11	37.87	187
2_05791	55	11	39.88	126
2_22012	55	11	42.35	170
2_16297	50	11	55.36	211
2_52960	55	11	59.6	218

*Ta = Annealing temperature; LG = Linkage group; cM = CentiMorgan

extension at 72 °C for 30 seconds and end with final extension at same temperature for 2 min. The PCR products were resolved for 3 hrs at 300 V on 6 % Polyacralamide gel (PAGE) in 0.5 × TBE buffer using a vertical gel electrophoresis apparatus (Model# DPC-5000). The gels were stained with ethidium bromide and the bands visualized with UV light. The size of the DNA bands in base pairs were determined using the 1 kb DNA standard ladder. The SNPs were scored as “A” if the RIL had a band length equal to IT97K-499-35 and “B” if the RIL had a band length equal to SARC-LO2. SNP scores were exported into QTL IciMapping for further processing.

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Marker-trait association

QTL IciMapping (Meng, Li, Zhang & Wang, 2015) version 4.1 was used to perform bi-parental mapping analysis using the Single Marker Analysis (SMA) program for seed size (based on 100 seed weight, seed thickness, seed length, and seed width) and *Striga* resistance data from population one. A SNP marker with an LOD score greater than 3.0 was considered to be significantly associated with the agronomic trait. The dendrogram was constructed using PowerMarker version 7 software based on five polymorphic markers with UPGMA tree method.

The quantitative data were subjected to analysis of variance (ANOVA) using Minitab version 17. Means were compared using Least Significant Difference (LSD) at 5% level of probability. Pearson's correlation coefficient was used to compute correlation between number of emerged *Striga* and number of cowpea flowers.

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CHAPTER FOUR

RESULTS

In this chapter, the results obtained from the experiments have been presented in full. Each result is accompanied by a brief description giving an account on the trend of the result.

SSR Markers Linked to *Striga gesnerioides* Resistance Among F_8 and F_9 Progenies

Out of 17 SSR markers screened (Appendix B), 27.4 % (5) polymorphic markers could distinguish the parental genotypes IT97K-499-35 and SARC-LO2. The SSR-1, C42-2B, CLM1320, LRR9 and LRR11 markers segregated with the *Striga*-resistant allele (s) and successfully discriminated 84 F_8 progenies of IT97K-499-35 and SARC-LO2 into *Striga*-resistant cowpeas and susceptible cowpeas. The presence of any of the five markers in a cowpea was an indication of the presence of the *Striga* resistant allele (s). The presence of a marker (+), denotes resistant and absence of a marker (-), denotes susceptible. The resolution of the five markers (SSR-1, C42-2B, CLM1320, LRR9 and LRR11) on agarose gel across the cowpea genome are illustrated in Figures 5-13. The product size of the five markers, SSR-1, C42-2B, CLM1320, LRR9 and LRR11 across the cowpea genome were 150 bp, 280 bp and 380 bp, 820 bp and 550 bp respectively. All DNA bands that corresponded to the product size of the markers indicate *Striga*-resistant cowpea genotypes.

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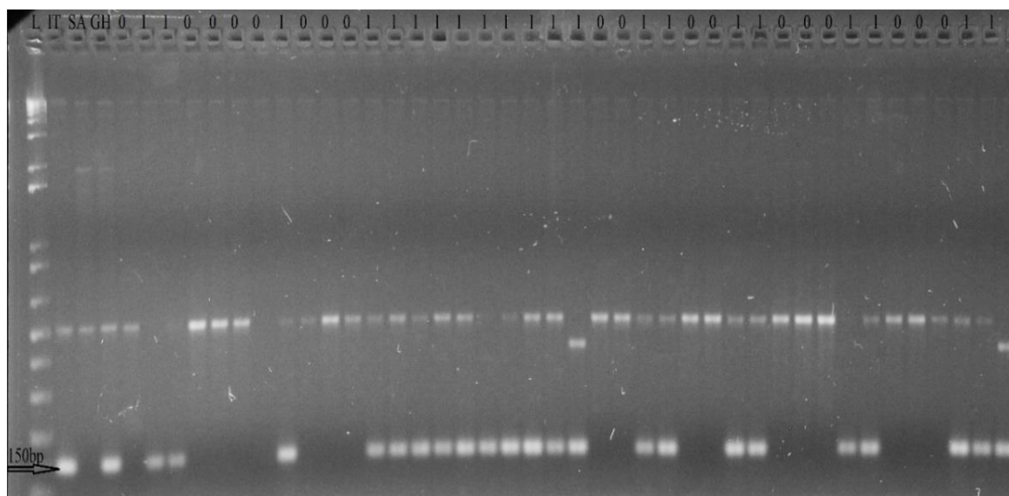

Figure 4: DNA bands from PCR amplification products of SSR-1 for some F_8 RILs of cowpea (Population 1) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 150bp DNA band indicates resistant cowpea genotype and absence of this band indicates susceptible genotype. L, IT, SA, GH represent the standard 1kb+ ladder, IT97K-499-35 (Resistant parent), SARC-LO2 (Susceptible parent), GH3684 (Local resistant check) respectively

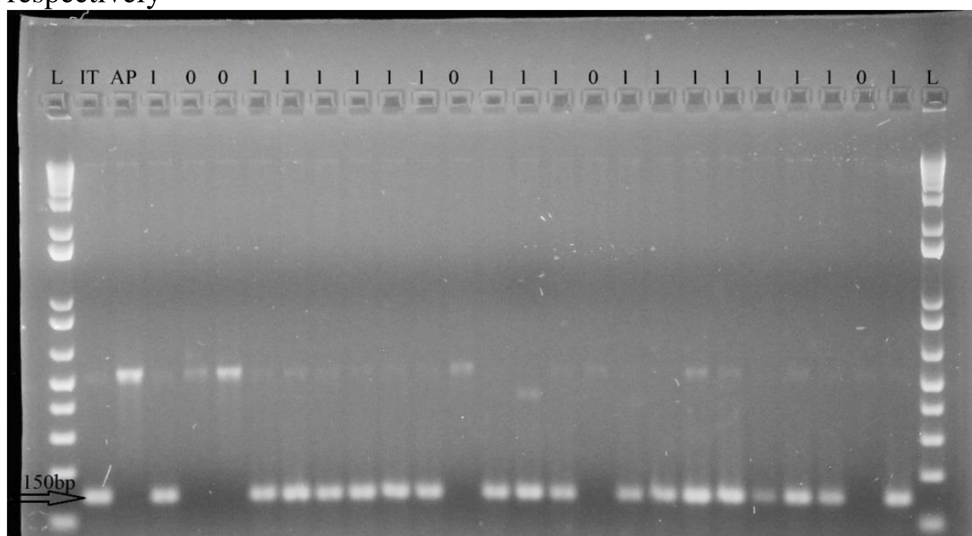

Figure 5: DNA bands from PCR amplification products of SSR-1 for some F_9 RILs of cowpea (Population 2) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 150bp DNA band indicates resistant genotype and absence of this band indicates susceptible genotype. L, IT, AP, represent the standard 1kb+ ladder, IT97K-499-35 (Resistant parent) and Apagbaala (Susceptible parent) respectively

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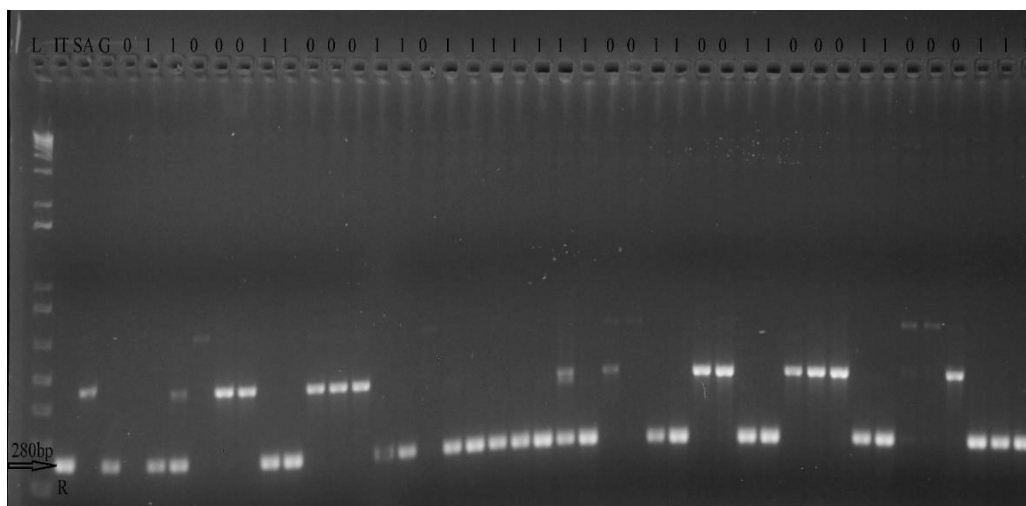

Figure 6: DNA bands from PCR amplification products of C42-2B for some F_9 RILs of cowpea (Population 1) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 280bp DNA band indicates resistant genotype and absence of this band indicates susceptible genotype. L, IT, SA, G and L represent and the standard 1kb+ ladder, IT97K-499-35 (Resistant parent), SARC-LO2 (Susceptible parent), GH3684 (Local resistant check) respectively

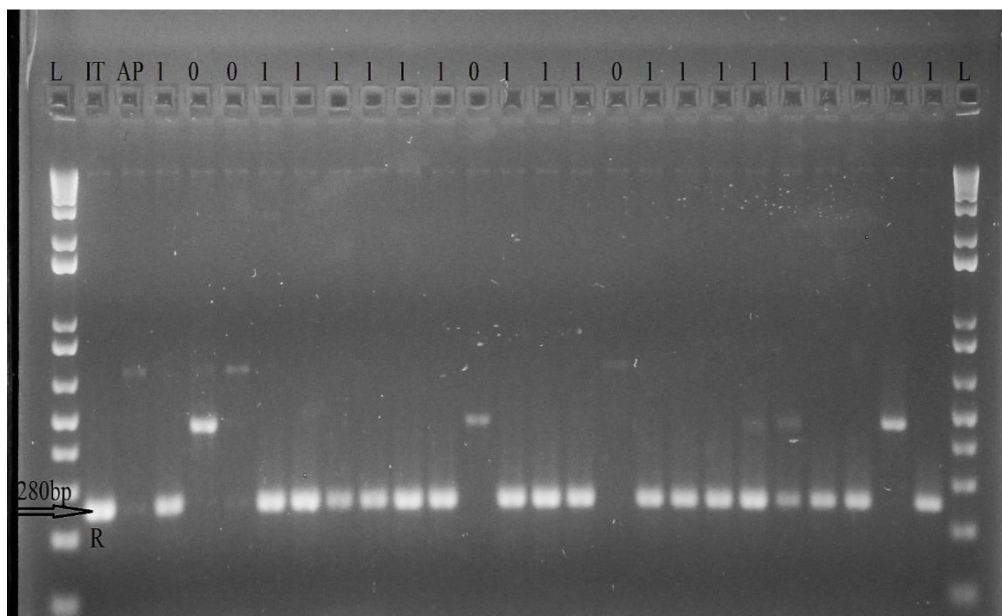

Figure 7: DNA bands from PCR amplification products of C42-2B for some F_8 RILs of cowpea (Population 2) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 280bp DNA band indicates resistant genotype and absence of this band indicates susceptible genotype. L, IT, AP, represent the standard 1kb+ ladder, IT97K-499-35 (Resistant parent), Apagbaala (Susceptible parent) respectively

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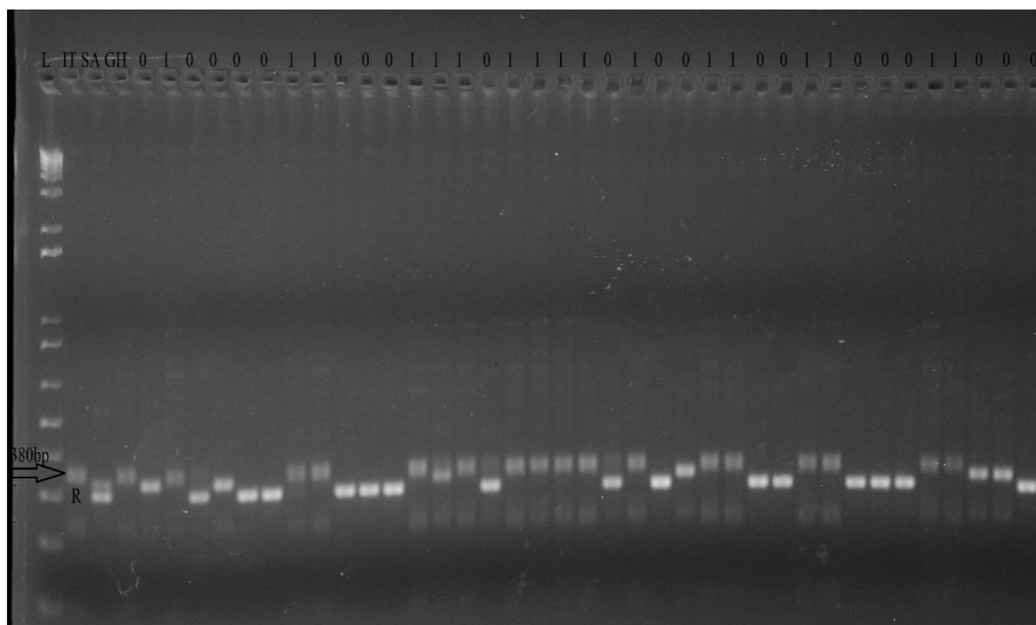

Figure 8: DNA bands from PCR amplification products of CLM1320 for some F_9 RILs of cowpea (Population 1) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 380bp DNA band indicates resistant genotype and absence of this band indicates susceptible genotype. L, IT, SA and G represent and the standard 1kb+ ladder, IT97K-499-35 (Resistant parent), SARC-LO2 (Susceptible parent), GH3684 (Local resistant check) respectively

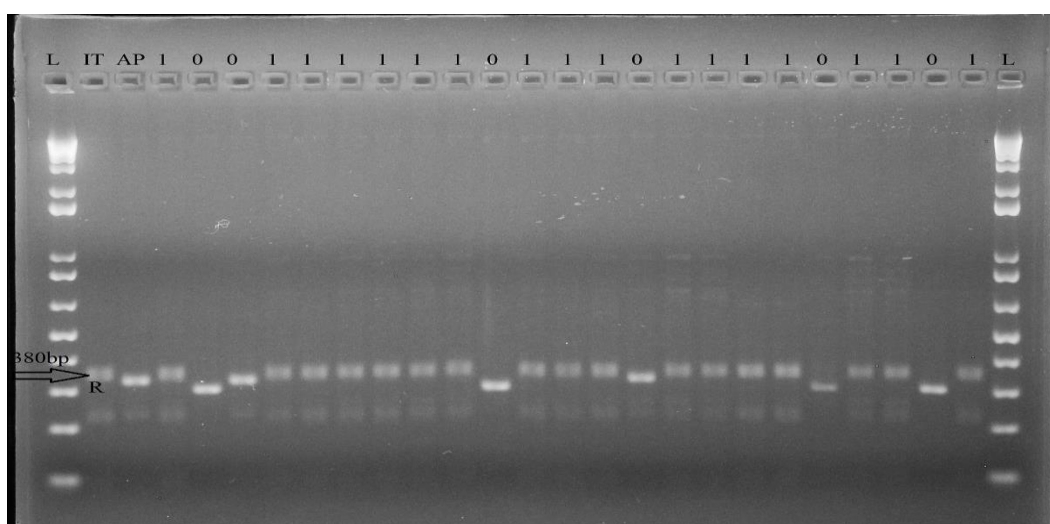

Figure 9: DNA bands from PCR amplification products of CLM1320 for some F_8 RILs of cowpea (Population 2) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 380bp DNA band indicates resistant genotype and absence of this band indicates susceptible genotype. L, IT and AP represent the standard 1kb+ ladder, IT97K-499-35 (Resistant parent), Apagbaala (Susceptible parent) respectively

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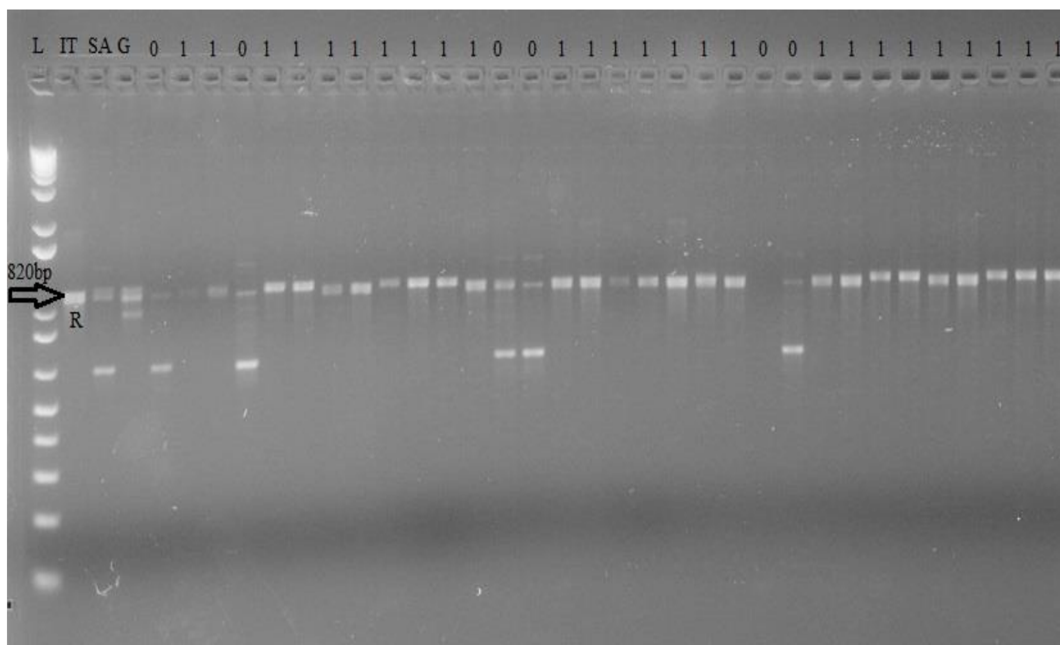

Figure 10: DNA bands from PCR amplification products of LRR9 for some F_9 RILs of cowpea (Population 1) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 820bp DNA band indicates resistant genotype and absence of this band indicates susceptible genotype. L, IT, SA and G represent the standard 1kb+ ladder, IT97K-499-35 (Resistant parent), SARC-LO2 (Susceptible parent), GH3684 (Local resistant check) respectively

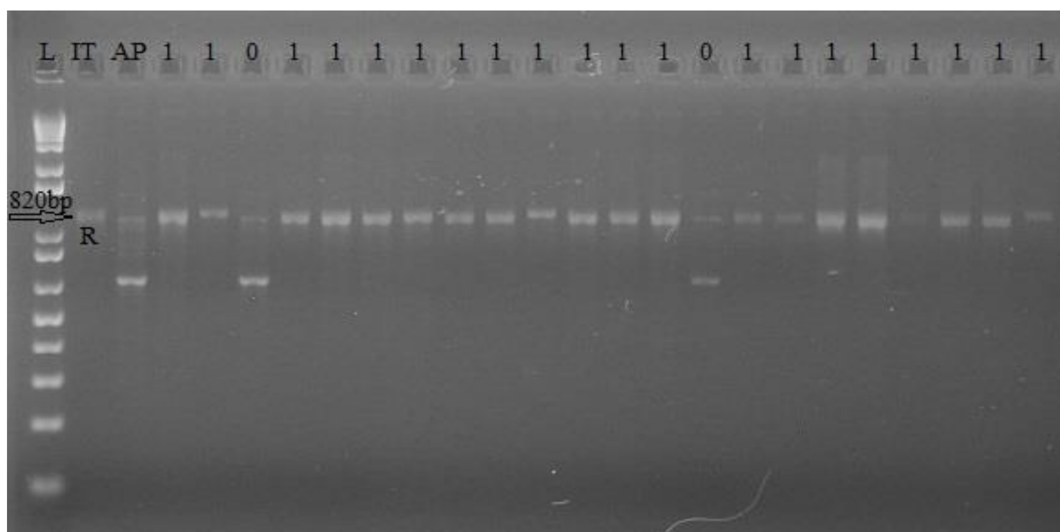

Figure 11: DNA bands from PCR amplification products of LRR9 for some F_8 RILs of cowpea (Population 2) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 820bp DNA band indicates resistant genotype and absence of this band indicates susceptible genotype. L, IT and AP represent the standard 1kb+ ladder, IT97K-499-35 (Resistant parent), Apagbaala (Susceptible parent) respectively

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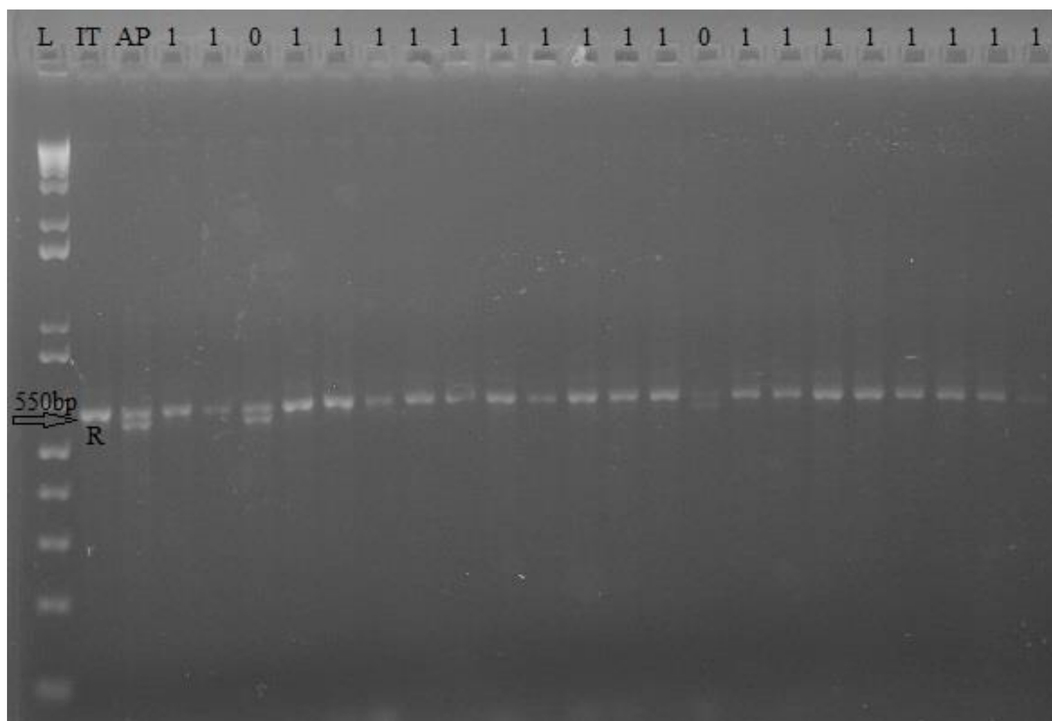

Figure 12: DNA bands from PCR amplification products of LRR11 for some F_8 RILs of cowpea (Population 2) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 580bp DNA band indicates resistant genotype and absence of this band indicates susceptible genotype. L, IT and AP represent the standard 1kb+ ladder, IT97K-499-35 (Resistant parent), Apagbaala (Susceptible parent) respectively

Based on the five markers used, the levels of polymorphism within the RILs were determined and are presented in Table 10 (population one) and Table 11 (population two). The marker LRR9 had the highest number of individuals (57 or 68 %) having it in population one. This was followed closely by SSR-1 (56 or 66.7 %), C42-2B (54 or 64.3 %), LRR11 (54 or 64.3 %) and CLM1320 (49 or 58.3 %). In population two, the marker LRR11 had the highest number of individuals (26 or 83.9 %) with this marker. This was also followed by LRR9 (24 or 77.4 %), SSR-1 (21 or 67.7 %), C42-2B (20 or 64.5 %) and CLM1320 (20 or 64.5 %).

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Table 10: *Polymorphism in F_8 progenies (Population 1) as revealed by the five markers*

Marker	Sample Size	Number of Individuals with the marker	Number of individuals without the marker
SSR-1	84	56	28
C42-2B	84	54	30
CLM1320	84	49	35
LRR9	84	57	27
LRR11	84	54	30

Table 11: *Polymorphism in F_9 progenies (Population 2) as revealed by the five markers*

Marker	Sample Size	Number of Individuals with the marker	Number of individuals without the marker
SSR-1	31	21	10
C42-2B	31	20	11
CLM1320	31	20	11
LRR9	31	24	7
LRR11	31	26	5

Cluster Analysis and Identification of F_8 (population 1) and F_9 (Population 2) Individuals with Marker

To identify the number of markers each of the F8 and F9 individuals possesses, a cluster analysis was performed. It was expected that those that had similar genotypes with respect to the presence or absence of a given marker combination would be clustered together.

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Cluster analysis of F_8 progeny of a cross between IT97K-499-35 and SARC LO2 (Population 1)

A dendrogram constructed using the combined data of all five polymorphic markers delineated the 84 F_8 progenies into twelve clusters I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and XII (Figure 14). The twelve clusters had varied proportions of cowpea genotypes (Table 12).

Table 12: *Marker combinations of the twelve clusters in population 1*

Cluster	Markers	Frequency of genotypes
I	SSR-1, C42-2B, CLM1320, LRR9 and LRR11	39
II	SSR-1, C42-2B, LRR9, and LRR11	5
III	SSR-1, C42-2B, CLM1320, and LRR9	5
IV	SSR-1, C42-2B, CLM1320, and LRR11	3
V	SSR-1, CLM1320, and LRR11	1
VI	SSR-1, CLM1320, and LRR9	1
VII	SSR-1, C42-2B, and LRR11	1
VIII	LRR9 and LRR11	4
IX	SSR-1 and C42-2B	1
X	LRR11	1
XI	LRR9	3
XII	None	20

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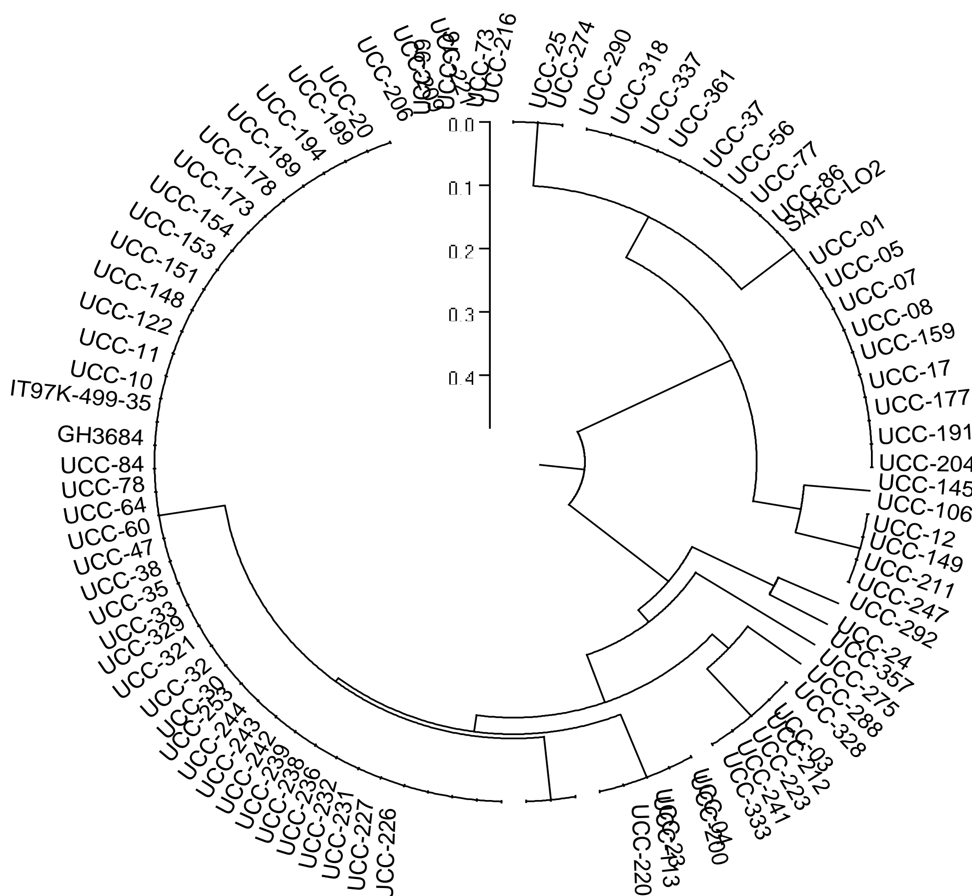

Figure 13: A dendrogram of 84 F_8 progenies (population 1) constructed from PowerMarker using five polymorphic markers with UPGMA tree method.

Cluster analysis of F_9 progeny of a cross between IT97K-499-35 and Apagbaala (Population 2)

A dendrogram constructed using the combined data of all five polymorphic markers delineated the 31 F_9 progenies into nine clusters I, II, III, IV, V, VI, VII, VIII, and IX (figure 15) The nine major clusters were associated with varied genotypes of cowpea as shown in Table 13.

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Table 13: *Marker combinations of the twelve clusters in population 2*

Clusters	Markers	Number of Individuals
I	SSR-1, C42-2B, CLM1320 and LRR11	1
II	SSR-1, CLM1320 and LRR11	1
III	SSR-1, CLM1320, LRR9, and LRR11	1
IV	SSR-1, C42-2B, LRR9 and LRR11	1
V	SSR-1, C42-2B, CLM1320, LRR9 and LRR11	17
VI	LRR9	1
VII	LRR9 and LRR11	4
VIII	C42-2B and LRR11	1
IX	None	4

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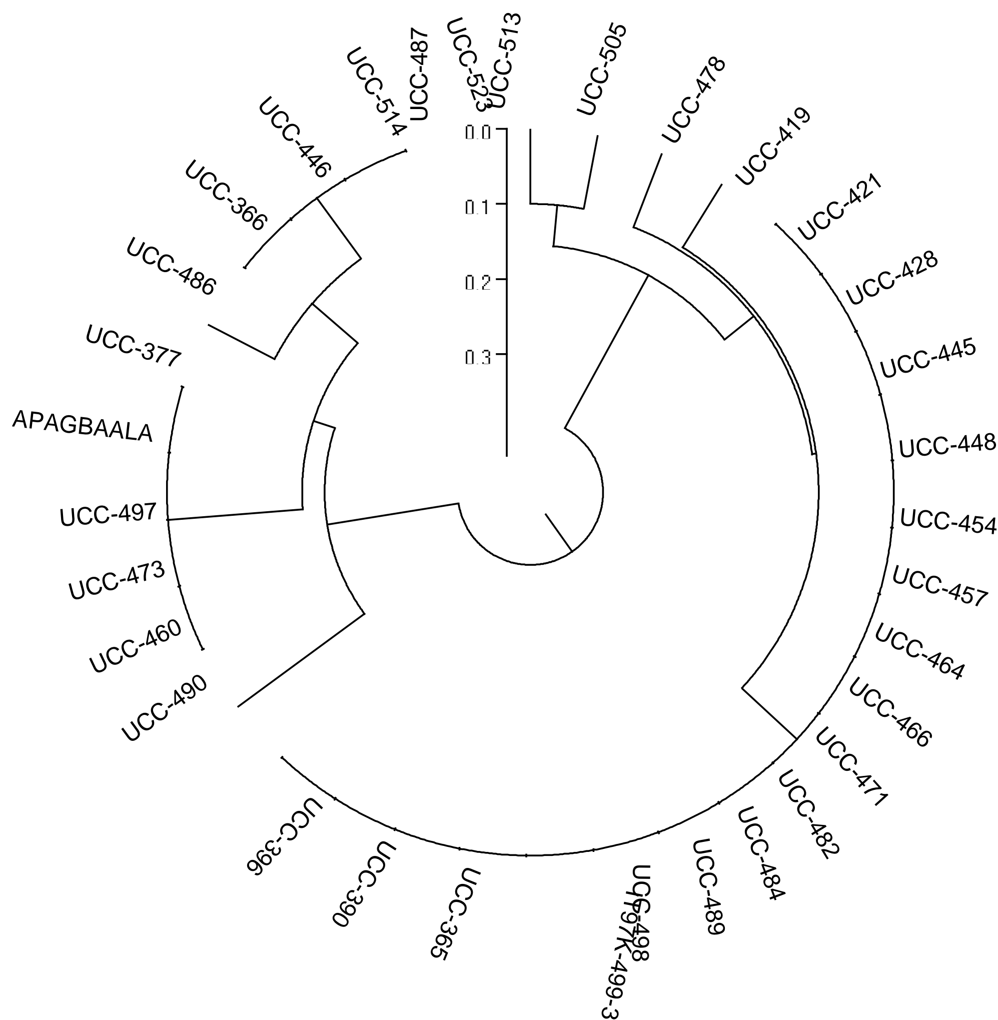

Figure 14: A dendrogram of 31 F_9 progenies (population two) constructed from PowerMarker using five polymorphic markers with UPGMA tree method.

Allele Frequency, Segregation Ratio, Gene Diversity and Polymorphism Information Content

The major allele frequencies for the five markers within the F_8 population (Cross between IT97K-499-35 and SARC-LO2) are given Table 14. The major allele frequencies yielded by the five markers ranged from 0.5833 to 0.6786 with the mean of 0.6429 and the mean number of allele was two. The gene diversity was low ranging from 0.4362 – 0.4861 with the mean of 0.4570. Based on the genetic diversity, each locus for allelic polymorphism

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information content (PIC) was calculated and the values ranged from 0.3411 to 0.3680 with the mean of 0.3524 (Table 14)

The major allele frequency yielded by the five markers in population 2 ranged from 0.6452 to 0.8387 with the mean of 0.7161 (Table 15), which was higher than that obtained in population one. The gene diversity ranged from 0.2706 to 0.4570 with a mean of 0.3946. The polymorphic information content also recorded a range from 0.2340 to 0.3530 with an average of 0.3140. There was strong negative correlation (-0.992) between the allele frequency and PIC (Table 15).

The average segregation ratios from all the five primers in population one was 1:2 and 1:3 in population two. However, the marker CLM1320 segregated in 1:1 ratio in population one while the marker C42-2B and CLM1320 also segregated in 1:1 ratio in population two (Table 14 and 15).

Table 14: *Major Allele Frequency, Segregation ratio, Gene Diversity and PIC of the Five Markers used in Population one (Cross between SARC-LO2 and IT97K-499-35)*

Marker	Major Allele Frequency	SR	Sample Size	Allele Number	Gene Diversity	PIC
SSR-1	0.6670	1:2	84	2	0.4444	0.3457
C42-2B	0.6429	1:2	84	2	0.4592	0.3538
CLM1320	0.5833	1:1	84	2	0.4861	0.3680
LRR11	0.6429	1:2	84	2	0.4592	0.3538
LRR9	0.6786	1:2	84	2	0.4362	0.3411
MEAN	**0.6429**	**1:2**	**84**	**2**	**0.4570**	**0.3524**

*PIC = Polymorphism Information Content; S.R = Segregation ratio

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Table 15: *Major Allele Frequency, Segregation Ratio, Gene Diversity and PIC of the Five Markers used in Population two (Cross between Apagbaala and IT97K-499-35)*

Marker	Major Allele Frequency	S.R	Sample Size	Allele Number	Gene Diversity	PIC
SSR-1	0.6774	1:2	31	2	0.4370	0.3415
C42-2B	0.6452	1:1	31	2	0.4579	0.3530
CLM1320	0.6452	1:1	31	2	0.4579	0.3530
LRR11	0.8387	1:5	31	2	0.2706	0.2340
LRR9	0.7742	1:5	31	2	0.3496	0.2885
MEAN	**0.7161**	**1:3**	**31**	**2**	**0.3946**	**0.3140**

*PIC = Polymorphism Information Content; S.R = Segregation ratio

Responses of the Recombinant Inbred Lines (RILs) to *Striga gesnerioides* GH Race and SG3 Infestation

Pot tests determined the reactions of the RILs against the *Striga* race from Ghana and the most devastating *Striga* race (SG3) from Nigeria. In all 86.9 % of population one RILs were resistant to both *Striga* from Ghana and SG3 of Nigeria within population 1, only 7.14 % (6 RIls) comprising UCC-25, UCC-77, UCC-86, UCC-204, UCC-247 and UCC-274 were found to be resistant to the *Striga* Race from Ghana but susceptible to SG3. Similarly, only 7.14 % (6 RILs) made up of UCC-04, UCC-47, UCC-64, UCC-189, UCC-232 and UCC-292 were also found to be resistant to SG3 but susceptible to the Ghana race (Table 16). In population 2, 12.9 % (UCC-366, UCC-446, UCC-460 and UCC-490) were found to be resistant to the Ghana race but susceptible to the SG3 and another 9.67 % (UCC-471, UCC-484 and UCC-489) were resistant to SG3 but susceptible to the Ghana race (Table 17).

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Table 16: *Reactions of cowpea RILs derived from a cross between IT97K-499-35 and SARC-LO2 (population one) to Striga generioides infection*

Genotype	Genotypic Reaction					Phenotypic Reaction	
	SSR-1	C42-2B	CLM1320	LRR11	LRR9	(GH)	(SG3)
IT97K-499-35	+	+	+	+	+	R	R
SARC-LO2	-	-	-	-	-	S	S
GH3684	+	+	+	+	+	R	R
UCC-01	-	-	-	-	-	S	S
UCC-03	+	+	+	-	+	R	R
UCC-04	+	+	-	+	+	S	R
UCC-05	-	-	-	-	-	S	S
UCC-07	-	-	-	-	-	S	S
UCC-08	-	-	-	-	-	S	S
UCC-10	+	+	+	+	+	R	R
UCC-11	+	+	+	+	+	R	R
UCC-12	-	-	-	+	+	S	S
UCC-16	-	-	-	-	+	S	S
UCC-17	-	-	-	-	-	S	S
UCC-20	+	+	+	+	+	R	R
UCC-23	+	+	+	+	-	R	R
UCC-24	+	-	+	+	-	R	R
UCC-25	-	-	-	-	-	R	S
UCC-30	+	+	+	+	+	R	R
UCC-32	+	+	+	+	+	R	R

R: Resistant, S: Susceptible, +: Presence of marker, - : Absence of marker

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Table 16 Continued

Genotype	Genotypic Reaction					Phenotypic Reaction	
	SSR-1	C42-2B	CLM1320	LRR11	LRR9	(GH)	(SG3)
UCC-33	+	+	+	+	+	R	R
UCC-35	+	+	+	+	+	R	R
UCC-37	-	-	-	-	-	S	S
UCC-38	+	+	+	+	+	R	R
UCC-47	+	+	-	+	+	S	R
UCC-56	-	-	-	-	-	S	S
UCC-60	+	+	+	+	+	R	R
UCC-64	+	+	+	+	+	S	R
UCC-73	-	-	-	-	+	S	S
UCC-77	-	-	-	-	-	R	S
UCC-78	+	+	+	+	+	R	R
UCC-84	+	+	+	+	+	R	R
UCC-86	-	-	-	-	-	R	S
UCC-99	-	-	-	-	+	S	S
UCC-106	-	-	-	+	+	S	S
UCC-113	+	+	+	+	-	R	R
UCC-122	+	+	+	+	+	R	R
UCC-145	-	-	-	+	-	S	S
UCC-148	+	+	+	+	+	R	R

R: Resistant, S: Susceptible, +: Presence of marker, - : Absence of marker

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Table 16 Continued

Genotype	Genotypic Reaction					Phenotypic Reaction	
	SSR-1	C42-2B	CLM1320	LRR11	LRR9	(GH)	(SG3)
UCC-149	-	-	-	+	+	S	S
UCC-151	+	+	+	+	+	R	R
UCC-153	+	+	+	+	+	R	R
UCC-154	+	+	+	+	+	R	R
UCC-159	-	-	-	-	-	S	S
UCC-173	+	+	+	+	+	R	R
UCC-177	-	-	-	-	-	S	S
UCC-178	+	+	+	+	+	R	R
UCC-189	+	+	+	+	+	S	R
UCC-191	-	-	-	-	-	S	S
UCC-194	+	+	+	+	+	R	R
UCC-199	+	+	+	+	+	R	R
UCC-200	+	+	-	+	+	R	R
UCC-204	-	-	-	-	-	R	S
UCC-206	+	+	+	+	+	R	R
UCC-209	+	+	+	+	+	R	R
UCC-211	-	-	-	+	+	S	S
UCC-212	+	+	+	-	+	R	R
UCC-216	-	-	-	-	-	S	S
UCC-220	+	+	+	+	-	R	R

R: Resistant, S: Susceptible, +: Presence of marker, - : Absence of marker
Table 16 Continued

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Genotype	Genotypic Reaction					Phenotypic Reaction	
	SSR-1	C42-2B	CLM1320	LRR11	LRR9	(GH)	(SG3)
UCC-221	+	+	+	+	+	R	R
UCC-223	+	+	-	+	+	R	R
UCC-226	+	+	+	+	+	R	R
UCC-227	+	+	+	+	+	R	R
UCC-231	+	+	+	+	+	R	R
UCC-232	+	+	+	+	+	S	R
UCC-236	+	+	+	+	+	R	R
UCC-238	+	+	+	+	+	R	R
UCC-239	+	+	+	+	+	R	R
UCC-241	+	+	-	+	+	R	R
UCC-242	+	+	+	+	+	R	R
UCC-243	+	+	+	+	+	R	R
UCC-244	+	+	+	+	+	R	R
UCC-247	-	+	-	-	-	R	S
UCC-253	+	+	+	+	+	R	R
UCC-274	-	-	-	-	-	R	S
UCC-275	+	+	+	-	+	R	R
UCC-288	+	+	+	-	+	R	R
UCC-290	-	-	-	-	-	S	S
UCC-292	+	+	-	+	-	S	R

R: Resistant, S: Susceptible, +: Presence of marker, - : Absence of marker
Table 16 Continued

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Genotype	Genotypic Reaction					Phenotypic Reaction	
	SSR-1	C42-2B	CLM1320	LRR11	LRR9	(GH)	(SG3)
UCC-318	-	-	-	-	-	S	S
UCC-321	+	+	+	+	+	R	R
UCC-328	+	+	+	-	+	R	R
UCC-329	+	+	+	+	+	R	R
UCC-333	-	+	-	-	+	S	S
UCC-337	-	-	-	-	-	S	S
UCC-357	+	-	+	+	+	R	R
UCC-361	-	-	-	-	-	S	S

Table 17: *Reactions of cowpea RILs derived from a cross between IT97K-499-35 and Apagbaala (population two) to Striga generioides infestation*

Genotype	Genotypic Reaction					Phenotypic Reaction	
	SSR-1	C42-2B	CLM1320	LRR11	LRR9	GH	SG3
IT97K-499-35	+	+	+	+	+	R	R
APAGBAALA	-	-	-	-	-	S	S
UCC-365	+	+	+	+	+	R	R
UCC-366	-	-	-	+	+	R	S
UCC-377	-	-	-	-	-	S	S
UCC-390	+	+	+	+	+	R	R
UCC-396	+	+	+	+	+	R	R

R: Resistant, S: Susceptible, +: Presence of marker, - : Absence of marker

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Table 17 continued

Genotype	Genotypic Reaction					Phenotypic Reaction	
	SSR-1	C42-2B	CLM1320	LRR11	LRR9	GH	SG3
UCC-419	+	+	+	+	+	R	R
UCC-421	+	+	+	+	+	R	R
UCC-428	+	+	+	+	+	R	R
UCC-445	+	+	+	+	+	R	R
UCC-446	-	-	-	+	+	R	S
UCC-448	+	+	+	+	+	R	R
UCC-454	+	+	+	+	+	R	R
UCC-457	+	+	+	+	+	R	R
UCC-460	-	-	-	-	-	R	S
UCC-464	+	+	+	+	+	R	R
UCC-466	+	+	+	+	+	R	R
UCC-471	+	+	+	+	+	S	R
UCC-473	-	-	-	+	-	S	S
UCC-478	+	+	-	+	+	R	R
UCC-482	+	+	+	+	+	R	R
UCC-484	+	+	+	-	+	S	R
UCC-486	-	-	-	-	+	S	S
UCC-487	+	+	+	+	-	R	R
UCC-489	+	+	+	+	+	S	R
UCC-490	-	+	-	+	-	R	S
UCC-497	-	-	-	-	-	S	S

R: Resistant, S: Susceptible, +: Presence of marker, - : Absence of marker

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Table 17 continued

Genotype	Genotypic Reaction					Phenotypic Reaction	
	SSR-1	C42-2B	CLM1320	LRR11	LRR9	GH	SG3
UCC-498	+	+	+	+	+	R	R
UCC-505	+	-	+	+	+	R	R
UCC-513	+	-	+	+	-	R	R
UCC-514	-	-	-	+	+	S	S
UCC-523	-	-	-	+	+	*	S

R: Resistant, S: Susceptible, +: Presence of marker, - : Absence of marker

Within population 1, 58.33 % (49 RILs) showed resistance to both SG3 and the Ghana race while 24.69 % (20) showed susceptibility to both races. In population 2, 58.06 % (18) of the RILs showed resistance to both races and 16.12 % (5) were susceptible to both races.

Effects of *Striga* Infestation on Cowpea

Analysis of variance showed that significant difference ($P \leq 0.05$) existed between pod formations of the susceptible and resistant RILs (Table 20). Similarly, there was a significant difference ($P \leq 0.05$) between flowering and *Striga gesnerioides* emergence with a negative correlation (-0.221). There was negative correlation (-0.225) between the number of *Striga* and number of pods (Appendix E). Table 18 and 19 show the low growth characteristics (number of flowers and number of pod) recorded in this experiment.

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Table 18: *Average Plant Height, Number of Flowers, Number of Pods, Number of Striga Attachment and Height of Striga in population 1*

Population 1	No. of Flowers	No. of pods	No. of *Striga*
IT97K-499-35	3	2	0
SARC-LO2	2	1	3
GH3684	4	3	0
UCC-01	3	0	3
UCC-03	4	1	0
UCC-04	6	2	5
UCC-05	0	0	4
UCC-07	1	0	10
UCC-08	4	1	2
UCC-10	2	0	0
UCC-11	7	2	0
UCC-12	4	0	1
UCC-16	1	0	10
UCC-17	6	3	3
UCC-20	4	2	0
UCC-23	0	0	0
UCC-24	8	4	0
UCC-25	1	0	0
UCC-30	5	2	0
UCC-32	7	3	0
UCC-33	3	1	0
UCC-35	4	2	0

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Table 18 Cont'd

Population 1	No. of Flowers	No. of pods	No. of *Striga*
UCC-37	2	0	2
UCC-38	4	1	0
UCC-47	5	3	4
UCC-56	1	0	8
UCC-60	2	1	0
UCC-64	6	2	1
UCC-73	3	1	1
UCC-77	2	1	0
UCC-78	5	3	0
UCC-84	3	1	0
UCC-86	0	0	0
UCC-99	5	1	7
UCC-106	0	0	3
UCC-113	1	1	0
UCC-122	6	4	0
UCC-145	4	1	1
UCC-148	0	0	0
UCC-149	4	3	4
UCC-151	7	3	0
UCC-153	5	3	0

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Table 18 Continued

Population 1	No. of Flowers	No. of pods	No. of *Striga*
UCC-154	0	0	0
UCC-159	3	1	2
UCC-173	5	2	0
UCC-177	0	0	13
UCC-178	6	4	0
UCC-189	1	0	3
UCC-191	0	0	15
UCC-194	8	4	0
UCC-199	4	2	0
UCC-200	6	2	0
UCC-204	3	1	0
UCC-206	3	2	0
UCC-209	2	1	0
UCC-211	2	0	1
UCC-212	5	3	0
UCC-216	0	0	17
UCC-220	4	3	0
UCC-221	7	3	0
UCC-223	1	1	0
UCC-226	1	0	0
UCC-227	0	0	0
UCC-231	0	0	0
UCC-232	0	0	4
UCC-236	2	1	0

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Table 18 Continued

Population 1	No. of Flowers	No. of pods	No. of *Striga*
UCC-238	0	0	0
UCC-239	3	0	0
UCC-241	6	2	0
UCC-242	0	0	0
UCC-243	0	0	0
UCC-244	0	0	0
UCC-247	0	0	0
UCC-253	0	0	0
UCC-274	2	0	0
UCC-275	0	0	0
UCC-288	0	0	0
UCC-290	1	1	3
UCC-292	2	1	6
UCC-318	2	2	5
UCC-321	0	0	0
UCC-328	6	4	0
UCC-329	0	0	0
UCC-333	0	0	1
UCC-337	0	0	1
UCC-357	0	0	0
UCC-361	3	1	4

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Table 19: *Average Plant Height, Number of Flowers, Number of Pods, Number of Striga Attachment and Height of Striga in population two*

POP 2	No. of Flowers	No. of pods	No. of *Striga*
IT97K-499-35	4	2	0
APAGBAALA	5	2	5
UCC-365	0	0	0
UCC-366	5	3	0
UCC-377	2	1	7
UCC-390	0	0	0
UCC-396	0	0	0
UCC-419	0	0	0
UCC-421	0	0	0
UCC-428	0	0	0
UCC-445	7	4	0
UCC-446	3	1	0
UCC-448	0	0	0
UCC-454	0	0	0
UCC-457	0	0	0
UCC-460	0	0	0
UCC-464	0	0	0
UCC-466	6	3	0
UCC-471	0	0	4
UCC-473	0	0	3

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Table 19 continued

POP 2	No. of Flowers	No. of pods	No. of *Striga*
UCC-478	8	4	0
UCC-482	0	0	0
UCC-484	3	2	3
UCC-486	0	0	5
UCC-487	0	0	0
UCC-489	0	0	6
UCC-490	0	0	0
UCC-497	0	0	2
UCC-498	0	0	0
UCC-505	0	0	0
UCC-513	5	2	0
UCC-514	0	0	4
UCC-524	*	*	*

Table 18 shows mean pod formations between resistant and susceptible RILs. There was a significant difference ($P \leq 0.05$) between mean pod formations of susceptible and resistant RILs with an L.S.D score of 0.555. The resistant RILs had a mean pod formation of 1.339 as compared to the susceptible RILs (0.774). Pearson correlation analysis indicated that there was significant negative correlation (-0.225) between pod formation and number of emerged *Striga gesnerioides*. A negative correlation (-0.221) was again recorded between number of flowers and number of emerged *Striga gesnerioides*.

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Table 20: *Mean pod formation of resistant and susceptible RILs*

Types of genotypes	Mean pod formation
Resistant genotypes	1.339
Susceptible genotypes	0.774
L.S.D	0.555
P	0.046

Distances between Genes Controlling *Striga resistance* in Cowpea

Linkage map construction analysis revealed that all the *Striga*-resistant markers and genes were linked spanning a total length of 34.29 cM. The CLM1320 marker was closest at 8.0 cM to the genes controlling resistance to GH race. The gene controlling the SG3 resistance is located just 12.60 cM away from the genes controlling resistance in the GH race. SSR-1 marker was located at the same position (12.60 cM) as SG3 (Appendix F).The marker LRR9 was found to be the farthest located from the GH race resistance gene. Table 21 gives detail positions of the various markers used in this study on chromosome nine.

Table 21: *Linkage Map Construction output*

Marker name	**Chromosome**	**Position (cM)**
GH Race	9	0.00
CLM1320	9	8.85
SSR-1	9	12.60
SG3	9	12.60
C42-2B	9	14.87
LRR11	9	24.26
LRR9	9	34.29

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Differential Response of Some Cowpea Genotypes to Races of *Striga gesnerioides* Infestation

The multi-*Striga* race resistance test for the selected 16 RILs, 3 parental genotypes and GH3684 confirmed local susceptible parent Apagbaala to be susceptible to all the 7 known races of *Striga* in West Africa similar to that of Ghana. In contrast, the local resistant check, GH3684 showed resistance to all the 7 races of *Striga gesnerioides* in West Africa similar to that of Ghana. SARC-LO2 which was susceptible to local *Striga* race in Ghana (GH race) however showed resistance to races SG2, SG4z, SG5, and SG6. IT97K-499-35 showed resistance to all races but susceptible to SG4z (Table 21). On the whole, 77.8% of the F_8 progenies derived from IT97K-499-35 and SARC-LO2 which comprises UCC-11, UCC-24, UCC-32, UCC-122, UCC-221, UCC-241 and UCC-328 were resistant to all the 7 known races of *Striga gesnerioides* in West Africa including that of Ghana (Table 22). Besides, 18.8 % of the cowpeas, made up of UCC-153, UCC-478 and UCC-513 expressed resistance to all the *Striga* races except that of SG4z. The remaining RILs were resistant to 2-5 races of *Striga*.

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Table 22: *Reaction of the Selected Cowpea RILs and Parental Genotypes to Different races of Striga gesnerioides*

RIL	GH Race	SG1	SG2	SG3	SG4	SG4z	SG5	SG6
IT97K-499-35	R	R	R	R	R	S	R	R
SARC-LO2	S	S	R	S	S	R	R	R
APAGBAALA	S	S	S	S	S	S	S	S
GH3684	R	R	R	R	R	R	R	R
UCC-11	R	R	R	R	R	R	R	R
UCC-24	R	R	R	R	R	R	R	R
UCC-32	R	R	R	R	R	R	R	R
UCC-86	R	S	R	S	R	R	R	R
UCC-122	R	R	R	R	R	R	R	R
UCC-153	R	R	R	R	R	S	R	R
UCC-221	R	R	R	R	R	R	R	R
UCC-241	R	R	R	R	R	R	R	R
UCC-328	R	R	R	R	R	R	R	R
UCC-366	R	R	R	S	S	S	R	S
UCC-377	S	S	R	S	S	S	R	S
UCC-445	R	R	R	R	R	S	R	R
UCC-466	R	S	R	S	R	S	S	R
UCC-473	S	R	R	S	S	S	R	R
UCC-478	R	R	R	R	R	S	R	R
UCC-513	R	R	R	R	R	S	R	R

*R= resistant, S= susceptible

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Figure 15: Apagbaala showing heavy *Striga* infestation. Arrows pointing to emerged *Striga gesnerioides*

Figure 16: IT97K-499-35 showing susceptibility to SG4z. Arrows pointing to emerged *Striga* race SG4z

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Figure 17: Arrow pointing to *Striga* shoots attached to the roots of susceptible cowpea RILs

Figure 18: Resistant cowpea RILs showing no *Striga* infestation

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SNP Marker Association in F8 RIL Population (a cross between IT97K-499-35 and SARC-LO2

All RILs with the same band length as IT97K-499-35 were called as “A” and those with the same band length as SARC-LO2 as “B” (Figure 20-22).

Figure 19: DNA bands from PCR amplification products of 2_16636 for some F_8 RILs of cowpea (Population 1) resolved in 6 % Polyacralamide gel stained with ethidium bromide. A and B represent alleles from IT97K-499-35 and SARC-LO2 respectively. L represent 1000Kb ladder.

Figure 20: DNA bands from PCR amplification products of 1_0018 for some F_8 RILs of cowpea (Population 1) resolved in 6 % Polyacrylamide gel stained with ethidium bromide. A and B represent alleles from IT97K-499-35 and SARC-LO2 respectively. L represent 1000Kb ladder.

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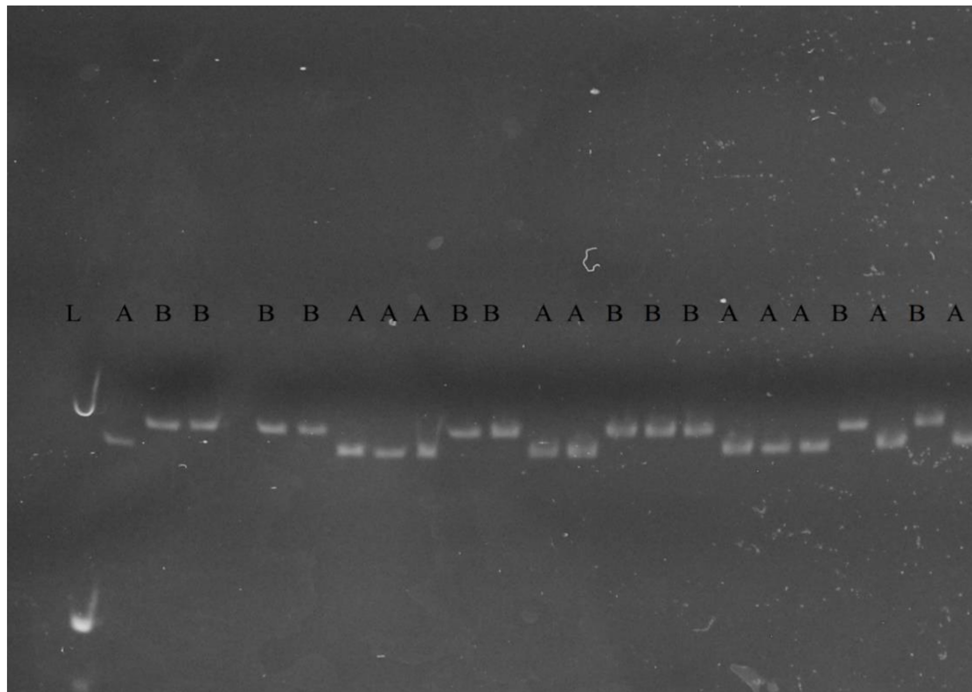

Figure 21: DNA bands from PCR amplification products of 1_0018 for some F_8 RILs of cowpea (Population 1) resolved in 6 % Polyacralamide gel stained with ethidium bromide. A and B represent alleles from IT97K-499-35 and SARC-LO2 respectively. L represent 1000Kb ladder.

From Table 23, the marker 2_21345 was linked with both 100 seed weight and seed length with LODs of 3.0508 and 3.0262 respectively. Five other markers (2_08679, 2_03702, 2_24219, 2_54689 and 2_21105 were linked with *Striga gesnerioides* resistances. Figure 23 shows significant SNP markers mapped onto chromosome.

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Table 23: *QTL mapping in biparental population output showing significant SNP marker linked with 100 Seed Weight (s.w) and Seed Length*

Trait name	Chromosome	Position	Marker name	LOD	PVE (%)	Add
100 S.W	11	2.980	2_21345	3.0508	16.13	-1.23
Seed length	11	2.980	2_21345	3.0262	16.01	-0.03
Striga resistance	9	51.1600	2_08679	8.8692	11.16	0.63
Striga resistance	9	57.0200	2_03702	12.1227	14.02	0.70
Striga resistance	9	58.880	2_24219	9.1428	11.42	0.63
Striga resistance	9	60.300	2_54689	5.6485	7.75	0.52
Striga resistance	9	63.7700	2_21105	9.1428	11.42	0.63

*Add = Additive

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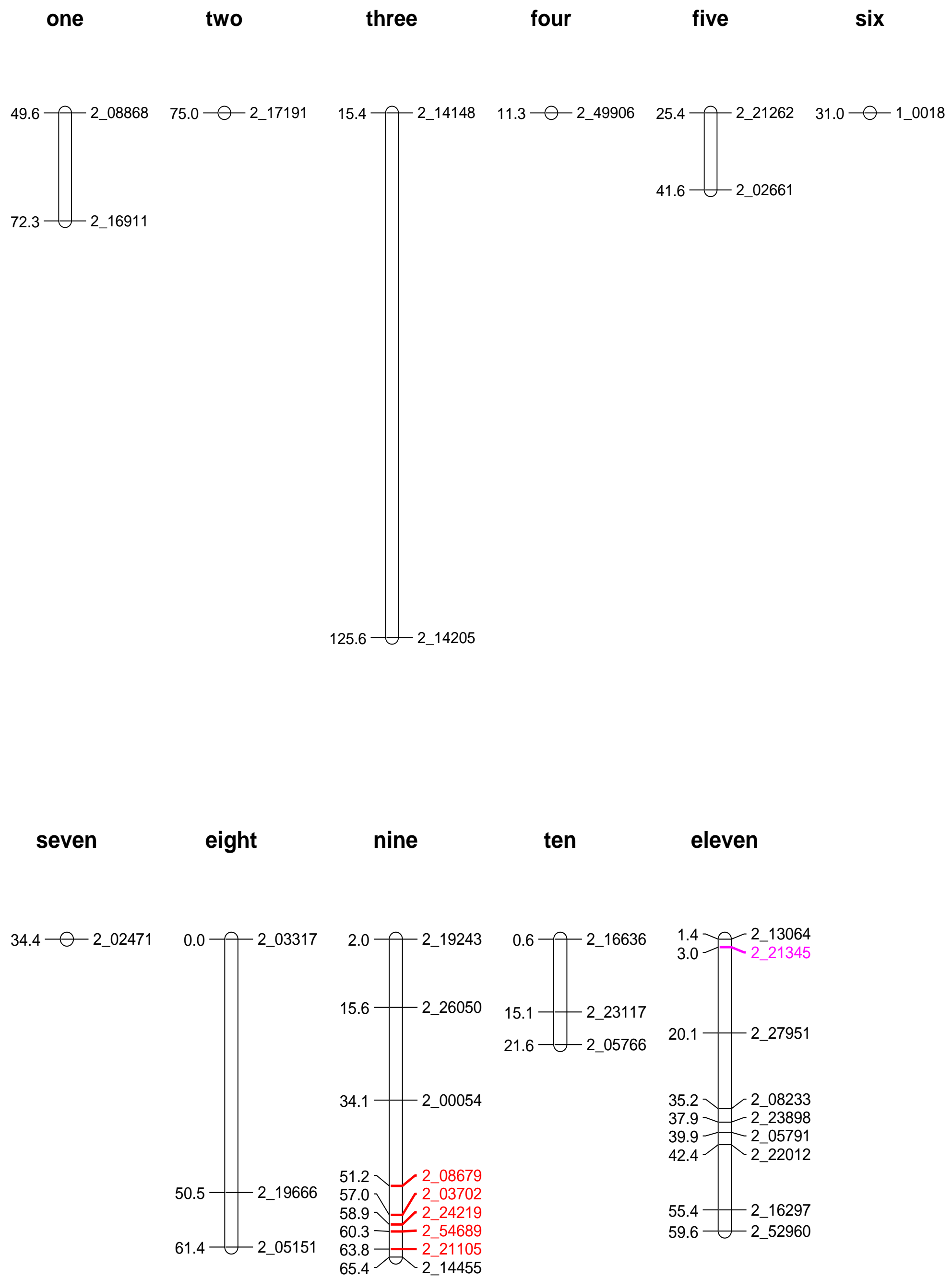

Figure 22: Chromosome map showing significant QTL in 100 S.W (violet), Seed length (violet) and *Striga gesnerioides* resistance (red)

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Genetic Relatedness of F_8 Progenies (population 1) as Revealed by SNP Markers

To successfully compare the effectiveness of SSR and SNP markers without being bias in the determination of genetic relatedness, five SNP markers linked with *Striga gesnerioides* resistance located on linkage group nine (same as the SSR markers used) were exported into PowerMarker programme, for further analysis.

Cluster analysis of F_8 progeny of a cross between IT97K-499-35 and SARC-LO2 (Population 1) Using the Five Polymorphic SNP Markers Associated with *Striga* Resistance

A dendrogram constructed using the combined data of all five polymorphic SNP markers delineated the F_8 progenies into twelve clusters (Figure 4.20).

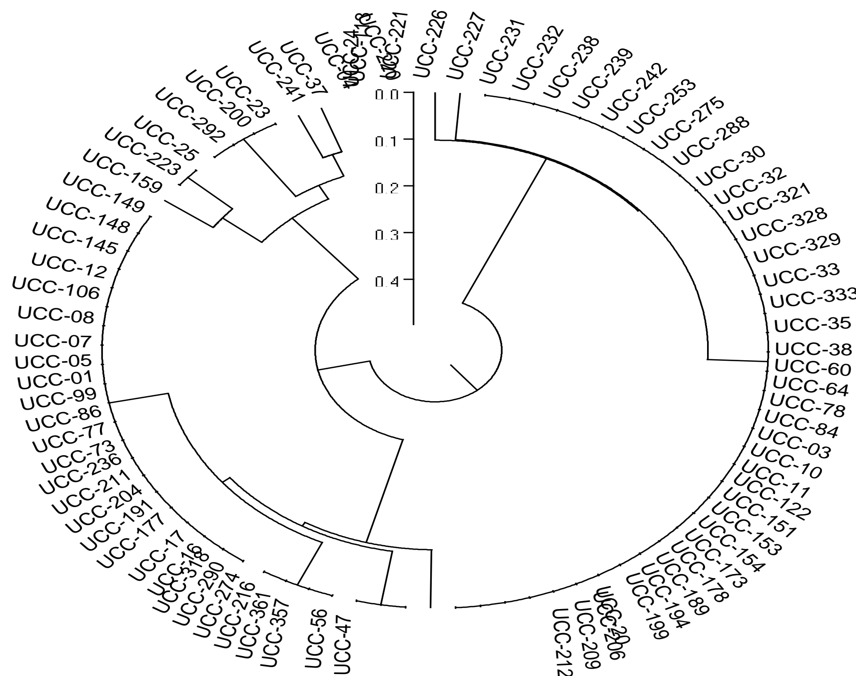

Figure 23: A dendrogram of F_8 progenies (population 1) constructed from PowerMarker using five polymorphic SNP markers with UPGMA tree method

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Table 24: *Major Allele Frequency, Gene Diversity and PIC of the Five SNP Markers associated with Striga resistance in population one (Cross between IT97K-499-35 and SARC-LO2)*

Marker	Major Allele Frequency	Sample Size	Allele Number	Gene Diversity	PIC
2_08679	0.6000	80	2	0.480	0.3648
2_03702	0.6125	80	2	0.4747	0.3620
2_24219	0.5250	80	2	0.5803	0.4976
2_54689	0.6000	80	2	0.4800	0.3648
2_21105	0.5250	80	2	0.5803	0.4979
Mean	**0.6429**	**80**	**2**	**0.5191**	**0.4174**

The average genetic diversity, PIC, major allele frequency from the SNP data analysis were 0.52, 0.42 and 0.6429 respectively (Table 24).

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CHAPTER FIVE

DISCUSSION

This chapter discusses the implications of the results obtained. Comparisons between the results of the current study and relevant previous researches are also made to ascertain the conformity of this work to previous knowledge or otherwise.

Marker Assisted Selection of *Striga* Resistant Genotypes in F_8 and F_9 Populations

Significant effort has been made to identify natural sources of genetic resistance within cowpea cultivars and to select and breed for improved lines with resistance to *S. gesnerioides* (Singh & Emechebe, 1997; Singh, Ehlers, Sharma & Freire Filho, 2002). However, the use of most resistant varieties is limited due to concerns about the potential adaptability and small or medium seed size as found in the variety IT97K-499-35 (Omoigui et al., 2007). IT97K-499-35 was a derivative of B301, a local landrace from Botswana, which produces small seeds but is multi-race resistant genotype to both *S. gesnerioides* and *Alectra vogelii* (Singh, 2002). IT97K-499-35 possess some important resistant and agronomic traits, but requires further improvement through hybridization with superior lines of cowpea to make the progenies more robust, high-yielding with increased seed size to meet farmers and consumers preferences.

The progenies of the cross between the local germplasm SARC-LO2 (with large seed, susceptible to *S. gesnerioides*) and the exotic *Striga*-resistant genotype IT97K-499-35 (developed in IITA, Nigeria) improved the crop towards *S. gesnerioides* resistance and high seed yield and quality adaptable to

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the dry savanna regions of Ghana. Based on the results of the current study, it is clear that the five markers used had discriminating power to distinguish between the *Striga*-resistant and susceptible genotypes for both Ghana race and SG3 resistance. These markers showed a clear association of the resistant parental genotype and the resistant phenotype. IT97K-499-35 showed the presence of the resistant allele for all the markers used indicating resistance to the parasitic weed. Apagbaala and SARC-LO2 however, lacked all the markers indicating their susceptibility to the parasitic weed. Asare, Galyuon, Padi, Otwe and Takrama (2013) recorded similar results when they used SSR-1 and C42-2B markers to test for association of the markers with resistance or susceptibility to IT97K-499-35, Apagbaala and SARC-LO2. This confirmed the phenotypic data where the susceptible cowpea genotypes were infested and had a number of *Striga* shoots emerged in a pot test while the resistant cowpea genotypes were completely devoid of *Striga* attachment or emergence.

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 in population one was 66 % suggesting that the resistant alleles associated with the SSR-1 is highly repeatable within the population. The same could be said for the same marker in population two which recorded allele frequency of 67 %. The marker LRR11 also had 64 % and 83 % of the allele frequencies in populations one and two respectively suggesting that such a marker can be very useful in discriminating resistant alleles from susceptible alleles within the populations. Overall, the mean allele frequencies for populations one and

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two were 64 % and 71 %. 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 Chi-square analysis showing a general segregation ratio, 1:2 and 1:3, in population one and two respectively as compared to the expected ratio of 1:1 in RILs suggested that there was a segregation distortion which was likely caused by selection pressure. Over generations, the RILs have been selected for *S. gesnerioides* resistance and hence the tilting of the populations towards the *S. gesnerioides* resistant allele.

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 of the markers across the cowpea genome in the present study were low, and ranged from 0.34 to 0.36 in population one and 0.23 to 0.35 in population two. The low PIC is an indication that there was little allelic variation within the populations used. The average PIC of 0.35 for population one and 0.31 for population two compared favourably with PIC of 0.38 obtained by Asare et al. (2010). The amount of PIC is a function of detected alleles and their frequency of distribution (Moghaddam et al., 2009). Thus, markers with higher allelic frequency for a particular allele had lower PIC.

The result of the cluster analysis based on five SSR markers revealed cowpea genotypes that possessed all five markers associated with resistance to *Striga*. The molecular data was consistent with the morphological data. In

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population one, cluster I, showed those individuals that had all the five markers present and also showed resistant phenotypes under pot conditions. Clusters II, III, IV, V, VI, VII, VIII, IX, X and XI were made up of cowpeas with either one or combinations of the markers present and resistant under pot condition. Cluster XII indicated individuals that did not have any of the three markers and were susceptible under pot conditions. In population two, cluster V showed individuals that had all the five markers present and also showed resistance to the parasitic weed under pot conditions. Clusters I, II, III, IV, VI, VII, and VIII contained cowpea genotypes with either one or combinations of the markers present and resistant under pot conditions. Cluster IX was made up of cowpea genotypes without any of the markers present and susceptible under field conditions. However, some cowpea genotypes lacked consistency between the marker and the phenotype. The marker may be present but cowpeas are susceptible to *Striga* under pot condition or cowpea genotypes are resistant to *Striga* but no marker expressed. This is indication that there might be epistatic interactions among the genes or the marker may have segregated away from the genes conferring the resistance.

All the markers distinguished between resistant and susceptible cowpea genotypes with different discriminating power. The SSR-1 and C42-2B markers have previously been found to co-segregate with *S. gesnerioides* race 3 or SG3 resistance gene (Li & Timko, 2009; Omoigui, et al., 2007). Both markers identified resistant cowpeas by amplification of DNA to produce bands in mainly cowpea resistant genotypes. In the current study, the SSR-1 marker was more efficient with 84.34 % discriminating ability in identifying resistant RILs to the Ghana race of *Striga* compared to that of C42-2B,

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CLM1320, LRR9 and LRR11 (81.73%, 82.60%, 73.04%, and 77.39% respectively). In addition, SSR-1 was found to be the most efficient with 100% discriminating ability followed by C42-2B (93.91%), CLM1320 (93.91%), LRR11 (85.21%) and LRR9 (82.60%) in identifying RILs resistant to *Striga* race 3. The implication is that, unlike other markers which sit closer to the resistance gene, SSR-1 marker is embedded in the SG3 resistance gene (Botanga & Timko, 2006). In other words, the marker is part of the gene itself.

Genetic Relatedness of the Recombinant Inbred Lines

The result of this study, produced two major clusters (A and B) with twelve subclusters in population one. Cluster "A" contained one parent (SARC-LO2) and cluster "B" contained the donor parent (IT97K-499-35). Only four subclasses comprising 29 RILs were observed in cluster "A" with cluster "B" being the most divergent with eight subclasses made up of 58 RILs.

Population two also produced two major clusters (A and B). Cluster "A" had only five subclusters (a total of 22 RILs) and cluster "B" contained four subclasses (with 11 RILs). IT97K-499-35 was found in Cluster A and Apagbaala was also found in cluster "B". In both populations, the genetic distances between most of the RILs considered under this study were similar. This was consistent with previous reports by Doebley (1989); Vaillancourt & Weeden, (1993); Fotso, Azanza, Pasquet & Raymond (1994); Fang, Chao, Roberts & Ehlers (2006); Simon, Benko-Iseppon, Resende, Winter & Kahl (2007). This is not surprising since it is well documented that cowpeas in general have a narrow genetic base due to the fact that a single domestication event is involved in the origin of the crop (Doebley, 1989; Pasquet, 2000; Coulibaly et al., 2002; Ba et al., 2004). Asare et al, (2010) also observed low

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genetic divergence among cowpea genotypes in Ghana. This low genetic variability has been attributed to the self-pollination nature of the crop (Padulosi, 1993). Given that RILs from this study were developed with only three parent involved, the low genetic variability was expected. However, some RILs had somewhat significant genetic difference between them as observed in UCC-490 and UCC-486. This could be attributed to outcrossing that occurred within the RILs as they progressed from generation to generation. The relatively low genetic distance among IT97K-499-35, Apagbaala and SARC-LO2 suggests that the cowpeas might have emerged from a common ancestral stock, though genetic exchange and recombination could occur.

Effect of *Striga* stress on Growth and Pod Formation

Crop yield losses due to stress imposed by *S. gesnerioides* ranges from 83 to 100% (Aggarwal & Ouédraogo, 1989; Alonge et al., 2005; Cardwell and Lane, 1995; Emechebe et al., 1991, Asare et al. 2013) depending on the extent of damage and level of infestation. The observed stunted growth, leaf necrosis, chlorosis, senescence, defoliation, reduced size of young leaves, poor flowering and poor pod formation in the pot culture emphasized the devastating effects of *Striga* parasitism on the crop. These observations were in line with the report by Asare et al (2010). The significantly ($P \leq 0.05$) low average number of pod (0.774) among susceptible genotypes compared to that of resistant genotypes (1.339) under laboratory conditions gave a total of 42.20 % loss in pod formation, which could be due to the parasite-induced damages. The decrease in the number of pods might also be the direct effect of reduction in photosynthesis and translocation of photosynthates in the

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cowpeas due to *Striga* stress. Competition between parasite and host for solutes and water coupled with lower rate of photosynthesis in the leaves may retard root and shoot growth and, consequently, yield. The evidence of the negative effect of *Striga gesnerioides* could also be seen in the negative correlation obtained between number of pod formation and number of *Striga* emergence. It was observed that as the number of *Striga* attachment increased, the number of pod formed reduced significantly ($P < 0.05$).

Linkage Analysis of the Genes Conferring *Striga* Resistance

Cowpea genotypes respond differently to the different races of the parasitic weed (Botanga & Timko, 2005). It is known that race specific resistance genes exist with few of the genes conferring resistance to multiple races. Botanga and Timko (2005) observed that SSR-1 and C42-2B markers were strongly linked with the resistance genes from

the *Striga* races three (SG3) and five (SG5) respectively. It is therefore assumed that, each of the markers used in this study is attributed to genes conferring resistance to at least one of the races of the parasitic weed. Knowledge of the genetic linkage between these genes will give the breeder an idea as to how likely these genes will be inherited together and indirectly confirming the number of races the new hybrid might be resistant to. Linkage analysis using the IciMapping program showed that all the markers fell on the same linkage group with the resistance genes from SG3 and Ghana race. The program pinned the distance between the Ghana race (GH) resistance gene and that of SG3 at 12.60 cM. This implies that there is at least 87.4% chance that these genes could be inherited together. The Ghana race resistance gene is 91.15% likely to be inherited with CLM1320. Overall, the programe pinned

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the distance between the first gene (Ghana race resistance gene) and the last gene (represented by LRR9) at 34.29 cM. This implies that there is 65.76% chance that all the markers in this study will be inherited together. The result again pinned SSR-1 and the resistance gene of SG3 at the same position (12.60 cM) buttressing the fact that, SSR-1 marker is indeed embedded in the SG3 resistance gene (Botanga & Timko, 2005).

Multi-Race *Striga* Resistance

Host plant resistance is the only known effective way to combat the effects of the parasitic weed *Striga gesnerioides* on cowpea. However, cowpea breeders are faced with the challenge of pyramiding genes into one genotype that will confer resistance to all known races of the parasite. At present, few successes have been recorded such as the report by Lane et al. (1996) and Singh and Emechebe (1997), that IT82D-849 and IT93K-693-2 were resistant to all know races of *Striga*. Similar results were obtained in this study involving more different cowpea genotypes than ever reported. The Multi-race *Striga* resistance study revealed that seven RILs (UCC-11, UCC-24, UCC-32, UCC-122, UCC-221, UCC-241 and UCC-328) were resistant to all known races of *Striga gesnerioides*. The local susceptible genotype, SARC-LO2, was found to be resistant to some races of the parasitic weed (i.e. SG2, SG4z, SG5 and SG6). This is a unique combination of resistance genes, not previously reported, suggesting SARC-LO2 may contain novel resistance genes or alleles. Further exploration of SARC-LO2 resistance genes is necessary to determine if resistance to SG2, SG5, and SG6 is conferred by the same resistance genes found in IT97K-499-35 and other resistance cowpea cultivars. Several RIL lines were found to be resistant to all of the *Striga* isolates tested

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in this study, confirming the SG4z resistance genes in SARC-LO2. For instance, where the IT97K-499-35 is unable to confer resistance (i.e. SG4z), SARC-LO2 conferred resistance. Therefore 77.8% (7 out of 9) of the recombinant progenies of IT97K-499-35 and SARC-LO2 in the multi-race resistance study have been established to have resistant to all the 7 known races of *Striga* in West Africa. The local resistant check, GH3684, was found to be resistant to all races of the parasitic weed. Botanga and Timko (2006) stated that no cowpea cultivar has been found to be naturally resistant to all races of *Striga*. The current finding contradicts this statement since the local landrace GH3684 showed natural resistance to all known races of the parasitic weeds. Though GH3684 and IT97K-499-35 may share similar genetic resistance to six known races of *Striga*, the gene expressing resistance to SG4z in GH3684 distinguished it from IT97K-499-35. Hence, GH3684 has a broader genetic base of resistance to combat *Striga* in West Africa than the previously improved IT97K-499-35. On the whole, Apagbaala alone was susceptible to all the races of *Striga*.

Identification of SNP Markers Associated with Cowpea Seed Sizes and *Striga gesnerioides* Resistance

A QTL located on linkage group 11 was found to have a link with seed weight and seed length. This result is consistent with previous work done by Egbazor et al. (2013) who also detected QTL on linkage group 11 associated with cowpea seed mass. The same QTL being linked with both agronomic traits (seed weight and length) is an indication that both traits might be controlled by the same locus and the strong positive correlation between these traits can only buttress this fact. The analysis gave an additive effect of -1.228 for 100 seed

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weight and -0.0284 for seed length which indicates that substitution of an IT97K-499-35 allele with a SARC-LO2 allele will result in an average gain in 100 seed weight and seed length of 1.228 g and 0.0284 cm more respectively. The relatively large additive effect of this QTL suggests selection for this genomic locus using MAS would be worthwhile for cultivar improvement.

Striga gesnerioides is an important economic parasitic weed which has received a lot of attention from crop scientist not only in West Africa but across the globe. The devastating effect on their host plant and their potential negative impact on food security has led scientists to investigate extensively on them in a bid to either combat or greatly reduce its impact through host plant resistance. The genes conferring host plant resistance have received great deal of attention since its discovery in wild cowpea types. Various works have been done on the resistance genes including mapping their location within the cowpea genome (Quedrago, Tignegre, Timko & Belzile, 2002). Research conducted by Quedrago, Tignegre, Timko & Belzile (2002) revealed that, the genes for *Striga* races 1 and 3 were located on linkage groups one and six in the cowpea genome using mostly Amplified Fragment Length Polymorphism markers. The cowpea linkage groups however changed from 2009 based on the work done by Muchero et al (2009). Linkage groups one and six became ten and nine respectively. This present study made use of SNP markers distributed across the cowpea genome and single marker analysis detected QTLs on linkage group nine associated with *Striga gesnerioides* resistance spanning the length of 19.89 cM. This was consistent with the observations made by Muchero et al. (2009).

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Effectiveness of SNP and SSR Markers in Revealing Genetic Relatedness among Cowpea

Two main major clusters (A and B) were observed with five SNP markers. Cluster "A" (with 3 subclasses) was closely related to the *Striga*-resistant parent IT97K-499-35. Cluster "B" (9 subclasses) showed relatedness to the *Striga*-susceptible parent SARC-LO2. Similar details could be observed in the genetic relatedness resolved with SSR markers in the current study. The type of DNA markers employed can also affect the level of polymorphism revealed among genotypes. Previous studies aimed at looking at genetic diversity of cowpea have used a variety of different molecular marker including isozymes, RAPDs, gene sequencing and SSRs. Li et al. (2001) used 12 cowpea-derived SSR primers to examine the genetic similarities and relationships among cowpea breeding lines developed at the IITA. Egbadzor et al. (2014) also used 458 SNP markers to assess the genetic diversity among 113 cowpea accessions. SNPs are known to be more effective in diversity assessment compared with other markers such as AFLPs and SSRs (Varshney *et al*. 2007). In the current study, the results of data analysis of both SSR and SNP markers showed similar clustering patterns (12 subclusters each) suggesting a substantial degree of association between origin and genotype. Similar result was obtained by Belayneh, Kifle, Gedil, Boukar & Christian (2017). The polymorphic information content was 0.3524 for SSR and 0.4977 for SNP showing moderate differentiation and high gene flow among cowpea accessions. Under normal conditions, SNP markers would be expected to distinguish populations made up of different cultivars better than SSR since the cultivars may have the potential to have more than 2 alleles for SSR and

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many varying haplotypes for SNPs. In RILs, there are potentially, only two alleles per locus for both SNPs and SSRs making it difficult to compare the effectiveness of SNPs and SSR markers in revealing genetic relatedness or variations within populations developed from the same parents.

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CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This chapter summarized the whole research and stated short but conclusive statements on the findings made and its implications. It also contained series of recommendations which seek to improve future researches and add knowledge to the existing one.

Summary

Five SSR markers separated all 116 RILs, Apagbaala, IT97K-499-35, SARC-LO2 and GH3684 into resistant and susceptible genotypes as far as *Striga* was concerned. These individual markers possessed varying degrees of discriminating power enabling them to distinguish between the genotypes. The molecular data was confirmed phenotypically in a pot trials with 3.44 % of the molecular data not conforming to the phenotypic data by way of the RILs possessing the markers but showing susceptibility to the GH race in pot trials. Conversly, 5.17 % of the 116 RILs also had none of the markers but showed resistance to the GH race in pot trials. On the whole, the genetic distances between the RILs were generally low which was expected considering the fact that only three parents were used to develop both populations and cowpeas generally have low genetic diversity.

Striga-resistance markers and genes were located on chromosome 9 spanning a total length of 34.29 cM and CLM1320 marker was closest at 8.0 cM to the GH race resistance gene. The gene controlling resistance in the race SG3 is located at 12.60 cM away from the genes controlling resistance in the GH race. SSR-1 marker was found to be occupying the same position (12.60 cM) as the resistance gene in SG3. Overall, the genes associated with the five

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markers were found to have a 65.76 % probability that they will segregate together.

SARC-LO2, which is known to be susceptible to *Striga* in Ghana was found to have resistance to four races of *Striga* including SG2, SG4z, SG5 and SG6. Hence, 77.8% of the RILs including UCC-11, UCC-24, UCC-32, UCC-122, UCC-221, UCC-241 and UCC-328 derived from IT97K-499-35 and SARC-LO2 proved resistant to all 7 known races of the parasite due to gene recombination and pyramiding. In addition, the local landrace *Striga*-resistant genotype, GH3684 was confirmed to be resistant to all the 7 known races of *Striga*. Hence, GH3684 may carry a robust resilient single dominant gene or gene complex to withstand all known races of *Striga* in sub-Saharan Africa in breeding programmes making it superior to IT97K-499-35 which is susceptible to 4SGz though resistant to all other races of *Striga*. However, Apagbaala was consistently susceptible to all the races of *Striga* and did not express any of the *Striga*-resistant markers. The current studies identified SNP QTLs associated with seed sizes (2_21345) and *Striga* resistance (2_08679, 2_03702, 2_24219, 2_54689 and 2_21105). Though both the SSR and SNP makers could discriminate all the cowpea RILs into varied clusters, there is close genetic relatedness due to low gene diversity and polymorphism information content.

Conclusions

On the whole, 5 SSR markers were informative to discriminate resistant and susceptible cowpea genotypes among RILs. The markers were 88.5 % consistent with the respective phenotypes and only 8.61 % inconsistency occurred. In all, 3.44 % of the RILs comprising UCC-47, UCC-

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232, UCC-471 and UCC-489 had all the markers present but showed susceptibility to GH race and resistant to SG3 in the pot test. Conversely, 5.17 % made up of UCC-25, UCC-77, UCC-86, UCC-204 UCC-274 and UCC-460 lacked the markers but were resistant to GH race while susceptible to SG3 under pot test.

In all, 54 (64.3 %) out of 84 RILs were resistant to the *Striga* GH race in population one and 22 (71 %) out of 31 RILs in population two were also found to have the same resistance.

The genes controlling *Striga* resistance in cowpea RILs may be located chromosome 9. The SG3 resistance gene is located 12.60 cM away from the gene controlling resistance in the GH race and SSR-1 marker occupies the same position (12.60 cM) as SG3. The resistance genes associated with the five markers were found to have a 65.76 % probability that they will segregate together.

The Multi-race-*Striga*-resistance testing coupled with 5 SSR markers confirmed the local resistant check, GH3684, to be resistant to all the known races of the parasitic weed in West Africa. In addition, 7 RILs derived from IT97K-499-35 x SARC-LO2 viz; UCC-11, UCC-24, UCC-32, UCC-122, UCC-221, UCC-241 and UCC-328 were also confirmed resistant to all known races of *Striga gesnerioides* in West Africa comparable to GH3684. However, IT97K-499-35 was susceptible to only SGz, though resistant to all other races.

The local susceptible cowpea genotype, SARC-LO2, was confirmed resistant to four known races of the parasitic weed (SG2, SG4z, SG5 and SG6) but the local variety Apagbaala was susceptible to all the races of *Striga*.

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The SNP marker, 2_21345 was found to be linked with 100 seed weight and seed length, and the QTL located on chromosome 11. Besides, five SNPs (2_08679, 2_03702, 2_24219, 2_54689 and 2_21105) were also found to be associated with *Striga gesnerioides* resistance and the QTL located on chromosome 9.

The RILs were closely related genetically and the genotypes clustered together in a dendrogram as shown in figures 14, 15 and 24.

Both SNPs and SSR markers were effective in discriminating the cowpea genotypes to reveal genetic relatedness among the RILs. The genetic diversity of 0.52 generated by SNP markers were higher than 0.46 (population one) and 0.39 (population two) generated by the SSR markers. This suggests that SNPs may have higher genomic sensitivity and discriminating power to generate information than SSR markers.

Recommendations

Base on this study, the following recommendations have been outlined for plant breeders and scientist alike to endeavor to execute the recommended suggestions.

1. The informative SSR markers associated with *Striga*-resistance should be incorporated into cowpea breeding in Ghana.
2. The RILs found to be resistant to all the 7 known races or multiple races of *Striga gesnerioides* should be further tested on-field towards release as varieties in Ghana and sub-Saharan African where the parasite exists.
3. The identified SNP markers associated with *Striga*-resistance and seed size traits should be further evaluated for use in breeding.

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4. GH3684 should be incorporated into breeding to generate more information on the genetics and mode of inheritance of the resistant gene to facilitate broader application to improve cowpea production.

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APPENDICES

APPENDIX A

DNA concentration and quality detection using NanoDrop

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	Sample Type	Factor
IT97K-499-35	10551.1	ng/µl	211.023	106.776	1.98	DNA	50
GH3684	10551.1	ng/µl	211.023	106.776	1.98	DNA	50
SARC-LO2	3470.3	ng/µl	69.406	34.555	2.01	DNA	50
APAGBAALA	6253.6	ng/µl	125.072	63.772	1.96	DNA	50
UCC-01	710.1	ng/µl	14.202	7.018	2.02	DNA	50
UCC-03	1012.7	ng/µl	20.253	9.965	2.03	DNA	50
UCC-04	712	ng/µl	14.24	6.93	2.05	DNA	50
UCC-05	747.9	ng/µl	14.957	7.552	1.98	DNA	50
UCC-07	881.1	ng/µl	17.622	8.865	1.99	DNA	50
UCC-08	1003.9	ng/µl	20.079	9.998	2.01	DNA	50
UCC-10	1097.2	ng/µl	21.944	10.916	2.01	DNA	50
UCC-11	744.8	ng/µl	14.895	7.534	1.98	DNA	50
UCC-12	1000.8	ng/µl	20.016	10.056	1.99	DNA	50
UCC-16	823.6	ng/µl	16.473	8.269	1.99	DNA	50
UCC-17	996.6	ng/µl	19.932	9.947	2	DNA	50
UCC-20	760.6	ng/µl	15.212	7.615	2	DNA	50
UCC-23	700.5	ng/µl	14.01	7.18	1.95	DNA	50
UCC-24	1046.1	ng/µl	20.922	10.443	2	DNA	50
UCC-25	740.2	ng/µl	14.805	7.432	1.99	DNA	50
UCC-30	1077.6	ng/µl	21.553	10.889	1.98	DNA	50
UCC-32	683.9	ng/µl	13.678	6.742	2.03	DNA	50
UCC-33	933.7	ng/µl	18.674	9.3	2.01	DNA	50
UCC-35	908.7	ng/µl	18.174	9.147	1.99	DNA	50
UCC-37	694.5	ng/µl	13.89	7.006	1.98	DNA	50
UCC-38	935.1	ng/µl	18.703	9.536	1.96	DNA	50
UCC-47	928.8	ng/µl	18.575	9.368	1.98	DNA	50
UCC-56	683.3	ng/µl	13.667	6.913	1.98	DNA	50
UCC-60	1105.9	ng/µl	22.117	11.06	2	DNA	50
UCC-64	793	ng/µl	15.86	8.071	1.97	DNA	50
UCC-73	942.5	ng/µl	18.849	9.444	2	DNA	50
UCC-77	868.3	ng/µl	17.367	8.727	1.99	DNA	50
UCC-78	901.6	ng/µl	18.032	9.01	2	DNA	50
UCC-84	891	ng/µl	17.821	8.967	1.99	DNA	50
UCC-86	921.5	ng/µl	18.431	9.121	2.02	DNA	50
UCC-99	744.6	ng/µl	14.892	7.439	2	DNA	50
UCC-106	890.7	ng/µl	17.814	8.682	2.05	DNA	50
UCC-113	841.9	ng/µl	16.839	8.34	2.02	DNA	50
UCC-122	869.1	ng/µl	17.382	8.675	2	DNA	50
UCC-145	1267.6	ng/µl	25.352	12.761	1.99	DNA	50
UCC-148	1203.6	ng/µl	24.072	12.051	2	DNA	50
UCC-149	728	ng/µl	14.561	7.399	1.97	DNA	50
UCC-151	930.5	ng/µl	18.61	9.914	1.88	DNA	50
UCC-153	2111.3	ng/µl	42.227	20.977	2.01	DNA	50
UCC-154	908.9	ng/µl	18.179	9.191	1.98	DNA	50
UCC-159	971	ng/µl	19.42	9.673	2.01	DNA	50
UCC-173	973.1	ng/µl	19.461	9.717	2	DNA	50
UCC-177	1021.1	ng/µl	20.422	10.091	2.02	DNA	50

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Appendix A continued

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	Sample Type	Factor
UCC-178	583.8	ng/µl	11.675	5.837	2	DNA	50
UCC-189	632.7	ng/µl	12.654	6.359	1.99	DNA	50
UCC-191	724.9	ng/µl	14.499	7.209	2.01	DNA	50
UCC-194	815.1	ng/µl	16.301	8.191	1.99	DNA	50
UCC-199	763.6	ng/µl	15.273	7.654	2	DNA	50
UCC-200	1200.6	ng/µl	24.013	12	2	DNA	50
UCC-204	704.8	ng/µl	14.096	7.019	2.01	DNA	50
UCC-206	907.1	ng/µl	18.141	9.019	2.01	DNA	50
UCC-209	843.1	ng/µl	16.862	8.407	2.01	DNA	50
UCC-211	698.5	ng/µl	13.97	7.004	1.99	DNA	50
UCC-212	914.2	ng/µl	18.284	9.06	2.02	DNA	50
UCC-216	982.5	ng/µl	19.649	9.722	2.02	DNA	50
UCC-220	1070.6	ng/µl	21.413	10.765	1.99	DNA	50
UCC-221	969.6	ng/µl	19.392	9.673	2	DNA	50
UCC-223	1020	ng/µl	20.4	10.126	2.01	DNA	50
UCC-226	881.3	ng/µl	17.625	8.677	2.03	DNA	50
UCC-227	809.1	ng/µl	16.182	7.997	2.02	DNA	50
UCC-231	1088.2	ng/µl	21.764	10.955	1.99	DNA	50
UCC-232	827.3	ng/µl	16.546	8.241	2.01	DNA	50
UCC-236	774.7	ng/µl	15.493	7.671	2.02	DNA	50
UCC-238	908	ng/µl	18.159	9.001	2.02	DNA	50
UCC-239	1043.4	ng/µl	20.868	10.521	1.98	DNA	50
UCC-241	972.1	ng/µl	19.442	9.738	2	DNA	50
UCC-242	1023.3	ng/µl	20.466	10.231	2	DNA	50
UCC-243	50.4	ng/µl	1.007	0.62	1.62	DNA	50
UCC-244	213.4	ng/µl	4.269	2.181	1.96	DNA	50
UCC-247	230.7	ng/µl	4.614	2.363	1.95	DNA	50
UCC-253	844.6	ng/µl	16.893	8.407	2.01	DNA	50
UCC-274	912.6	ng/µl	18.253	9.141	2	DNA	50
UCC-275	611.1	ng/µl	12.221	6.119	2	DNA	50
UCC-288	699.1	ng/µl	13.981	7.051	1.98	DNA	50
UCC-290	724.5	ng/µl	14.489	7.177	2.02	DNA	50
UCC-292	952	ng/µl	19.039	9.52	2	DNA	50
UCC-318	1012.4	ng/µl	20.248	10.039	2.02	DNA	50
UCC-321	886.6	ng/µl	17.731	8.876	2	DNA	50
UCC-328	936.8	ng/µl	18.737	9.302	2.01	DNA	50
UCC-329	856.3	ng/µl	17.126	8.512	2.01	DNA	50
UCC-333	2257.7	ng/µl	45.154	22.693	1.99	DNA	50
UCC-337	3223.1	ng/µl	64.461	32.473	1.99	DNA	50
UCC-357	1345.7	ng/µl	26.914	13.263	2.03	DNA	50
UCC-361	1505.5	ng/µl	30.11	14.854	2.03	DNA	50
UCC-365	1075.8	ng/µl	21.515	10.443	2.06	DNA	50
UCC-366	1153.8	ng/µl	23.075	11.499	2.01	DNA	50
UCC-377	1367.5	ng/µl	27.35	13.452	2.03	DNA	50
UCC-390	2151.9	ng/µl	43.037	21.365	2.01	DNA	50
UCC-396	1194	ng/µl	23.879	11.844	2.02	DNA	50
UCC-419	1532.2	ng/µl	30.643	15.223	2.01	DNA	50
UCC-421	1075.8	ng/µl	21.515	10.653	2.02	DNA	50

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Appendix A continued

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	Sample Type	Factor
UCC-428	1490.6	ng/µl	29.812	14.742	2.02	DNA	50
UCC-445	2481.3	ng/µl	49.626	24.767	2	DNA	50
UCC-446	2634.4	ng/µl	52.688	26.524	1.99	DNA	50
UCC-448	1297.6	ng/µl	25.952	12.891	2.01	DNA	50
UCC-454	1429.3	ng/µl	28.586	13.967	2.05	DNA	50
UCC-457	1497.7	ng/µl	29.954	14.756	2.03	DNA	50
UCC-460	2432.9	ng/µl	48.659	24.286	2	DNA	50
UCC-464	1820	ng/µl	36.401	18.156	2	DNA	50
UCC-466	4025.6	ng/µl	80.513	40.063	2.01	DNA	50
UCC-471	2172.5	ng/µl	43.45	21.718	2	DNA	50
UCC-473	2231.6	ng/µl	44.632	22.111	2.02	DNA	50
UCC-478	2242.7	ng/µl	44.855	22.433	2	DNA	50
UCC-482	1721.9	ng/µl	34.438	17.107	2.01	DNA	50
UCC-484	2787	ng/µl	55.74	27.868	2	DNA	50
UCC-486	2390.1	ng/µl	47.801	23.697	2.02	DNA	50
UCC-487	1109.7	ng/µl	22.194	10.924	2.03	DNA	50
UCC-489	1875.2	ng/µl	37.504	18.701	2.01	DNA	50
UCC-490	1679.7	ng/µl	33.594	16.783	2	DNA	50
UCC-497	2969.9	ng/µl	59.399	29.765	2	DNA	50
UCC-498	3052.1	ng/µl	61.042	30.495	2	DNA	50
UCC-505	2603.8	ng/µl	52.076	26.167	1.99	DNA	50
UCC-513	2284.6	ng/µl	45.693	22.548	2.03	DNA	50
UCC-514	1920.6	ng/µl	38.412	19.422	1.98	DNA	50
UCC-523	2608.8	ng/µl	52.176	25.93	2.01	DNA	50

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APPENDIX B

PCR Primers used to amplify markers associated with Resistance to *Striga gesnerioides* races SG1 (Burkina faso), SG2 (Mali), SG3 (Nigeria/Niger) and SG5 (Cameroon)

Maker	Forward Primer	Reverse Primer	
SSR1	cctaagcttttctccaactcca	caagaaggaggcgaagactg	Imbedded in RSG3-301 resistance gene (SG3)
61R	aattcacttatgactgagctatat	taacaaaaattgatttgtttggtt	Original marker tightly linked to SG1
61RM2	gatttgtttggtttccttaag	ggttgatcttggaggcatttt	Original marker tightly linked to SG1
C42-2B	cagttccctaatggacaacc	caagctcatcatcatctcgatg	Linked to SG5
C42-499	caatgagccaacaagtctagag	gccctaaactagaatcattgcc	
SSR-6799	tagaccagatgacattgtaattc	gtcgtaactgggcacaatag	
SSR-6547	aaactgacacttgaacacga	ctcatgcagagttcaagatc	
CP01038	ttttgacagaagaaacgtggtgga	ggggtatgtctgaaagttcaacgc	
B31	ctgcggcaacagctgaagtttc	ggaactggtttgaacactggaac	
B53	Cttctcttccatccgccgcc	gttgcattgccttgtcacctc	
SG25R	Ggagttgttgtatgagaagttgc	cgtaataatggatgtgtgttttctc	
LRR8	Cattcatccactctcttccc	cctttggtcattgaatacatg	
LRR9	Gttccataacatgctctgac	gctttctcaactctcatctctc	
LRR11	Ggtagctcctctgttgattcag	catatgtccaaccattgccacag	
LRR18	Gggatagtgagtattgatgctg	ccggtcttcgcctccttcttgg	
CLM1320	Cacaacttgcaacaacatgc	tacgtggatctggtctttcc	
C11-5R3	Ggtcttaccttctacaccct	cgtaataatggatgtgtgttttctc	

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APPENDIX C

Cowpea Seed Size Data

RILs	LENGTH (cm)	WIDTH (cm)	THICKNESS (cm)	100 S.W (g)
UCC-01	1.035	0.8	0.5	23
UCC-03	0.86	0.69	0.5	16.4
UCC-04	0.9	0.695	0.53	17.5
UCC-05	0.965	0.65	0.49	13.2
UCC-07	0.95	0.675	0.52	15.8
UCC-08	0.985	0.69	0.5	18.1
UCC-10	0.88	0.645	0.475	13.4
UCC-11	0.805	0.6	0.495	18.8
UCC-12	0.895	0.61	0.4	13
UCC-16	1.02	0.68	0.51	19
UCC-17	1.02	0.68	0.49	16.5
UCC-20	0.96	0.69	0.51	17.6
UCC-23	0.905	0.605	0.475	16.9
UCC-24	0.925	0.64	0.475	20.1
UCC-25	0.995	0.67	0.53	18.5
UCC-30	0.807	0.593	0.435	15.5
UCC-32	1.005	0.685	0.575	19.9
UCC-33	0.845	0.665	0.51	19
UCC-35	0.905	0.65	0.475	16
UCC-37	0.852	0.614	0.451	12.1
UCC-38	0.99	0.6	0.41	14.2
UCC-47	0.895	0.615	0.47	21.2
UCC-56	0.96	0.69	0.51	18.9
UCC-60	0.925	0.6	0.49	16.9
UCC-64	0.817	0.64	0.49	20.9
UCC-73	0.92	0.664	0.495	15
UCC-77	0.9	0.6	0.5	17.8
UCC-78	0.845	0.635	0.515	18
UCC-84	0.99	0.695	0.46	18.5
UCC-86	0.9	0.617	0.457	14.6
UCC-99	0.995	0.63	0.495	14.6
UCC-106	1.005	0.7	0.5	20.5
UCC-113	0.955	0.635	0.49	16.5
UCC-122	0.865	0.63	0.46	16.7
UCC-145	0.985	0.695	0.495	10.9
UCC-148	0.895	0.65	0.5	16.3
UCC-149	0.853	0.611	0.46	16
UCC-151	0.821	0.61	0.462	16.6
UCC-153	0.93	0.655	0.51	17.7

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Appendix C continued

RILs	LENGTH (cm)	WIDTH (cm)	THICKNESS (cm)	100 S.W (g)
UCC-154	0.846	0.603	0.502	21.4
UCC-159	0.675	0.565	0.43	12
UCC-173	0.877	0.608	0.469	16.2
UCC-177	0.905	0.65	0.48	18.4
UCC-178	0.886	0.582	0.471	16.5
UCC-189	0.861	0.592	0.422	15.5
UCC-191	0.89	0.591	4.708	16.8
UCC-194	0.856	0.622	0.487	16
UCC-199	0.86	0.6	0.5	16.6
UCC-200	0.951	0.644	0.451	16.5
UCC-204	0.905	0.59	0.425	17.9
UCC-206	0.849	0.62	0.459	14.1
UCC-209	0.902	0.594	0.464	16
UCC-211	0.897	0.65	0.509	14
UCC-212	0.802	0.579	0.453	13.1
UCC-216	0.891	0.627	0.44	17.3
UCC-220	0.879	0.597	0.444	16
UCC-221	0.862	0.63	0.452	16.9
UCC-223	0.879	0.603	0.448	15.8
UCC-226	0.875	0.7	0.5	23
UCC-227	0.875	0.629	0.451	16.4
UCC-231	0.879	0.607	0.476	16.6
UCC-232	0.787	0.602	0.455	16
UCC-236	0.85	0.61	0.4	17.1
UCC-238	0.807	0.62	0.44	16.5
UCC-239	0.815	0.595	0.44	18
UCC-241	0.9	0.61	0.5	21.3
UCC-242	0.833	0.621	0.443	13.9
UCC-243	0.867	0.598	0.495	13.7
UCC-244	0.913	0.627	0.464	17
UCC-247	0.775	0.625	0.435	17.5
UCC-253	0.826	0.588	0.488	15.3
UCC-274	0.806	0.622	0.485	17.5
UCC-275	0.855	0.61	0.445	18.5
UCC-288	0.855	0.573	0.455	15.8
UCC-290	0.8	0.61	0.469	17.6
UCC-292	0.842	0.599	0.436	13.5
UCC-318	0.873	0.632	0.445	17.2
UCC-321	0.871	0.618	0.515	16.2

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Appendix C continued

RILs	LENGTH (cm)	WIDTH (cm)	THICKNESS (cm)	100 S.W (g)
UCC-328	0.87	0.615	0.46	15.6
UCC-329	0.858	0.613	0.45	16
UCC-333	0.837	0.565	0.491	14.6
UCC-337	0.916	0.614	0.489	18.5
UCC-357	0.898	0.621	0.433	15.4
UCC-361	0.822	0.573	0.432	16.5

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APPENDIX D

SNP Markers Distributed Across the Genome of Cowpea

Marker Name	Ta	mgcl2	LG	Consensus Map Location (cM)	Fragment Size (not always accurate)	Sequence	SNP
1_0018F	55	0.5	6	30.95	143	TAACCTCGGCTGCGAGATAC	
1_0018R1						AGATAGATAATTTCTCGATCACAGAAAACC	G
1_0018R2						TTTCTCGATCACAGAAATCG	C
1_0647F	55	0.5	1	45.88	150	GTAGCCCGCTACAAAAATGC	
1_0647R1						TATATATAAAACCAAATTGAAGACAGAGGTC	G
1_0647R2						ACCAAATTGAAGACAGAGCTT	A
1_1129R	55	0	2	0	221	TGCATTTGCATTGTTAGTTTTG	
1_1129F1						GAAGAAAGGTCTCCCATATGTTCATGGTAGTG	G
1_1129F2						CTCCCATATGTTCATGGTACTA	A
2_00616F	55	0	10	9.68	200	CACCGAGTTCCACATACAACA	
2_00616R1						CAATACCACTTTTGGTAATGC	G
2_00616R2						ATTACTACTACAATACCACTTTTGGTAAAGA	T
2_01068R	50	0.5	6	10.48	181	GCAAAAACCCATAGGCACTT	
2_01068F1						GAAGAAAGGTTGTAGAGAAAAGTAGATGCGAG	G
2_01068F2						TGTAGAGAAAAGTAGATGCCAA	A
2_01258R	50	0	6	34.92	134	GCACCAGAGTATGCAATCCA	
2_01258F1						GGGCGAAGGAGGAATTCACCATTAACG	G
2_01258F2						GAAGAAAGGTGGGAGAAGGAGGAATTCACCATTATCA	A
2_01793R	55	0.5	7	21.42	230	CTCACAAACAGCTCCACCAA	

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2_01793F1						TATATAAATAATTGTTTGGGTGTGGAAAGG	G
2_01793F2						ATTGTTTGGGTGTGGAATGA	A
2_02085R	55	0	3	120.69	212	TGATCCCGTCACTGATGAAA	
2_02085F1						ATTACTACTAGACGGGTCAAGACACAGAAGTCATCCT	T
2_02085F2						ATCAAGTCAAGACACAGAAGTCATGCC	C
2_02367F	55	0	2	5.07	112	TTTGATGGGTTGATCCAAAA	
2_02367R1						ACCATACATTACATACTCGTACTCTTCCCCACCAC	G
2_02367R2						CGAGCCTCGTACTCTTCCCCACGAT	A
2_02471F	55	0	7	34.40	264	TTGGCAGGTTCATTGGATTT	
2_02471R1						AGTACATGCGTGACAACCGC	G
2_02471R2						ATATAACTTAAGTACATGCGTGACAACGGT	A
2_02661F	45	0	5	41.61	198	CAAACTGACAGGAATGCACAA	
2_02661R1						ATTACTACTAGACGGAATTCGTGACATAGAATCAGTA	T
2_02661R2						ATCAAAATTCGTGACATAGAATCACTG	C
2_02870F	55	0	11	30.56	160	TTCCTCGGATGAAGGTGCT	
2_02870R1						GGGCGTTTGTTTATCTAAATGTGCGGC	G
2_02870R2						GAAGAAAGGTGGGAGTTTGTTTATCTAAATGTGCCGA	T
2_02911F	55	0	8	23.65	176	CCCAATGCGTAAATCTTCAA	
2_02911R1						CGAGCGCTGACTGTTTCAGTTCTCGAC	G
2_02911R2						ACCATACATTACATAGCTGACTGTTTCAGTTCTCCAT	A
2_03317R	55	0.5	8	0	144	GGTCCTTTTTCAGGAAGTTCG	
2_03317F1						GGGCGAAGCAACTGCATCACCGG	G
2_03317F2						GAAGAAAGGTGGGAGAAAGCAACTGCATCACGGA	A
2_03535R	55	0	7	41.15	200	AAGAACATCCAGGGTCAGTG	
2_03535F1						ATCAAGAAAGAAGGGTCAAGCTCCTG	G

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2_03535F2						ATTACTACTAGACGGGAAAGAAGGGTCAAGCTCGTA	A
2_03748F	60	0.5	11	5.02	191	TACAACCCAGAAACCGATCC	
2_03748R1						CCATACATTACATAGTGGTAGTGTTGGCGGAGA	T
2_03748R2						CGAGCTGGTAGTGTTGGCGGTGG	C
2_04048F	55	0.5	2	50.10	204	TTTTGGGAAAGGCCATGATA	
2_04048R1						ATTACTACTAGACGGGGCAGGTAATGATGCAAAACAA	T
2_04048R2						CGAGCGGCAGGTAATGATGCAAAAGAG	C
2_04147F	60	0	4	40.30	218	AGACCCCACTTCTTGTTCCA	
2_04147R1						ACCATACATTACATACTATCTCTACTAACCGACAGCC	
2_04147R2						ATCAACTATCTCTACTAACCGACACCT	
2_04164F	55	0.5	5	46.27	215	ACAGTGGCTCCAAAATCCAC	
2_04164R1						GAAGAAAGGTGGGAGTACATTTCTTTGGGCCGC	
2_04164R2						CGAGCTGTACATTTCTTTGGGCGGT	
2_04189R	50	0	7	0	214	TGAAAGTAGCAATGCCTACCC	
2_04189F1						ACCATACATTACATAATTGCACATGCTATGAAATGAG	
2_04189F2						ATCAAATTGCACATGCTATGAAATCAT	
2_04224F	55	0	2	70.73	156	AACCAATCTCTTTCGTTTGGAA	
2_04224R1						ACCATACATTACATAGGTGAGAAGAGTTCAGTTGGC	
2_04224R2						TGCGGGGGTGAGAAGAGTTCAGTTCGT	
2_04710F	60	0	2	75.04	217	GTCGTCGGCAGTCTGAGAA	
2_04710R1						GAAGAAAGGTGGGAGCAAAGGGTAAAGGGAAACGA	
2_04710R2						CGAGCCAAAGGGTAAAGGGAAAGGG	
2_04994R	55	0.5	8	61.42	206	ACGCCTTCGTTGTTTCAAAT	
2_04994F1						AGAAAGGTGGGAGATTTTCCGGACTTGTGATTGT	
2_04994F2						ATCAATTTCCGGACTTGTGATAGC	

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2_05151F	55	0	8	12.79	197	ATAGGCCTGTGGACTGGTGA	
2_05151R1						GAAGAAAGGTGGGAGCGGATGGAGAACAAACCAA	
2_05151R2						TGCGGCGGATGGAGAACAAACGAG	
2_05766F	55	0	10	21.62	162	TGGCCATTGATCAAGTTTTT	
2_05766R1						GAAGAAAGGTGGGAGCAATTCAAACACAGAAAACACC	
2_05766R2						ATCAACAATTCAAACACAGAAAACTCA	
2_06268R	55	0	11	15.28	211	GGCTCTACCGAACGTGATGT	
2_06268F1						GAAGAAAGGTGGGAGTGATCAAGATAAGAGTTACGAT	
2_06826R	60	1.5	6	39.26	178	GCTCAATGAGTACGCCATTTC	
2_06826F1						ATTACTACTAGACGGTCCTTCATTTTGGTGGCG	G
2_06826F2						CGAGCCTCCTTCATTTTGGTGCCA	A
2_06829R	55	0.5	6	57.25	166	CGAGGAAACTTGAGGATACCA	
2_06829F1						GAAGAAAGGTGGGAGCTTTACCCTGGAGCCCCT	T
2_06829F2						CGAGCCTTTACCCTGGAGCCGCC	C
2_07872R	55	0.5	11	24.78	183	ATGGGAAAAGGCATAAAAGC	
2_07872F1						GAAGAAAGGTGGGAGTTATCTGAAAACAGTCAAAGGG	G
2_07872F2						TGCGGTTATCTGAAAACAGTCAAACGA	A
2_07903R	60	1	3	109.76	207	AAGAAACCTTGGCAGGAGAA	
2_07903F1						CCATACATTACATACCTTTGCAGGTTGATAAGGGTA	A
2_07903F2						CGAGCCTTTGCAGGTTGATAAGGCTC	C
2_08233F	55	0.5	11	35.16	213	TCTGCTTTAGGGACTCCGAAT	
2_08233R1						ACCATACATTACATACAGTGCCCATCTTCTTTCAC	G
2_08233R2						ATCAAGCAGTGCCCATCTTCTTTGAT	A
2_08249R	50	0	1	82.20	200	CGGAACGATGACAAGGAAGA	
2_08249F1						ATTACTACTAGACGGGACTGGTGAAATTCCTGTGAAG	G

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2_08249F2						ATCAAGACTGGTGAAATTCCTGTGTAA	A
2_08782F	55	0	1	17.39	219	CCTTTTCCTTACCGTGTTCG	
2_08782R1						GAAGAAAGGTGGGAGATTACACAAACAGGGTCCTTCC	G
2_08782R2						CGAGCATTACACAAACAGGGTCCTACA	T
2_08868R	55	0.5	1	49.56	163	CCTTACCAAGGCAAGACCAA	
2_08868F1						ATTACTACTAGACGGGGTATCTTACGGCAACTACCT	T
2_08868F2						ATCAAGGTATCTTACGGCAACTAGCC	C
2_08889F	60	0.5	9	11.00	147	TTGTGTTTCGTTTCCTTATGTT	
2_08889R1						GAAGAAAGGTGGGAGACAACCCCTCGCCAACAC	G
2_08889R2						CGAGCACAACCCCTCGCCAAGAT	A
2_09140F	50	0	4	16.16	173	TTGGACTGTGTGAAAGGCTCT	
2_09140R1						ACCATACATTACATAAATGCCTGCTTTGAATCACTC	G
2_09140R2						ATCAAAATGCCTGCTTTGAATCAGTA	T
2_09924R	55	0.5	3	74.66	204	TTTGATTTACCAAACCCACCT	
2_09924F1						TTACTACTAGACGGATCAATTTCCATGAGTGCGTT	T
2_09924F2						TGCGGTCAATTTCCATGAGTGCCTC	C
2_09959F	55	0	5	38.07	216	GCCGCATTTCAGAAACCTT	
2_09959R1						ATTACTACTAGACGGACCTTTGAGTGGTACAATGCC	G
2_09959R2						TGCGGGACCTTTGAGTGGTACAATCCT	A
2_10843F	55	0.5	5	61.66	102	ACGGCGTCTTTCTGATGCTA	
2_10843R1						GAAGAAAGGTGGGAGAATGGCTTATGCTTTTACCGTC	G
2_10843R2						CGAGCAATGGCTTATGCTTTTACCCTT	A
2_13136R	55	0.5	7	35.90	198	ATGCAGTCAAGCGGATTTTC	
2_13136F1						GAAGAAAGGTGGGAGAGCTGTGGACACAGTGAGAGTT	T
2_13136F2						CGAGCAGCTGTGGACACAGTGAGACTC	C

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2_14997BF	55	0	7	18.05	166	GAAACTAGGGCTGAGCATCG	
2_14997BR1						TTTAACTGTTCTCCCACGCC	G
2_14997BR2						ATATAGACTATTTAACTGTTCTCCCACCCT	A
2_21071R	45	0	7	22.84	175	CCCAATGTTCAGTTTCTAACCTTT	
2_21071F1						ATCAATCGTGTGATAGAAAAGAAGTTG	G
2_21071F2						ATTACTACTAGACGGTCGTGTGATAGAAAAGAAGATA	A
2_23395F	55	0	7	25.99	207	AAGGAATGTCCTCACCCAGA	
2_23395R1						ACCATACATTACATAATACAGTTTAGGGTTTGGTGTT	A
2_23395R2						CGAGCATACAGTTTAGGGTTTGGTCTG	C
2_32056F	50	0	7	11.97	180	AATGTGGGGTAATGCAAGGA	
2_32056R1						GAAGAAAGGTGGGAGAGCAAAAATCACATAAGCAGTC	G
2_32056R2						ATCAAAGCAAAAATCACATAAGCACTT	A
2_10882R	50	0.5	3	62.67	189	CAACCACTGCCTCAAAACAA	
2_10882F1						GAAGAAAGGTGGGAGTCACCCCCCTAATTTTATCTTG	G
2_10882F2						ATCAATCACCCCCCTAATTTTATCATA	A
2_10954F	55	0.5	3	54.31	153	ATGTGCTGCTCAAGATGCTC	
2_10954R1						ATTACTACTAGACGGCCCAGCCTTACCAATGAGAC	G
2_10954R2						ATCAACCCAGCCTTACCAATGACAT	A
2_12229F	50	0.5	8	25.58	211	CTGCAATCACCATCTCATTGTT	
2_12229R1						ACCATACATTACATAAGCTGAGATGACGAATAGACAA	T
2_12229R2						ATCAAAGCTGAGATGACGAATAGAGAG	C
2_13124F	55	0.5	5	70	180	TATGCCACTGCCAGATTTGA	
2_13124R1						GAAGAAAGGTGGGAGGGCATATACGAAGGCCATATAT	A
2_13124R2						TGCGGGGCATATACGAAGGCCATAAAG	C
2_13382F	55	0.5	3	97.2	183	TCCCTCGGTTAAAATCTTGG	

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2_13382R1						ACCATACATTACATACCACCTTCACCTTTTGAATCC	G
2_13382R2						ATCAACCACCTTCACCTTTTGAAACT	A
2_13411R	50	0.5	1	20.59	220	CTGCATGAGCAAGGCTGTAA	
2_13411F1						ATTACTACTAGACGGGTGAGTGTACTTTGAGTTTGGT	T
2_13411F2						ATCAAGTGAGTGTACTTTGAGTTTCGC	C
2_14148F	50	0.5	3	15.4	194	CTGAAGGAGGCAAAAACAGC	
2_14148R1						GAAGAAAGGTGGGAGCTTGCTCTAACCAACGTATCTA	T
2_14148R2						CGAGCCTTGCTCTAACCAACGTATGTG	C
2_14205R	55	0.5	3	125.60	167	CACGTCAGCATGGGAACTC	
2_14205F1						ATCAAGCTCCCGAAGGGTTTTGG	G
2_14205F2						CCATACATTACATAGGCTCCCGAAGGGTTTAGA	A
2_15029R	55	0.5	2	60.11	201	ATCTTGCAGGCTGATCTTGG	
2_15029F1						CGAGCGTTTACATGCTTCCCCCG	G
2_15029F2						CCATACATTACATATGTTTACATGCTTCCCGCA	A
2_16297F	50	1	11	55.36	211	GCTTATGACATGCACCAAGG	
2_16297R1						ACCATACATTACATAGGCACATCTTTGCCATTTTC	G
2_16297R2						ATCAAGGCACATCTTTGCCATTATT	A
2_16636R	55	0.5	10	0.64	126	TTCTCCGCAACTTTCTCAGC	
2_16636F1						ACCATACATTACATAGCTTCGTGCTGGTAACCAT	T
2_16636F2						ATCAAGCTTCGTGCTGGTAACGAC	C
2_16708F	50	0.5	5	15.26	195	TTTCGGACAGTGAACTGCAT	
2_16708R1						ACCATACATTACATAATGCATAGAAAAGTAGGCTGAA	T
2_16708R2						ATCAAATGCATAGAAAAGTAGGCTCAG	C
2_16911F	45	0.5	1	72.30	220	TGCACTCTCCTCTGCTAATGC	
2_16911R1						ACCATACATTACAACTACACCACCCTTGTGATAA	T

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2_16911R2						ATCAATACACCACCCTTGTGAAAG	C
2_17191R	55	0	2	75.04	174	TGCTAGCCAGTGATCTTCCA	
2_17191F1						ACCATACATTACATATCGTCTTTCCCTGCTTCCTAT	T
2_17191F2						ATCAATCGTCTTTCCCTGCTTCCAAC	C
2_17476R	55	0.5	3	50.81	170	AGACACAGACATCTGGTGAAGC	
2_17476F1						ACCATACATTACATACCTTGTCTCCCAATCACGG	G
2_17476F2						ATCAACCTTGTCTCCCAATCAGGA	A
2_17704R	55	0.5	8	13.34	162	TGGCCCTTCAGTTTTTCAAC	
2_17704F1						ACCATACATTACATAGACAACTTGAAACTTCTACCAT	T
2_17704F2						ATCAAGACAACTTGAAACTTCTACGAC	C
2_19666R	50	0.5	8	50.49	146	TGAGTAACGGGAGGTACGAGA	
2_19666F1						ACCATACATTACATAACAATGGTTTTTCCTTCTCTAA	A
2_19666F2						CGAGCACAATGGTTTTTCCTTCTCAAC	C
2_20296R	60	0.5	4	31.43	179	CCTAAGCCTGCCATTTCAAG	
2_20296F1						ACCATACATTACATACTTTTCTTCACCGCCGTT	T
2_20296F2						ATCAACTTTTCTTCACCGCCCTC	C
2_20826F	50	0.5	3	20.25	158	CCCCTTGAATGAGTGTCCAT	
2_20826R1						ACCATACATTACATAAAAGGTCCCCCTAGAAGGC	G
2_20826R2						ATCAAAAAGGTCCCCCTAGAACGT	A
2_20995F	55	0	3	25.51	189	CAGGGTGATCCCTCACATTA	
2_20995R1						ACCATACATTACATAGTTGTGCTGAAAGATGCCAA	T
2_20995R2						ATCAAGTTGTGCTGAAAGATGCGAG	C
2_21226R	50	0	1	5.29	210	GATCGCTGAAAGCTGTGATG	
2_21226F1						ACCATACATTACATAGTACATGCCAAAATACAGACAG	G
2_21226F2						ATCAAGTACATGCCAAAATACAGAGAA	A

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2_21262R	55	1	5	25.41	151	TCTCCAAATCCAAACAACTCG	
2_21262F1						ATCAAATGATAACGGAATTGAAACCG	G
2_21262F2						AACCATACATTACATATGATAACGGAATTGAAAGCA	A
2_21345F	55	0.5	11	2.98	107	CGGTCTCAGTTTCTGTTTCCA	
2_21345R1						ATCAACAATGGCACAGACATCAGAC	G
2_21345R2						ACCATACATTACATCCAATGGCACAGACATCACAT	A
2_22099F	55	1	6	16.70	215	TTTTTCCCTGCCCTCTTTTT	
2_22099R1						CCATACATTACATAGGAGTTCGAATTGGTCAGCTA	T
2_22099R2						ATCAAGAGTTCGAATTGGTCAGGTG	C
2_22541R	45	0.5	5	20.86	216	GGTACGTTTTAAAATTGATATGACCA	
2_22541F1						ACCATACATTACATAGATGTTCACAGATGTACTGATG	G
2_22541F2						ATCAAGATGTTCACAGATGTACTGTTA	A
2_22565F	55	0.5	3	106.52	204	GTCCAGAAGCTCCACGAATG	
2_22565R1						ACCATACATTACATTTGGTTTTGGCTACCTTCAATA	T
2_22565R2						ATCAATGGTTTTGGCTACCTTCATTG	C
2_22946F	55	0	4	50.57	203	ATATGGGTCACACTGCCACA	
2_22946R1						ACCATACATTACATAGTAATACGGTTTCCACTTGTGC	G
2_22946R2						ATCAAGTAATACGGTTTCCACTTGAGT	A
2_23058R	55	0.5	2	10.68	215	TGGATATTTGGGAGCACATTC	
2_23058F1						ATCAAGTCCCGAAATCACTAATCGTG	G
2_23058F2						ACCATACATTACATTGTCCCGAAATCACTAATCCTA	A
2_23117R	50		10	15.14	200	ACGTGCTGTAGGACCAATCC	
2_23117F1						ACCATACATTACATAAACACAACAGAACAGAAACGTG	G
2_23117F2						ATCAAAACACAACAGAACAGAAACCTA	A
2_23610R	55	1	8	3.42	211	TCAGAACATGCACCTTTCCA	

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2_23610F1						ATCAATGGATTACCGGTGGTGTAGTG	G
2_23610F2						GAAGAAAGGTGGGATTGGATTACCGGTGGTGTACTA	A
2_23898R	55	0.5	11	37.87	187	GAGAAGGGGTGGTGTCCATA	
2_23898F1						CCATACATTACATATGCTTCAATACAGACATTAGCG	G
2_23898F2						ATCAAGCTTCAATACAGACATTACCA	A
2_25640R	55	0.5	10	29.86	197	AAATTTGGCATGTCTCTTGC	
2_25640F1						GAAGAAAGGTGGGAGGGTTTCGGGATAAAAACATACG	G
2_25640F2						CGAGCGGTTTCGGGATAAAAACATTCA	A
2_25785R	55	0.5	8	71.07	217	TGAGGTTGAGACCTATAGGCAAG	
2_25785F1						GAAGAAAGGTGGGAGTGTAAAAGTTCATCTTCCAG	G
2_25785F2						ATCAATGTAAAAGTTCATCTTCGAA	A
2_26364F	55	0	3	70.39	218	GCAAGGTGGGCTAGAACGTA	
2_26364R1						ACCATACATTACATATCTAGAAGCAAACAAATCCTCC	G
2_26364R2						ATCAATCTAGAAGCAAACAAATCCACT	A
2_27367R	55	0.5	6	19.14	210	TTCTTTCCGATTTCCACCAG	
2_27367F1						ATCAAGGGATGGGAAGAGGTTAGG	G
2_27367F2						ACCATACATTACATAGGGATGGGAAGAGGTTTGA	A
2_27735F	50	0.5	6	4.94	203	AGCCAAGGACGTTGACTTGT	
2_27735R1						ATTACTACTAGACGGAAATAAATCCAGTCAAACGC	G
2_27735R2						TGCGGAAATAAATCCAGTCAAAGGA	T

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2_27946R	55	1	9	13.99	207	TTGCTGCACCTTCATACTGC	
2_27946F1						ACCATACATTACATAGTGACGGAGGCCAGAGAG	G
2_27946F2						ATCAAGTGACGGAGGCCAGACAA	A
2_28580F	55	0.5	2	21.05	89	TGCAATATGCGAAGATGGTT	
2_28580R1						ACCATACATTACATATGATTTCTGATTGATTGGCA	T
2_28580R2						ATCAATGATTTCTGATTGATTGCCG	C
2_29720F	55	0.5	6	45.79	185	TGTTACAAAAGAGCTTTGATGTCC	
2_29720R1						GAAGAAAGGTGGGAGTGTAATGTTTCTCTCACCAGTC	G
2_29720R2						CGAGCTGTAATGTTTCTCTCACCACTT	A
2_31444F	55	0.5	8	73.00	217	AACCAAACGAATCCACATGA	
2_31444R1						GAAGAAAGGTGGGAGTCGATCTTTGCCTAGACGTT	A
2_31444R2						ATCAATCGATCTTTGCCTAGACCTG	C
2_31831F	55	0.5	3	87.32	180	TGCCTCCAATCTGAACTCAA	
2_31831R1						ACCATACATTACATATAACTACTGTTACGCCCTTGTC	G
2_31831R2						ATCAATAACTACTGTTACGCCCTTCTT	A
2_31978F	55	0.5	1	12.89	214	TTGGGAGAGTCGGAAGTGTT	
2_31978R1						ACCATACATTACATAATGGAGATTCATTGCTTTTACA	T
2_31978R2						ATCAAATGGAGATTCATTGCTTTTTCG	C
2_32586R	55	0	11	63.76	163	TTTCCCTCTTGGACGCTATG	
2_32586F1						ACCATACATTACATATTAACCAAGCGGAAGAGACG	G
2_32586F2						ATCAATTAACCAAGCGGAAGAGTCC	C
2_32890F	60	0.5	7	14.71	201	GGTCTCGGACCTGGGAAAC	
2_32890R1						ACCATACATTACATACGCCGTGACACACAGGAC	G
2_32890R2						ATCAACGCCGTGACACACAGCAG	C

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2_33297R	55	0	10	38.89	177	GGCAATGAGCCACCATAGAT	
2_33297F1						ACCATACATTACATAGAACTAAGACAAACAACCAACA	A
2_33297F2						ATCAAGAACTAAGACAAACAACCATCT	T
2_33400F	55	2	1	57.10	186	AGGTCCCACTTACGGTCAGA	
2_33400R1						ACCATACATTACATACACCATGACCAACGACAATC	G
2_33400R2						ATCAACACCATGACCAACGACATTG	C
2_33548R	55	0	2	45.08	204	CCATTTTGCAAACAGGATCA	
2_33548F1						ACCATACATTACATACCAGAACTCCTCTCCGCA	A
2_33548F2						ATCAACCAGAACTCCTCTCCCCT	T
2_34044R	55	0.5	3	92.14	223	GTTTTGCGGGGTATGGAAT	
2_34044F1						ACCATACATTACATAAAATGTTCCTTTGGTCGGG	G
2_34044F2						ATCAAAAATGTTCCTTTGGTCCGC	C
2_40097F	55	0	10	46.81	194	GCTTGCATTTGAATGGTGAA	
2_40097R1						ACCATACATTACATACACTCATCTTGAGTTCTCCGTT	A
2_40097R2						ATCAACACTCATCTTGAGTTCTCCCTG	C
2_44318R	55	0.5	10	58.54	183	TAATCCGCTAAGGCCCTTTT	
2_44318F1						ACCATACATTACATATGGTGCCTCTTGGTTACCTG	G
2_44318F2						ATCAATGGTGCCTCTTGGTTACGTT	T
2_44580F	60	0	9	25.56	189	GAGAACATTTTCGCCTGAGC	
2_44580R1						ACCATACATTACATACACGATTTAATTCATACCACAA	T
2_44580R2						ATCAACACGATTTAATTCATACCAGAG	C
2_45089R	55	1	4	56.14	217	ACCTCAATTTGTTGTGGTCAA	
2_45089F1						ACCATACATTACATATCTTCCTTTACATCTTCCTACG	G
2_45089F2						ATCAATCTTCCTTTACATCTTCCTTCT	T
2_48181F	55	0.5	4	22.59	183	ATTTCGCGGACTCGTTTTC	

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2_48181R1						ACCATACATTACATAGATCGTCACATCACACAACA	T
2_48181R2						ATCAAGATCGTCACATCACACATCG	C
2_50243F	55	0.5	3	68.71	188	AACTCAACAAATTTGCGATCC	
2_50243R1						ACCATACATTACATATTCACTCCATTGTTTGTGTCTT	A
2_50243R2						ATCAATTCACTCCATTGTTTGTGTGTG	C
2_51032R	55	1.5	2	14.46	103	TCATTCTCAAAACAAACAGGGTA	
2_51032F1						ACCATACATTACATACCTCCACAAAAGACACAGGAT	T
2_51032F2						ATCAACCTCCACAAAAGACACAGCAC	C
2_51968R	55	0.5	3	78.86	210	CAACAGGCTCTGGCTGAAAT	
2_51968F1						ATTACTACTAGACGGCCATCCCTATGTAATGCTTGTG	G
2_51968F2						ATCAACCATCCCTATGTAATGCTTCTA	A
2_52960R	55	1	11	59.60	218	TCCAGGGCCAGTTTCTACTC	
2_52960F1						GAAGAAAGGTGGGAGTGTTATTATCCTTGTTTGACGG	G
2_52960F2						ATCAATGTTATTATCCTTGTTTGAGGA	A
2_53921R	55	0.5	10	24.43	198	CTCACACACACACCCGAAAC	
2_53921F1						ACCATACATTACATAGAGCACAGGTTTTCACAGACAT	T
2_53921F2						ATCAAGAGCACAGGTTTTCACAGAGAC	C
2_54013R	60	0	10	54.18	122	TATCTGCAGCGTTCCATCTG	
2_54013F1						ACCATACATTACATACATTTCTAAAGGCCAAATTCCA	A
2_54013F2						ATCAACATTTCTAAAGGCCAAATTGCC	C
2_54765F	55	0.5	9	45.05	185	TGCACAAGTCTTTTGGGAAA	
2_54765R1						ACCATACATTACATATCTCTTCTTCTGCCTTTGGC	G
2_54765R2						ATCAATCTCTTCTTCTGCCTTTCGT	A
2_55168F	55	0.5	6	49.62	208	TGAGAGGCATATTGTGTTCCA	
2_55168R1						ACCATACATTACATACTCACTCTTAGATTGTGACGTA	T

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2_55168R2						ATCAACTCACTCTTAGATTGTGACCTG	C
2_55423R	55	1.5	10	59.53	220	CCTGAACTTGGACCACCAGT	
2_55423F1						ACCATACATTACATAATCAAATTCTACCCACTTACAG	G
2_55423F2						ATCAAATCAAATTCTACCCACTTAGAA	A
2_01176R	55	0.5	6	0	163	GGGCATTCGTCGTTCTGTAT	
2_01176F1						CGAGCGCGTTGGAGAACGACTGAAAC	T
2_01176F2						GAAGAAAGGTGGGAGGCGTTGGAGAACGACTGATAA	C
2_02374F	55	0	6	11.01	158	GGGCATTCGTCGTTCTGTAT	
2_02374R1						ATCAAGCGTTGGAGAACGACTGAAAC	G
2_02374R2						GAAGAAAGGTGGGAGGCGTTGGAGAACGACTGATAA	T
2_04951F	55	0.5	5	29.27	165	TGGGTTAACCAAACCATCTT	
2_04951R1						ACCATACATTACATATCCACTTATCCAAACAGAGAAC	G
2_04951R2						ATCAATCCACTTATCCAAACAGAGTAT	A
2_05791R	55	0	11	39.88	126	TCATGAAACCAATTCCTCCA	
2_05791F1						ACCATACATTACATATTTGCCCAGAAGTTCCACAG	G
2_05791F2						ATCAATTTGCCCAGAAGTTCCAGAA	A
2_11123F	55	0.5	8	20.55	169	TTTTGAGGAACCAGACAACAGA	
2_11123R1						ACCATACATTACATAGAGTCAGCCAAACATTATCAC	G
2_11123R2						ATCAAGAGTCAGCCAAACATTATGAA	T
2_11663F	55	1	1	1.68	175	TGGTTACATGGTTTGTTCTGC	
2_11663R1						ACCATACATTACATATCAGACTGGCCTCAGATAACAA	T
2_11663R2						ATCAATCAGACTGGCCTCAGATAAGAG	C
2_11687R	50	1	9	54.42	150	ACTCCAACAAGGAGGTGGTG	
2_11687F1						ACCATACATTACATACCTCCTGCTTACTTTCCAAAT	T
2_11687F2						ATCAACCTCCTGCTTACTTTCCATAC	C

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2_12433F	55	0.5	3	13.98	120	CGAGTGAGGCGAGTGAAATC	
2_12433R1						ACCATACATTACATACTCTCTCCCAAACCCTAGCC	G
2_12433R2						ATCAACTCTCTCCCAAACCCTACCT	A
2_15712F	55	0.5	2	66.32	172	TCTGCAAATACAAAATGCATCA	
2_15712R1						ACCATACATTACATATCCCACTCTGCAGGTATTACAA	T
2_15712R2						ATCAATCCCACTCTGCAGGTATTAGAG	C
2_16448R	55	0.5	3	35.30	164	AAGGTGATTCATGGCTCTGG	
2_16448F1						ACCATACATTACATACTCCAAGGAGGGGAGAGACG	G
2_16448F2						ATCAACTCCAAGGAGGGGAGAGTCA	A
2_19370R	55	1	10	43.32	143	TGTTCTCACTGCGGAACAGT	
2_19370F1						ACCATACATTACATATGTTTCCATTTTCACACAAG	G
2_19370F2						TGCGGTGTTTCCATTTTCACACTAA	A
2_19626F	55	0.5	3	131.58	168	TGGCACTTCAGAATAGCTCTAGG	
2_19626R1						ATTACTACTAGACGGCAGGTATTACTCTCCCTCGCC	G
2_19626R2						CGAGCCAGGTATTACTCTCCCTCCCT	A
2_22012F	55	0.5	11	42.35	170	ATCCTGTGAATCCTCGAAGC	
2_22012R1						ACCATACATTACATATCAACCCTGACAATGGACTC	G
2_22012R2						ATCAATCAACCCTGACAATGGAGTT	A
2_22741R	55	0.5	8	34.09	166	GTCTGGTTCAGGTCCCAATG	
2_22741F1						ATCAACCCTGGACAACCTTCACAAAG	G
2_22741F2						ACCATACATTACATACCCTGGACAACCTTCACATAA	A
2_24787R	60	0	1	10.55	148	TGGTTCTGATTACGCAGTCG	
2_24787F1						ACCATACATTACATATAGACACGCAATTAGGGCGG	G
2_24787F2						ATCAATAGACACGCAATTAGGGGGA	A
2_24988F	55	0	3	39.57	92	TCAGCTATCAATCTGGTCAGGA	

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2_24988R1						ATCAATAAGCCAATTGAAGCTCCCC	G
2_24988R2						ACCATACATTACATATAAGCCAATTGAAGCTCGCA	T
2_28435F	55	1	2	3.08	173	TCGAAGAAGAAGAAGAAGAAGAAAA	
2_28435R1						ATCAATGTTGCTAATAACCCATGGC	G
2_28435R2						ATTACTACTAGACCCTGTTGCTAATAACCCATCGT	A
2_31400F	55	0.5	3	129.37	109	GGTGGAAGCAGTTCCTGAAA	
2_31400R1						ACCATACATTACATAGCTTGTCTCATCTCATGGGTC	G
2_31400R2						ATCAAGCTTGTCTCATCTCATGGCTA	T
2_32479R	55	1	8	78.36	175	GCAAAGCTTAGCAGGGAGAA	
2_32479F1						ACCATACATTACATACAAAGCAGAGACACCCAAGAG	G
2_32479F2						ATCAACAAAGCAGAGACACCCAACAC	C
2_39135R	55	1.5	1	78.26	129	AAGCAACAACGACACACAGC	
2_39135F1						ACCATACATTACATACATTGCCATCTGTGTCCCTT	T
2_39135F2						ATCAACATTGCCATCTGTGTCCGTC	C
2_46163F	55	0	6	59.44	164	TTTGTGCATTTTACTCCTTTGC	
2_46163R1						ATTACTACTAGACGGCAATTCCCCTTCCATTATATTC	G
2_46163R2						ATCAACAATTCCCCTTCCATTATAATT	A
2_48173F	55	0.5	1	24.68	160	TTAGTGTGGCAGTGACAGGTG	
2_48173R1						ATTACTACTAGACGGCATGTGCAGAAGAAAGAGGA	T
2_48173R2						ATCAACATGTGCAGAAGAAAGACGG	C
2_51037F	55	1.5	2	29.85	133	TTTTTGTTTTGCTTTGGTCA	
2_51037R1						ATTACTACTAGACGGTCAAGCTTAAAACGAAGAACAC	G
2_51037R2						ATCAATCAAGCTTAAAACGAAGAAGAT	A
2_55199F	55	0.5	1	37.89	159	TGGTCTCCTTGTGCTACCTCT	
2_55199R1						ACCATACATTACATATTTACCTTGGGCTTTTTGAA	T

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2_55199R2						ATCAATTTACCTTGGGCTTTTTCAG	C
1_0473F	55	1	9	40.55	154	TTTCTGCTGAAGTGTGTGGAA	
1_0473R1						ACCATACATTACATATATTAACCTCCTCAACCGCC	G
1_0473R2						ATCAATATTAACCTCCTCAACCCCT	A
2_00243F	55	0.5	3	30.75	125	AGTTGGCTGGGAAAGAAAGC	
2_00243R1						ATCAACATTCCCAGAATCCCGCC	G
2_00243R2						ACCATACATTACAATCATTCCCAGAATCCCCCA	T
2_00323R	55	0.5	6	1.70	176	CCAATCACCCTCGTACACTG	
2_00323F1						ATCAATCTCTTTACTTTTAGCTTGCGG	G
2_00323F2						ACCATACATTACATATCTCTTTACTTTTAGCTTGGGA	A
2_04705R	55	0.5	11	48.85	166	TGGTGAACCATGGGATTGAT	
2_04705F1						ATCAAGAAGCTTGGAGGAGAAGCG	G
2_04705F2						ACCATACATTACATGGAAGCTTGGAGGAGAACCT	T
2_06275R	55	0.5	5	33.97	160	AATCAGTTGTGTCCCGTGCT	
2_06275F1						ATTACTACTAGACGGTGTGAACCCAAGATGAGACAT	T
2_06275F2						ATCAATGTGAACCCAAGATGAGAGAC	C
2_14455F	55	0.5	9	65.44	170	GGATCGTGATGATGGTGTGA	
2_14455R1						ACCATACATTACATAAGCATAGCTTTCTTCATCAAAC	G
2_14455R2						ATCAAAGCATAGCTTTCTTCATCATAT	A
2_15464F	55	0	3	2.56	100	CCCATATCTCCTGGCCAAC	
2_15464R1						ACCATACATTACATAAGGCAAGGCTAAGGGTGTAA	T
2_15464R2						ATCAAAGGCAAGGCTAAGGGTGAAG	C
2_16425F	55	0.5	10	51.96	169	TGAAGGGGTATGCACCTAGC	
2_16425R1						ACCATACATTACATACACCAGCAGATTCAACCTAA	T
2_16425R2						ATCAACACCAGCAGATTCAACCAAG	C

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2_16837R	55	0.5	7	48.98	173	ATCAGCAGCCGAAATGCTAT	
2_16837F1						CCATACATTACATATGTCCAGCTGTGGAAATCCT	T
2_16837F2						ATCAAGTCCAGCTGTGGAAATGCC	C
2_16995R	55	0.5	1	56.56	168	CGATTTTGGGAGTGCAAAAG	
2_16995F1						ACCATACATTACATATGAGAACAAATCCTAAAAGGGA	A
2_16995F2						ATCAATGAGAACAAATCCTAAAAGCGC	C
2_17172F	60	1.5	7	33.75	135	GATTGTGGGACAGAGTTTGC	
2_17172R1						ACCATACATTACATATTCCTGACCCTTTTATTGGA	T
2_17172R2						ATCAATTCCTGACCCTTTTATTCGG	C
2_19698F	50	0.5	4	62.36	162	TTAAAGTGCCAAAGCCTTCC	
2_19698R1						ATTACTACTAGACGGGTGTTCTCCGCCGCTTTA	T
2_19698R2						ATCAAGTGTTCTCCGCCGCTATG	C
2_21023R	55	1	7	3.65	163	CTTTCCTCTTTGGTACTGTGCTC	
2_21023F1						ACCATACATTACATAAGGTGGGAGAACAGTGGCTG	G
2_21023F2						ATCAAAGGTGGGAGAACAGTGGGTA	A
2_22017R	55	0.5	8	38.39	113	TCAACGGTTGTGACAGTGGT	
2_22017F1						ACCATACATTACATAGCAAATTCATAACCAAGCCG	G
2_22017F2						ATCAAGCAAATTCATAACCAAGGCA	A
2_24923R	55	0.5	8	6.43	148	TCCAATACAAAACCTTTCTAACCA	
2_24923F1						ATCAAAAGGGTATCCAACCTTTCCT	T
2_24923F2						ACCATACATTACATAAAGGGTATCCAACCTTTGCC	C
2_31767R	60	1	1	29.37	173	CAGGGATGGCAGGAAACTTA	
2_31767F1						ACCATACATTACATACATAGAATGTTAGTGCCCGAT	T
2_31767F2						ATCAACATAGAATGTTAGTGCCCCAC	C
2_53682R	55	0.5	5	6.07	128	GCTGTGTTTATTTTAAAACTGTTGGA	

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2_53682F1						ACCATACATTACATTTTTGCAAACTTAGACTTTCCT	T
2_53682F2						ATCAATTTGCAAACTTAGACTTTGCC	C
2_00054R	55	0.5	9	34.08	168	CAAGCTTTGGCAAAAACCTATT	
2_00054F1						ATTACTACTAGACGGGAAATAAAGCATTATGGGCG	G
2_00054F2						ATCAAGAAATAAAGCATTATGGCCA	A
2_03016R	55	0.5	4	54.95	193	CATTGCAATAAGCCTCCACA	
2_03016F1						ATTACTACTAGACGGGATTCTCGGCAATGGATGG	G
2_03016F2						ATCAAGATTCTCGGCAATGGAAGA	A
2_04477R	55	0.5	2	36.98	198	TGTTAATGGAGCCTGAGTCG	
2_04477F1						ACCATACATTACATATCTTCCCAGAATACGCAACTT	T
2_04477F2						ATCAATCTTCCCAGAATACGCAAGTC	C
2_08039F	55	0.5	5	12.00	144	TCCTTTTAGTCCCTTTGCTGA	
2_08039R1						TTACTACTAGACGGTTTTTCATGTGCTTCAATGTT	A
2_08039R2						ATCAATTTTCATGTGCTTCAATCTG	C
2_13064F	55	0.5	11	1.41	165	TGCCTCACCAAAATTCTCCT	
2_13064R1						ACCATACATTACATACCACTGGATGCCTAGAACAA	T
2_13064R2						ATCAACCACTGGATGCCTAGAAGAG	C
2_20667F	55	1	7	51.86	174	AGAGATCCGCGCTCTATCAA	
2_20667R1						ACCATACATTACATAATTCACTTGCAGGGGATGA	T
2_20667R2						ATCAAATTCACTTGCAGGGGAAGG	C
2_22775R	50	0	5	47.79	189	TCCAAGGCCATTCTTATCGT	
2_22775F1						ACCATACATTACATACCATTTAGCCTTTCATTTCCTG	G
2_22775F2						ATCAACCATTTAGCCTTTCATTTCGTA	A
2_24145F	55	0	3	101.58	133	GCCTGGAATTTGTGGTTGTT	
2_24145R1						GAAGAAAGGTGGGAGTACACTTCCAGAGTTCCGCC	G

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2_24145R2						ATCAATACACTTCCAGAGTTCCCCA	T
2_24387F	55	0	2	40.49	137	TTTGCAGCAATTGAGAAAACA	
2_24387R1						ACCATACATTACATATCATCCTATTCACCAAGCTCC	G
2_24387R2						ATCAATCATCCTATTCACCAAGCACT	A
2_27951F	55	0.5	11	20.14	173	CCTGAGTATGCAATGCGAAA	
2_27951R1						ATCAATGGACGGAAAAAACATATCC	G
2_27951R2						ACCATACATTACATCTGGACGGAAAAAACATAACT	A
2_32753F	55	1.5	4	37.20	172	GATACGTCCCTTTCCCATCA	
2_32753R1						ACCATACATTACATACGGCAGCTAGATTATTGCGT	A
2_32753R2						ATCAACGGCAGCTAGATTATTGGGA	T
2_49906F	55	0.5	4	11.25	190	GAATCATTCGGTGCATTTCA	
2_49906R1						ACCATACATTACATATCAAACTCCTAAAAATTGGCA	T
2_49906R2						ATCAATCAAACTCCTAAAAATTGCCG	C
2_05322F	55	0.5	3	47.34	167	AAAAGAAGGCCGACAGGACT	
2_05322R1						CCATACATTACATAGCTCTGCCAACACTTCAGCA	T
2_05322R2						ATCAACTCTGCCAACACTTCACCG	C
2_42732R	55	1	6	23.82	182	CGCATTGTGACCTCAGAAGA	
2_42732F1						ATCAAAACATTCAGGTCCACTTCCG	G
2_42732F2						ACCATACATTACATGAACATTCAGGTCCACTTGCT	T
2_17305R	55	0.5	8	86.64	193	CCAACACTAATGCTAAAGAGTGAGA	
2_17305F1						ACCATACATTACATATCACATTTACAGGTTCAAGCAT	T
2_17305F2						ATCAATCACATTTACAGGTTCAAGGAC	C
2_09144R	55	0.5	5	3.12	173	TCACTCAATTGATTTCCCCTTC	
2_09144F1						ACCATACATTACATAGGTTTCTCCCAGCAAAGGA	A
2_09144F2						ATCAAGGTTTCTCCCAGCAAACGC	C

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2_24046R	55	0	2	56.92	152	CACCTTCAAAGCTTCATCTGC	
2_24046F1						ACCATACATTACATAGTTTTGTGGATGCTCTTTTAAT	T
2_24046F2						ATCAAGTTTTGTGGATGCTCTTTTTAC	C
2_24897R	55	0.5	2	16.88	199	CGACCAAAGTTGGCTTATCA	
2_24897F1						ATCAAAGTCTTATTGGTCAAAGCCG	G
2_24897F2						ACCATACATTACACAAGTCTTATTGGTCAAAGGCA	A
2_11056R	55	0.5	1	69.29	189	AGGGGTGACCCTTCCATTAG	
2_11056F1						ATTACTACTAGACGGTGTGAATCCAACACAAACCTT	T
2_11056F2						ATCAATGTGAATCCAACACAAACGTC	C

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APPENDIX E
Statistical Analysis of Obtained data
One-way ANOVA: Pod formation between Resistant and Susceptible RILs

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	2	P. Resistant, P. Susceptible

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	1	6.372	6.372	4.10	0.046
Error	85	131.973	1.553		
Total	86	138.345			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.24604	4.61%	3.48%	0.44%

Means

Factor	N	Mean	StDev	95% CI
P. Resistant	56	1.339	1.366	(1.008, 1.670)
P. Susceptible	31	0.774	0.990	(0.329, 1.219)

Pooled StDev = 1.24604

Correlation: Number of flowers formed vs Number of emerged *Striga*

Pearson correlation of No. of Flowers and No. of Striga = -0.221
P-Value = 0.040

Correlation: Number of pods vs Number of emerged *Striga*

Pearson correlation of No. of pods and No. of Striga = -0.225
P-Value = 0.036

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