Deployment of the cowpea aphid resistance gene for cowpea improvement in Ghana

By

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Declaration of Originality

I certify that the substance of this thesis has not been submitted for any degree and it is not being submitted for any other degree. I certify that to the best of my knowledge any help received in preparing this thesis and all sources used have been acknowledged in this thesis.

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Dedication

This work is dedicated to my wife, Lydia and our children Prince, Emmanuel, Francis and Bright for their love and support during my studies.

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Abbreviations

ANOVA	Analysis of Variance
APS	Ammonium persulphate
BC	Backcrossing
bp	Base pair
CABMV	Cowpea aphid-borne mosaic virus
CGIAR	Consultative Group on International Agricultural Research
CRIG	Cocoa Research Institute of Ghana
CSIR	Council for Scientific and Industrial Research
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of Congo
EtBr	Ethidium Bromide
FAO	Food and Agriculture Organization of the United Nations
FTA	Fast technology for analysis
GPS	Geographic position system
h-PAGE	horizontal-Polyacrylamide gel electrophoresis
IITA	International Institute of Tropical Agriculture
MAS	Marker-assisted selection
NARS	National agricultural research system
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PLABSIM	PLAnt Breeding SIMulation
QTL	Quantitative trait loci
SARI	Savannah Agricultural Research Institute
SAT	Semi Arid Tropics

SSA	Sub Sahara African
SSR	Single sequence repeat
TBE	Tris/Borate/EDTA
TEMED	Tetramethylethylenediamine
UV	Ultraviolet

Abstract

Resistance to the cowpea aphid is important component of integrated pest management of cowpea cropping systems most especially at the vegetative stage. The objective of this study was to demonstrate the effectiveness of the aphid resistance locus identified in advanced breeding line SARC 1-57-2 in reducing damage from the cowpea aphid in Ghana. Using an F_2 population developed from Apagbaala x SARC 1-57-2, the resistance locus was tagged with the SSR marker CP 171F/172R with a recombination fraction of 5.91%. Based on the CP 171F/172R, recurrent marker assisted backcrossing was carried out to introduce the resistance locus into the susceptible cultivar, Zaayura. This led to the development of several BC_4F_3 lines that are isogenic except for the region of the resistance locus. In field tests under no insecticide protection, the BC_4F_3 lines carrying the dominant marker allele suffered 3% loss of biomass and 4% loss of grain yield compared with plots protected with recommended insecticides. The BC_4F_3 lines carrying the recessive marker allele recorded 12% loss of biomass and 33% reduction in grain yield compared with the sprayed plots. The resistance locus did not influence the number of days to flowering or maturity and no pleiotropic effects were observed in terms of plant morphology or seed characteristics. In all segregating populations analysed, the locus segregated as a single Mendelian gene. Stability of the resistance locus was conducted at 18 locations covering six important cowpea growing Regions in Ghana. The range of damage by the pest on resistant and susceptible progenies were consistent across locations, and did not support the hypothesis of existence biotypes of the insect (based on differences in feeding damage on different varieties) in Ghana. This stability in performance places a premium on the resistance locus in improving cowpea cultivars developed for different agro-climatic regions of the country for resistance to the pest. The study has demonstrated the effectiveness of an insect resistance locus in significantly reducing insect damage under typical cowpea production conditions in Ghana.

CHAPTER ONE

1.0. Introduction

Cowpea, *Vigna unguiculata* (L.) Walp is an important source of protein for human nutrition in many parts of the semi-arid tropics (SAT) (Rachie, 1985; Githiri *et al*., 1996; Bashir *et al*., 2002). It is eaten in the form of dry seeds, green pods, green seeds, and tender green leaves (Githiri *et al*., 1996). Cowpea is also an important source of protein for animal nutrition; it is used for pasture, hay, silage, or green manure (Singh, 1990). Nigeria, Brazil, Niger and Burkina Faso are among the major producers and account for over 70 % of the world crop (FAO, 2008). Nigeria is the largest producer and consumer of cowpea, with about 5 million ha and over 2 million mt production annually, followed by Niger (650,000 mt) and Brazil (490,000 mt) (Timko *et al*., 2008: FAO, 2008; Asare, 2012). However, yields at farmers level are low (Jackai and Dacoust, 1986; Motimore *et al*., 1997: Asare, 2012). The major cause of the low yields are insect pests, diseases, drought and low soil fertility, of which insect pests constitute the major constraint (Nampala *et al*., 1999; Asare, 2012).

Cowpea suffers serious insect pest infestation from the time of planting through harvesting and during storage (Obeng-Ofori, 2007). The crop therefore suffers severe attack of pre-harvest and post-harvest infestation which if not controlled could lead to total crop failure. The major field pests of cowpea are aphids (*Aphis craccivora* Koch*)*, flower bud thrips (*Megalurothrips sjostedti* Trybom), the legume pod borer (*Maruca vitrata* Fab), pod-sucking bugs including *Clavigralla tomentosicollis* Stål, *Anoplycnemis curvipes* Fab., *Mirperus jaculus* Thunbeng and *Nezera viridula* Linnaeus (Singh and Jackai, 1985; Jackai and Adalla, 1997; Obeng-Ofori, 2007: Egho, 2011). The cowpea aphid, *A. craccivora*, is an important pest of cowpea in Africa (Singh and Jackai, 1985; Kusi *et al*., 2010a; Souleymane *et al.*, 2013). The pest primarily infests the seedlings of cowpea and causes direct damage to the crop by sucking plant sap, resulting in stunted plants and distorted leaves (Bohlen, 1978; Jackai and Daoust, 1986; Ofuya, 1997a). Aphids are usually found in clusters around stems, young leaves and on young shoots. The infested leaves are often cupped or distorted and become more or less yellow (Singh and Jackai, 1985). In heavy infestation the plant dies, especially under water stress (Ofuya, 1995). High numbers of cowpea aphids can produce a significant amount of honeydew and sooty mould which reduce the photosynthetic ability of the leaves (Baute, 2004). Indirectly, cowpea aphid transmits aphid-borne cowpea mosaic viruses (Singh and Jackai, 1985; Thottappilly and Rossel, 1985; Shoyinka *et al*., 1997). Estimated yield losses of 20% to 40% in cowpea due to *A. craccivora* infestation in Asia and up to 35% in Africa have been reported (Singh and Allen 1980; Kusi *et al.*, 2010b). In eastern region of the Democratic Republic of Congo, *Aphis craccivora* (Hemiptera: Aphididae) is a major pest of cowpea and groundnut (Munyuli *et al.*, 2007) where about 35-65% of yield losses are associated with this pest species (Munyuli *et al.*, 2008; Munyuli, 2009).

The cowpea aphid can be controlled by various methods including the use of insecticides, cultural practices and biological control (Singh and Jackai, 1985). However, growing of aphid resistant cultivars offers one of the simplest and most convenient methods of pest control for the resource-poor farmers (Dent, 1991; Orawu *et al*., 2013).

Host plant resistance as indicated by Painter (1951) is a relationship between the plant feeding insects and their host-plants. It is the property that enables a plant to avoid, tolerate or recover from injury by insect populations that will cause greater damage to other plants of the same species under similar environmental conditions (Kogan, 1975; Tingey, 1986). Kumar (1984) and Dent (1991) defined host plant resistance as the inherent ability of crop plants to restrict, retard or overcome pest infestation and thereby improve yield and/or quality of the harvested product. Host plant resistance has proved to be a successful tool against insect pests that attack many crops (van Emden, 1991; Thomas and Waage, 1996; Felkl *et al.*, 2005; Orawu *et al.*, 2013). Plant genotypes, either due to environmental stress or genetic makeup, possess physiological and biochemical differences which alter the nutritional value (primary metabolites) and may also cause changes in the levels of secondary metabolites that could affect the behaviour of phytophagous insects (Eckey-Kaltenbach *et al.*, 1994; Karban and Baldwin, 1997: Siemens *et al.*, 2002; Städler, 2002; Theis and Lerdau, 2003).

Three mechanisms of plant resistance originally defined by Painter (1951) are non-preference, antibiosis and tolerance. The non-reference has since been replaced by antixenosis (Kogan and Omar, 1978). Antixenosis is the inability of a plant to serve as host to an insect herbivore. The basis of this resistance mechanism can be morphological (e.g. leaf hairs, surface waxes, tissue thickness) or chemical (e.g. repellents or antifeedants) (Kogan and Omar, 1978). These plants would have reduced initial infestation and/or higher emigration rate of the insect than susceptible plants.

Antibiosis is the mechanism that describes the negative effects of a resistant plant on the biology of an insect which has colonized the plant (e.g. adverse effect on development, reproductive and survival) (Painter, 1951; Kogan and Omar, 1978). Both chemical and morphological plant defences can induce antibiosis effects (Painter, 1951; Kogan and Omar, 1978). The consequences of antibiosis resistance may vary from mild effect that influences fecundity, development time and body size to acute direct effect resulting in death (Painter, 1951; Kogan and Omar, 1978).

Plant tolerance is the degree to which a plant can support an insect population that under similar conditions would severely damage a susceptible plant (Painter, 1951; Kogan and Omar, 1978). When two cultivars are equally infested the less tolerant one will produce low yield.

Cowpea aphids are easily controlled by the use of aphid resistant varieties (Singh, 1977; Obeng-Ofori, 2007). Several aphid-resistant cowpea lines have been identified at the IITA and have been tested against aphid populations from several locations in Africa and Asia (Chari *et al*., 1976; Dhanorkar and Daware, 1980; Karel and Malinga, 1980; MacFoy and Dabrowski, 1984; Manawadu, 1985; Ofuya, 1988a; 1993). Antibiosis has been shown as the main mechanism responsible for aphid resistance in cowpea (Singh, 1977; Ansari, 1984; Ofuya, 1988b,) and is controlled by a single dominant gene (Singh and Ntare, 1985; Bata *et al.*, 1987; Ombakho *et al.*, 1987; Singh, *et al*., 1987; Pathak, 1988). Additionally, a large number of aphid-resistant lines have been developed, and have been evaluated in international yield trials (MacFoy and Dabrowski, 1984; Manawadu, 1985; Ofuya, 1988a; 1993).

1.1. Justification

Farmers have over-relied on chemical insecticides over the years to control cowpea aphid which has resulted in misuse of chemicals, high cost of production, poisoning of human beings, the environment and development of resistance to most of the insecticides leading to resurgence of the aphids (Dent, 1991; Singh and Jackai, 1985).

More recently, Kusi *et al.* (2010a) identified new sources of cowpea genotypes (SARC1-57-2) resistant to *A. craccivora*. Segregation ratio in F_2 population generated between a resistant line and Apagbaala (a susceptible parent) suggested that a single dominant gene controlled resistance to the aphid in the breeding line. This presents a valuable source of resistance for developing cowpea cultivars with resistance to the cowpea aphid in the field. Ongoing efforts at mapping the cowpea genome presents an opportunity to tag the resistance locus with co-dominant PCR based markers to facilitate marker-based selection of aphid resistant progenies in large segregating populations.

1.2. Objectives

The main objective of the study was to demonstrate the effectiveness of an aphid resistance locus in the cowpea line SARC 1-57-2 at controlling aphid in Ghana and specifically:

1. Identify DNA marker(s) tightly linked to locus controlling resistance to the cowpea aphid in a resistant breeding line, SARC 1-57-2.
2. Deploy the DNA marker(s) to improve at least one cowpea cultivar through marker-assisted backcrossing.
3. Assess the stability of the cowpea aphid resistant line in the major cowpea growing regions in Ghana.
4. Determine yield loss due to aphid infestation in near isogenic lines developed from the resistant line SARC 1-57-2 and Zaayura

CHAPTER TWO

2.0. Literature Review

2.1. Origin and Cultivation of Cowpea

Cowpea (*Vigna unguiculata* (L.) Walp), is said to have originated in Africa, where it has become an integral part of traditional cropping systems, particularly in the semi-arid West African savannah (Steele, 1972). The history of cowpea dates to ancient West African cereal farming, 5 to 6 thousand years ago, where it was closely associated with the cultivation of sorghum and pearl millet (Davis *et al*., 2003). Cowpea is an annual legume and is also commonly referred to as southern pea, blackeye pea, crowder pea, lubia, niebe, coupe or frijole. Worldwide cowpea production has increased dramatically in the last 25 years (Davis *et al*., 2003). It is widely grown in Africa, Latin America, Southeast Asia and in the Southern United States (Fery, 1985; Mishra *et al*., 1985; Singh and Ntare, 1985).

2.2. Production Level

Cowpea, a native crop of West Africa, is one of the most important food legume crops now grown in the semi-arid tropics covering Asia, Africa, southern Europe and Central and South America (Akibode and Maredia, 2011). Total cowpea area harvested has risen by 38% between 1994-06 and 2006-08 (Akibode and Maredia, 2011; FAO, 2011). World cowpea production has increased 88% and yields have increased by 35% in the same time period. This increase in area, production and yield has been made possible by a similar trend in Sub Sahara African (SSA), which dominates the world scene (Akibode and Maredia, 2011; FAO, 2011). Despite the dramatic increase in production in SSA, cowpea yields remain one of the lowest among all food legume crops, averaging at 450 kg/ha in 2006-08 (Akibode and Maredia, 2011; FAO, 2011).

The top five cowpea growing countries in African are all in West Africa. Nigeria and Niger have maintained the top first and second position over the past 14 years, together covering more than 80% of total cowpea area in the world (FAO, 2011). Other important cowpea growing countries include Burkina Faso (6%), Mali (2%) and Senegal (2%). These five West African countries share more than 90% of the world cowpea area harvested in 2006-08 (Akibode and Maredia, 2011; FAO, 2011). While area cultivated has stayed stable in Burkina Faso, Mali and Senegal over the last 14 years, it has fluctuated significantly in Niger and Nigeria with drops and increments at the scale of more than 1 million ha (FAO, 2011). The average yields in Nigeria have steadily increased since mid-1990s and have reached around 700 kg/ha in recent years (Akibode and Maredia, 2011: FAO, 2011). Compared to Nigeria, all the other top cowpea growing countries in West Africa have significantly lower yields (almost by 200-300 kg/ha). Except, for Nigeria and Niger, these countries have either experienced a decline in average yields or yields have remained stagnant over the past 14 years (Akibode and Maredia, 2011; FAO, 2011). Cowpea is one of the most widely grown grain legumes in Ghana but, commercial production is restricted to some parts of the Volta, Northern, Upper East, Upper West and Brong-Ahafo regions (Tweneboah, 2000). In Ghana the estimated researcher-managed on-farm yields of 1.8 t ha^{-1} are more than double the average farm level yields (SARI, 1999). Reasons for the low yields in most countries include use of low yielding traditional varieties, poor soil fertility, unfavorable weather, and insect pest and disease attack (Diehl and Sipkins, 1985; Montimore *et al*., 1997; Blade *et al*., 1997; Asare, 2012).

2.3. Uses and Nutritional Value of Cowpea

Cowpea is one of the five most important legumes in the tropics and provides the source of protein for most people in the region. According to Jackai and Singh (1983), 100 g raw mature seeds typically contain 11.4 g moisture, 338 kcal (1415kj) of energy, 22.5 g protein, 1.4 g fat, 61.0 g total carbohydrate, 5.4 g fiber, 3.7 g ash, 104 mg Ca, 416 g P, 0.08 mg thiamine, 0.09 mg riboflavin, 4.0 mg niacin, and 2 mg ascorbic acid. Cowpea has many uses, in fresh form, the young leaves, immature pods and peas are used as vegetables, while several snacks and main meal dishes are prepared from the grain (Jackai and Singh, 1983). All parts of the plant that are used for food are nutritious, providing protein, vitamins (notably vitamin B) and minerals. The cowpea haulm is also an important source of livestock feed, and therefore of great value to farmers.

2.4. Insect Pests of Cowpea

Cowpea suffers serious insect pest infestation from the time of planting through harvesting and during storage (Obeng-Ofori, 2007). Several pre-harvest and post-harvest insect pests are associated with the crop (Tables 2.1 and 2.2), which if not controlled could lead to total crop failure. In general, cowpea suffer more damage as a monocrop than as a mixed crop which is the traditional method of production (Singh and Jackai, 1985). Studies have shown that in unprotected monocrops, yield losses due to the major field pests may range from 20-100% (Youdeowei, 1989). The pest problem is more serious in Africa than in Asia or Latin America (Singh and Jackai, 1985).

In Ghana, Agyen-Sampong (1978) reported that, there were more than 150 species of insects recorded to be associated with cowpea in both field and storage, but only few were of major economic importance. The pest complex of cowpea in Ghana includes leafhoppers, *Empoasca spp*, aphids, *Aphis craccivora* Koch, flower bud thrips, *Megalurothrips sjostedti* (Trybom), pod borers, *Maruca vitrata* (Fab), pod-sucking bugs *Clavigralla tomentosicollis* Stål, *Nezera viridula* Linnaeus, *Leptoglossus spp* and bruchids *Callobruchus spp*. In Africa the major field pests of cowpea are aphid (*A. craccivora*), legume flower thrips *(M. sjostedti)*, legume pod borer (*M. vitrata*) and pod-sucking bugs (*C. tomentosicollis, Leptoglossus spp* and *N. viridula*) (Singh and Jackai, 1985; Jackai and Adalla, 1997; Obeng-Ofori, 2007).

The principal storage pest of cowpea grain in Sub-Saharan Africa is the cowpea beetle *Callosobruchus maculatus* Walp (Taylor, 1981). In low resource farms, *C. maculatus* infestation starts in the field and continues in storage. Another bruchid pest of cowpea is *C. chinensis* L. (Taylor, 1981). Other storage pests of cowpea include *Acanthoselides obtectus* (Say), *A. clandestinus* (Mots), *C. analis* (F), *C. rhodesianus* Pic and *Zabrotis subfasciatus* (Boheman), all in the family Bruchidae (Obeng-Ofori, 2007).

Table 2.1. Major Insect Pest Species Found on Cowpea Worldwide

Pest Species (Order: Family)	**Geographical Distribution**	**Plant Part Attacked**	**Importance**
Callosobruchus sp. (Coleoptera: Bruchidae)	Cosmopolitan	Seed (Storage)	Key
Chalcodermus sp. (Coleoptera : Curculionidae	USA South America	Pods	Key
Ophiomyia phaseoli (Trybom) (Diptera: Agromizidae)	Asia, Africa	Leaves, Stem	Key, Sporadic
Clavigralla tomentosicollis Stål (Hemiptera : Coreidae)	Africa	Pods	Key
	Asia	Pods	Minor
	South America	Pods	Minor
Crinocerus sanctus (Fab) (Hemiptera: Coreidae)	South America	Pods	Key
Leptoglossus sp. (Hemiptera : Coreidae)	USA	Pods	Sporadic
Lygus hysperus (Hemiptera : Miridae)	USA	Pods, Leaves	Key
Nezera viridula Linnaeus (Hemiptera : Pentatomidae)	USA	Pods	Key
	Africa	Pods	Sporadic
	Asia	Pods	Sporadic
	South America	Pods	Sporadic
Aphis craccivora Koch (Homoptera: Aphididae)	Cosmopolitan	Foliage, flowers, pod	Key
Empoasca biguitula (Shiraka)	Asia	Leaves	Unknown

Table2.1. Continued

Empoasca dolichi Paoli (Homoptera: Cicadelidae)	Africa	Leaves	Key
Empoasca kraemri Ross and Moore (Homoptera:Cicadelidae)	South America	Leaves	Key
Amsacta moorei (Butler) (Lepidoptera: Arctiidae)	Africa (Senegal)	Leaves	Sporadic
Elasmopalpus lignosellus (Zeller) (Lepidoptera: Pyralidae	South America	Stem	Key
Etiella zinckenella (Treitschke) (Lepidoptera : Pyralidae)	Asia	Pods, Flowers	Sporadic
Maruca vitrata (Fab) (Lepidoptera : Pyralidae)	Cosmopolitan (Rare in America)	Stems, Flowers, Pods	Key
Megalurothrips sjostedti (Trybom) (Thysanoptera: Thripidae)	Africa,	Floral structures	Key
	Asia,	Floral structures	Not important
	Americas	Floral structures	Unknown
Thrips palmi (Thysanoptera: Thripidae)	Asia	Floral structures	Sporadic
Thrips tabasi Lindeman (Thysanoptera: Thripidae)	Asia South America	Floral structures	Sporadic

Source: Jackai and Adalla, (1997) (re-arranged according to the order of the pests in alphabetical order)

Table 2.2. Cowpea growth stages and pest incidence

Growth stages	Days after planting						Insect pests
	20	30	40	50	60	70	
Foliage	▬	▬					Aphis, Leafhoppers, Foliage Beetle
Flower Budding		▬	▬				Flower Thrips
Flowering			▬	▬			Flower Thrips, pod borers
Podding				▬	▬		Pod sucking bugs, pod borers
Late Podding				▬	▬		Pest population decline due to crop senescence
Spraying by growth stage	•		•	•	•		

Source: Jackai and Adalla (1997).

2.4.1 Aphids

Aphids are small soft-bodied insects of the order Hemiptera and sub-order Homoptera that feed on the fluid in the plant phloem (Summers *et al*., 2006). Aphids are plant sucking bugs which occur throughout the world (Jackai and Adalla, 1997). The greatest number of species is in the temperate regions, where few higher plants are free from aphid attack (Dixon, 1977). They differ from other plant sucking bugs of the Aphidoidea in that the females of at least a few generations are parthenogenetic and viviparous (Dixon, 1977). Although many species are small and inconspicuous, they frequently become abundant. As many as 2000 million aphids per acre (0.4 ha) may live on the above-ground parts of plants, and the roots may support a further 260 million (Dixon, 1977). Aphids, like many other insects, are capable of migrating great distances (up to 1300 km) by means of wind (Dixon, 1977). Aphids exhibit polymorphism. Asexual aphids of some species can either possess wings (in which case they are termed alatae), or lack wings (these morphs are called apterae). Typically, there are several structurally different morphs in a species, including both sexual and asexual forms (Dixon, 1977).

Aphids exhibit complex life cycles. It is estimated that approximately 10% of the species alternate between a primary (usually woody) host plant and a secondary (herbaceous) host plant (Blackman and Eastop, 1994). Non-host alternating species are usually monophagous but may feed on a range of related host plants (Blackman and Eastop, 1994). Aphids display high reproductive peculiarities in their reproductive biology (Braendle *et al*., 2006). First, female aphid reproduces parthenogenetically, obviating the need for males. Secondly, during their parthenogenic generations, the embryos initiate development immediately after the budding of the oocyte from the germarium and are born as fully developed first-instar nymphs (i.e. they are viviparous). Finally, the oldest embryos also contain embryos, so that adult parthenogenetic aphids carry not only their daughters but also some of their granddaughters within them (Braendle *et al*., 2006).

2.4.1.1 The Cowpea Aphid, *Aphis craccivora* Koch.

The cowpea aphid is cosmopolitan in distribution, occurs in the temperate, subtropical and tropical regions of the world (Jackai and Adalla, 1997). The cowpea aphid is a soft-bodied, pear-shaped insect, has antennae which are shorter than the body length (about two third as long as the body) and a pair of cornicles (tailpipe-like appendages) (Summers *et al*., 2006).

They may be winged (alate) or wingless (apterous) but the wingless forms are most common (Summers *et al.*, 2006). It has a shiny black body with white appendages and blackish tips and ranges from 1.5 to 2.5 mm long. In general, the cowpea aphid is a relatively small aphid, less than 2 mm long. Though smaller than adults, nymphs resemble the apterous forms in shape. Cowpea aphid nymphs are pale green to grey with powdery coating (Summers *et al.*, 2006).

2.4.1.2. Host Plants of *A. craccivora*

The host plants of the cowpea aphid include alfalfa, apple, carrot, cotton, cowpea, kidney bean, lettuce, lima bean, pinto bean, peanut, pepperweed, pigweed, red clover, wheat, white sweet clover and yellow sweet clover (A'Brook, 1964; Hamid *et al.*, 1977; Kumar, 1984; Ofuya, 1989). Studies also indicate that a common pattern in the tropics is for *A. craccivora* to spend the dry season on wild hosts and weedy species such as *Medicago spp.*, *Melilotus spp.*, *Trifolium spp.*, *Euphorbia spp.*, *Boerhaavia sp*p as well as volunteer species of other legume crops (A'Brook, 1964; Hamid *et al.*, 1977; Kumar, 1984; Ofuya, 1989).

2.4.1.3. Damage and Economic Importance

Cowpea aphid is an important pest of cowpea in Africa (Singh and Jackai, 1985). The pest primarily infests the seedlings of cowpea and causes direct damage on the crop by sucking plant sap, resulting in stunted plants and distorted leaves. The aphids are usually found in clusters around stems, young leaves and on young shoots. The infested leaves are often cupped or distorted and become more or less yellow (Singh and Jackai, 1985). In heavy infestation the plant dies, especially under water stress conditions. High numbers of cowpea aphids can produce a significant amount of honeydew, excreted by aphids which lead to the development of sooty mold, a dark coloured fungus. This covers the surface of the leaves, affecting photosynthesis and results in yield loss (Baute, 2004). Ultimately these feeding effects of aphid result in reduced vegetative biomass and reduced grain yield. Estimated yield losses of 20% to 40% in cowpea due to *A. craccivora* infestation in Asia and up to 35% in Africa have been reported (Singh and Allen, 1980; Kusi *et al*., 2010b). In eastern region of the Democratic Republic of Congo, *Aphis craccivora* (Hemiptera: Aphididae) is a major pest of cowpea and groundnut (Munyuli *et al.*, 2007) where about 35-65% of yield losses are associated with this pest species (Munyuli *et al.,* 2008; Munyuli, 2009).

Aphis craccivora causes indirect damage by transmitting aphid-borne cowpea mosaic viruses. The cowpea aphid-borne mosaic virus (CABMV) is a cosmopolitan, economically significant seed-borne virus of cowpea (Thottappilly and Rossel, 1985; Shoyinka *et al*., 1997). It can cause a yield loss of 13-87% under field conditions depending upon crop susceptibility, virus strain and the environmental conditions (Thottappilly and Rossel, 1985; Shoyinka *et al*., 1997). CABMV has spread world-wide through the exchange of virus-infected germplasm materials. The virus-infected seed provides the initial inoculum and aphids are responsible for the secondary spread of the disease under field conditions (Obeng-Ofori, 2007).

2.4.1.4. Reproduction of *A. craccivora*

The reproductive system of the parthenogenetic aphid consists of ovarioles (the number of which is determined prior to birth) that contain the developing embryos (Lees, 1959; Osteo and Helms, 1971). Each ovariole usually contains several embryos at different stages of development (Dixon, 1985). There are many factors, both intrinsic and extrinsic, that can affect an aphid's reproductive capacity. The reproductive capacity is correlated positively with adult weight (Murdie, 1969; Dixon, 1970; 1971; Dixon and Wretten, 1971; Taylor, 1975; Kempton *et al*., 1980; Wellings *et al*., 1980). It may either increase or decline with nutritional quality (Wellings *et al*., 1980), and may be correlated positively (Dixon and Oharma, 1980; Leather and Welling, 1981) or negatively (Dixon, 1977) with ovariole number.

Fecundity has been used to measure aphid's responses to environmental conditions. showed that *Uroleucon jacae*, L. that fed on high quality host plants had more embryo that were larger and more sclerotised than when fed on low-quality plants (host plant grown in a mixture of sand, gravel and compost in equal volumes (low quality treatment or in compost only (high quality treatment. Stadler (1995) also found that aphids feeding on low-quality plants selectively controlled the development of only a few old embryos. Ward and Dixon (1982) and Leather *et al*. (1983) showed that if adult aphid (*Megoura viviae* and *Aphis fabae* Scoop.) were starved, they would resorb their smallest embryo and concentrate their effort on producing a few large embryos that were more likely to survive than the smaller embryos.

2.4.1.5. Growth, Development, Fecundity and Longevity of *A. craccivora*

The growth, development, fecundity and longevity of *A. craccivora* vary with weather conditions, soil fertility, soil moisture and host plants. The adult may live up to 15 days or less. Daily progeny production can be as high as 20, and female fecundity may reach 100 or more (Suranyi *et al*., 1998; Mackean, 2006). Developmental time from the first instar to adulthood takes an average of 3 to 5 days (Ofuya, 1997b; Mackean, 2006). Feeding and reproduction increase with warm weather. At temperatures of about 11.5°C, nymphs develop into adults in about 22 days. At warmer temperatures of about 28.5°C, development takes only 5 days. Under conditions of abundant food and favourable climate, parthenogenetic apterous adult females are successively produced (Ofuya, 1997b; Dagg, 2002; Mackean, 2006). Most nymphs mature into wingless females, but periodically, winged females (alatae) develop and migrate to new host plants (Dixon, 1977). The alatae immigrants reproduce parthenogenically and thereby colonize new plants (Dagg, 2002). Many generations are produced each year. The insect passes through four nymphal instars before reaching adulthood.

2.4.1.6. Management of *A. craccivora*

Aphis craccivora can be controlled by various methods including use of insecticides, cultural practices, biological control and host plant-resistance (Singh and Jackai, 1985: Erbaugh *et al*., 1995; Jackai and Adalla, 1997; Omongo *et al*., 1997; Stoddard *et al*., 2010). The majority of African farmers still rely on indigenous pest control approaches to manage pest problems, although many government extension programs encourage the use of pesticides. However, current pest management research activities carried out by national and international agricultural research programmes in Africa focus on biological control and breeding of resistance host plants (Abate *et al*., 2000). Aphids are generally susceptible to most insecticides (Hill, 1983). Insecticides that have been reported to be effective against *A. craccivora* include: carbofuran granules which when applied to the soil gives good control of *A. craccivora* infestation at the cowpea seedling stage (Jackai and Dacoust, 1986). Foliar application of phosphamidon, dimethoate, thiometon and pirimicarb are effective against the pest (Jackai and Dacoust, 1986). Lambda cyhalothrin, a synthetic pyrethroid, at the rate of 20 g active ingredient ha^{-1} has been one of the most common insecticides used in Ghana for the control of cowpea aphid (Kusi *et al*., 2010b).

Generally, the use of natural enemies for the control of the insect pests of cowpea has not been given adequate attention (Singh *et al*., 1990). The potential of biological control is much higher in tropical than in temperate countries due to the high arthropod diversity and year-round activity of natural enemies (Gullan and Cranston, 1994). In nature, *A. craccivora* is attacked by many parasitoids, predators and pathogens (Booker, 1963; Bohlen, 1978; Jang and Yun, 1983; Singh and Jackai, 1985; Ofuya, 1991).

Coccinellid adults and larvae, and syrphid larvae are the most numerous predators. Parasitoids encountered include species in the genera Aphidius, Trioxys and Psyllaephagus. Entomophagous fungi are the main pathogens (Ofuya, 1990; 1995). Ofuya and Akingbohungbe (1988) have shown that *Cheilomenes lunata* (Fabricius) and *Cheilomenes vicina* (Mulsant) could be good candidates for the biological control of the aphid in Nigeria. *Trioxys indicus* (Shubba Rao and Sharma), a hymenopterous endoparasitoid of aphids, has also been reported to show promise as an effective control agent for *A. craccivora* in India. In the green house, *Neozygites fresenii* (Nowakowski) Remaudiere and Kellet, an entomophagous fungus, has been observed to kill effectively *A. craccivora* in all instars especially high densities of aphids under high humidity (Singh and Jackai, 1985).

Two common aphid parasites, *Lysiphlebus* spp. and *Diaraetiella* spp. have been identified from both the high and low desert areas of California (Summers *et al.*, 2006). Although parasitism as high as 95% has been documented, fields with high aphid infestation can cause significant injury to the plants. From observations made in Malawi, Farrell (1976a, 1976b) concluded that the natural enemies of *A. craccivora* reduce population densities only after the aphid population starts to decline towards the end of the season, as a result of the deterioration of the host plants. Ofuya (1997a) recommended the conservation approach of judicious use of insecticides to avoid significant natural enemy mortality. This method may include use of less toxic chemicals to the natural enemies, reduction of frequency of applications and reduction of dosage levels. Pirimicarb has been reported as a selective aphicide (Jackai and Dacoust, 1986).

2.4.2. Host-Plant Resistance

Host-plant resistance has been variously defined by different authors. Snelling (1941), defined resistance as “including those mechanisms which enable a plant to avoid, tolerate or recover from attacks of insects under conditions that will cause great injury to other plants of the same species”. Painter (1951), defined plant resistance to insect as the amount of heritable qualities possessed by the plant, which influences the ultimate degree of damage done by the insect. Beck (1965), defined plant resistance as “collective heritable characteristics by which a plant species, race, clone or individual may reduce the probability of successful utilization of the plant as a host by insect species, race, biotype or individual. Kumar (1984), also defined resistance as the inherent ability of crop plant to restrict, retard or overcome pest infestation and thereby improve the yield and/or the quality of the harvestable crop product.

Crop plants may also avoid damage from a pest species through the mechanism of escape where the sensitive phases of development do not coincide with the optimum conditions for the pest’s development (Cuartera *et al*., 1999). When a pest cannot establish a compatible relationship under any condition with a certain plant genotype, then the genotype is said to be immune or absolutely resistant to the pest. Resistance shown by non-host plants is termed non-host resistance, basic resistance, or basic incompatibility. Non-host resistant plants can exhibit resistance to their specific pests. If a plant expresses some resistance to all isolates or races of a pest, it has non-race-specific resistance. If it expresses resistance to only one isolate or pest race it has race-specific resistance.

From the point of view of the farmer, horticulturalist and others, the use of resistant cultivars represents one of the simplest and the most convenient methods of insect pest control provided that the cultivar does not require expensive input of fertilizer in order to guarantee high yields.

2.4.2.1. Advantages and Disadvantages of Host-Plant Resistance

Some of the many advantages of pest control by use of resistant cultivars over control by application of pesticides include (i) the technique is easy to apply because the grower only has to buy seeds of resistant cultivars; (ii) it is relatively inexpensive. In the majority of cases, seed of resistant cultivars is not more expensive than seed of non-resistant cultivars; (iii) completely resistant cultivars need no chemicals for pest control and even partially resistant cultivars need much less to control pests; (iv) resistant cultivars can be incorporated into integrated pest management programmes and when combined with biological control they give a cumulative effect; (v) adverse environmental effects are minimal or nil, pollution is much reduced; and (vi) resistant cultivars, except transgenic cultivars, are acceptable to the public.

Some of the disadvantages of resistant cultivars are: (i) it takes a long time to develop a resistant cultivar; (ii) resistant cultivars may control only one pest, while pesticides are often effective against several pests; (iii) resistance must be introduced in each new cultivar; and (iv) the pest may adapt to the resistance and this limits the durability of resistant cultivars. The disadvantages of resistant cultivars are, however, much less than their advantages.

Public concerns about the effects of pesticides have compelled governments to make laws to reduce the use of pesticides. The best way to avoid or reduce the use of pesticides in crop production is to introduce integrated pest management techniques that include the use of resistant cultivars. Consequently the prospects for the future development of many more resistant cultivars appear promising.

2.4.2.2. Classification of Host-Plant Resistance

In terms of infestation levels and degree of damage, resistance can be classified as Immunity**:** a variety that cannot be infested or injured at all by specific insect species under any known condition, anything less than immunity is resistance. High resistance: varieties which suffer little damage by a specific insect under a given set of conditions. Low level of resistance: varieties of species which are damaged less by a pest than the average damage for the crop. Susceptible: varieties which show average or more than average damage by an insect pest. Highly susceptible: varieties which are readily infested and suffer considerably more damage than the average by an insect pest under consideration (Painter, 1951; Obeng-Ofori, 2007). Johnson and Law (1975) proposed the term durable resistance to describe long-lasting resistance. Durability does not imply that resistance is effective against all variants of a pest, but that the resistance has merely given effective control for many years in environmental conditions favourable to the pest (Russell, 1978).

Where susceptible cultivars are grown, the pest population comprises a set of races in dynamic equilibrium, but one or two of the races will tend to predominate. If a resistant cultivar is introduced, the predominant races either will not propagate, or their propagation rate will be substantially less than normal. In both cases, if one or some races can propagate effectively on the resistant cultivar, their proportions in the pest population will increase because they no longer have competition from the other races. A new outbreak of the pest will occur because the resistance will have been effectively “broken” (Cuartera *et al*., 1999).

It is difficult to determine whether a pest population is composed of a mixture of races, some present in very small proportions, or whether the pest produces virulent mutants that disappear from the pest population unless there is a compatible resistant host plant in which they can propagate (Cuartera *et al*., 1999). In theory, when the introduced resistance is complete, the predominant races will disappear and more virulent races will spread. The spread will be faster than when the introduced resistance is only partial because the virulent and dominant races will compete (Cuartera *et al*., 1999).

2.4.2.3. Mechanisms of Resistance

Three mechanisms of plant resistance originally defined by Painter (1951) are non-preference (interference with insect behaviour), antibiosis (interference with insect biology) and tolerance. The non-preference has since been replaced by antixenosis (Kogan and Omar, 1978). Antixenosis is the inability of a plant to serve as host to an insect herbivore. The basis of this resistance mechanism can be morphological (e.g. leaf hairs, surface waxes, tissue thickness) or chemical (e.g. repellents or antifeedants). These plants would have reduced initial infestation and/or higher emigration rate of the insect than susceptible plants. Some plant morphological characteristics that can interfere with or modify the behaviour of the insect are colour, shape, type of cuticle wax and the hairiness of plant stalks and leaves (Kogan and Omar, 1978).

Antibiosis is the mechanism that describes the negative effects of a resistant plant on the biology of an insect which has colonized the plant (e.g. adverse effect on development, reproduction and survival). Both chemical and morphological plant defences can induce antibiosis effects. The consequences of antibiosis resistance may vary from mild effect that influences fecundity, development time and body size through to acute direct effect resulting in death (Kogan and Omar, 1978).

Plant tolerance is the degree to which a plant can support an insect population that under similar conditions would severely damage a susceptible plant. When two cultivars are equally infested, the less tolerant one produces low yield. A tolerant plant may be colonized by a pest to the same extent as susceptible plants, but there is no reduction in yield both in quantity and quality.

The usual patterns of insect approach, landing, probing, feeding and egg-laying on a susceptible plant can be disturbed by resistance and induce non-preference or non-acceptance. These disturbances modify the behaviour of the insect and so protect a plant in the initial phase of an attack. Many examples of plant substances with repellent, deterrent or anti-feedant properties are known (Cuartera, *et al.*, 1999). Several groups of toxic, secondary plant compounds like alkaloids, flavonoids and terpenoids may adversely affect the growth, development, generation-time and fertility of the insects.

The gene-for-gene interaction produces absolute resistance, or absolute susceptibility, of the host plant against a race of the pest. This race-specific response is termed vertical resistance and is very effective, but only against certain biotypes of a particular pest species. If the resistance is effective against all genotypes of the pest species without differential interaction, the resistance would be race-non-specific or horizontal resistance (Cuartera *et al.*, 1999).

2.4.2.4. Resistance of Cowpea to *A. craccivora*

Cowpea aphids are easily controlled by the use of aphid resistant varieties (Obeng-Ofori, 2007). Several aphid-resistant cowpea lines have been identified at the IITA and had been tested against aphid populations from several locations in Africa and Asia (Chari *et al*., 1976; Dhanorkar and Daware, 1980; Karel and Malinga, 1980; MacFoy and Dabrowski, 1984; Manawadu, 1985; Ofuya, 1988a; 1993). Antibiosis has been shown as the main mechanism responsible for aphid resistance in cowpea (Singh, 1977; Ansari, 1984; Ofuya, 1988b) and is controlled by a single dominant gene (Singh and Ntare, 1985; Bata *et al.*, 1987; Ombakho *et al.*, 1987; Singh *et al*., 1987; Pathak, 1988).

A large number of aphid-resistant lines have been developed and evaluated in international yield trials. These lines, which need no insecticide protection against aphids include, IT8S-728-5, IT83S-728-13, IT83S-742-2, IT84E-1-108 (Obeng-Ofori, 2007). Others include TVu 36, TVu300, TVu 310, TVu408, TVu410, TVu2996, TVu3000, IT 84S-2246, IT87S-1459, IT 84S-2049 and IT 93K-503-1 (Bata, *et al*., 1987; Ofuya, 1997b). Other aphid resistant genotypes include: IT90K-59, IT90K-76, IT97K-499-35 and IT00K-1251 (Singh, 2004). The resistance in genotype IT84S-2246 is the source of resistance in genotypes IT90K-59, IT90K-76, IT97K-499-35 and IT00K-1251 (Singh, 2004).

2.4.2.5. Antibiosis

The antibiosis category of plant resistance occurs when the negative effects of a resistant plant affect the biology of an arthropod attempting to use that plant as a host. The antibiotic effects of a resistant plant range from mild to lethal, and may result from both chemical and morphological plant defensive factors (Smith *et al*., 2004). The effects on an arthropod feeding on a plant with antibiosis mechanism of resistance may be death of the neonate (larva or nymph), reduced food consumption resulting in a lower weight, increased development time, low food reserves, death in pre-pupal or pupal stages, reduced weight of pupae and/or reduced fecundity (Wiseman, 1999).

According to Schultz (2002), antibiosis mechanism of resistance is offered by certain endogenously produced compounds like phenolics, jasmonic acid, oxilipins, terpenoids, etc. These compounds are essential for resistance to pests and diseases in plants. The author identified that the resistance of certain varieties of sugarcane to woolly aphid was due to the presence of large quantities of phenolic acid and terpenoids in these varieties.

Dahms (1972), illustrated the antibiotic effects of resistant plant on differential rate of aphid development. Nymphs matured in 5 days (susceptible variety), 10 days (intermediate antibiosis) and 20 days (high antibiosis). Mortality of immature arthropods was one of the most important factors limiting the increase of arthropod populations, which was also illustrated by Dahms (1972).

2.5. Application of Molecular Markers in Crop Improvement

Many agriculturally important traits such as yield, quality and some forms of disease resistance are controlled by many genes and are known as quantitative traits (also ‘polygenic,’ ‘multi-factorial’ or ‘complex’ traits). The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs). The identification of QTLs based only on conventional phenotypic evaluation is not possible. A major breakthrough in the characterization of quantitative traits that created opportunities to select for QTLs was initiated by the development of DNA (or molecular) markers in the 1980s (Mc Couch and Doerge, 1995; Mohan *et al.*, 1997; Paterson, 1996a,b).

One of the main uses of DNA markers in agricultural research has been in the construction of linkage maps for diverse crop species. Linkage maps have been utilised for identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis (reviewed by Mohan *et al.*, 1997). The process of constructing linkage maps and conducting QTL analysis to identify genomic regions associated with traits is known as QTL mapping (also ‘genetic,’ ‘gene’ or ‘genome’ mapping) (Mc Couch and Doerge, 1995; Mohan *et al.*, 1997; Paterson, 1996a,b).

DNA markers that are tightly linked to agronomically important genes (called gene ‘tagging’) may be used as molecular tools for marker-assisted selection (MAS) in plant breeding (Ribaut and Hoisington, 1998). MAS involves using the presence/absence of a marker as a substitute for, or to assist in phenotypic selection, in a way which may make it more efficient, effective, reliable and cost-effective compared to the more conventional plant breeding methodology. The use of DNA markers in plant (and animal) breeding has opened a new realm in agriculture called ‘molecular breeding’ (Rafalski and Tingey, 1993).

DNA markers are widely accepted as potentially valuable tools for crop improvement in rice (Mackill *et al.*, 1999; McCouch and Doerge, 1995), wheat (Eagles *et al.*, 2001; Van Sanford *et al.*, 2001; Koebner and Summers, 2003), maize (Stuber *et al.*, 1999; Tuberosa *et al.*, 2003), barley (Thomas, 2003; Williams, 2003), tuber crops (Fregene *et al.*, 2001; Gebhardt and Valkonen, 2001; Barone, 2004), pulses (Weeden *et al.*, 1994; Svetleva *et al.*, 2003; Kelly *et al.*, 2003), oilseeds (Snowdon and Friedt, 2004), horticultural crops (Mehlenbacher, 1995; Baird *et al.*, 1996, 1997) and pasture species (Jahufer *et al.*, 2002). Some studies suggest that DNA markers will play a vital role in enhancing global food production by improving the efficiency of conventional plant breeding programs (Kasha, 1999; Ortiz, 1998). Although there has been some concern that the outcomes of DNA marker technology as proposed by initial studies may not be as effective as first thought, many plant breeding institutions have adopted the capacity for marker development and/or MAS (Lee, 1995; Kelly and Miklas, 1998; Eagles *et al.,* 2001). A thorough understanding of the basic concepts and methodology of DNA marker development and MAS, including some of the terminology used by molecular biologists, will enable plant breeders and researchers working in other relevant disciplines to work together towards a common goal – increasing the efficiency of global food production.

2.5.1. Success in the deployment of aphid resistance loci in soybean improvement

Soybean is the second highest cash crop following corn in the United States. Farmers annually produced on average nearly 2.8 billion bushels, valued at more than $15 billion, on 72.4 million acres during the 2000–2002 period (Kim *et al*., 2008). Most soybeans produced in the United States are used by domestic consumers and the livestock sector, with any remainder exported to foreign consumers. Exports from the 2003 crop were 887 million bushels out of a total crop of 2,454 million bushels, or 36 percent of production (World Agricultural Outlook Board, 2008). However, this valuable crop for U.S. farmers has come under attack by invasive species-the soybean aphid from the North and soybean rust from the South (Livingston *et al*., 2004, Lee *et al*., 2006). Soybean aphid is an economically damaging pest in most parts of the North Central United States (McCarville *et al*., 2013). They are capable of reaching densities of over 1,000 per plant in the field and can reduce soybean yields by 14–40%. Soybean aphids cause damage, including plant stunting, reduced pod and seed counts, and puckering and yellowing of plant leaves. Additionally, soybean aphids are capable of transmitting viruses, including alfalfa mosaic, soybean mosaic, and bean yellow mosaic (Grau *et al*., 2002).

Since its discovery in North America in 2000, economically damaging populations of soybean aphids have developed in parts of Iowa in the past twelve years. It is however not economical to treat soybean aphids with insecticides due to the high cost of insecticides and the high number of soybean aphids that can be found on a single plant (McCarville *et al*., 2013). Reports suggest that before soybean growers faced severe economic losses from this invasive insect, greater efforts were made to develop new high-yielding seed varieties that are resistant to the soybean aphid. However, without the successful development of soybean aphid resistant varieties through conventional breeding, soybean growers suffered greater economic losses from soybean aphid infestations. There was therefore the need to breed for resistance to soybean aphid through marker assisted selection. These varieties incorporate one or more genes conferring resistance to the soybean aphid. For instance, in 2004, scientists from USDA's Agricultural Research Service (ARS) and the University of Illinois collaborated on the discovery of genes (*Rag*1, *Rag2*, *Rag3* and *Rag4*) which confer resistance to soybean aphids (Suszkiw, 2005, Wang *et al*., 2005). These genes suppress aphid growth and reproduction causing their populations to develop much slower, often preventing them from reaching economically damaging levels. Soybean aphid-resistant varieties slow the rate at which soybean aphids populations increase. The resistant plants will not be aphid free, but they will have fewer aphids than susceptible plants. This development has set the stage for seed companies to breed for high-yielding cultivars that are resistant to the soybean aphid (Hill *et al*., 2006).

2.5.2. Genetic Markers

2.5.2.1. What are Genetic Markers?

Genetic markers represent genetic differences between individual organisms or species. Generally, they do not represent the target genes themselves but act as ‘signs’ or ‘flags’. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene ‘tags’. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or ‘linked’ to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called ‘loci’ (singular ‘locus’) (Winter and Kahl, 1995; Jones *et al.*, 1997). There are three major types of genetic markers: (1) morphological (also ‘classical’ or ‘visible’) markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA (Winter and Kahl, 1995; Jones *et al.*, 1997).

Morphological markers are usually visually characterized phenotypic characters such as flower colour, seed shape, growth habits or pigmentation. Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining. The major disadvantages of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995). However, despite these limitations, morphological and biochemical markers have been extremely useful to plant breeders (Weeden *et al.*, 1994; Eagles *et al.*, 2001).

DNA markers are the most widely used type of marker predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996a). These markers are selectively neutral because they are usually located in non-coding regions of DNA. Unlike morphological and biochemical markers, DNA markers are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant (Winter and Kahl, 1995). Apart from the use of DNA markers in the construction of linkage maps, they have numerous applications in plant breeding such as assessing the level of genetic diversity within germplasm and cultivar identity (Weising *et al.*, 1995; Winter and Kahl, 1995; Baird *et al.*, 1997; Henry, 1997; Jahufer *et al.*, 2003).

DNA markers may be broadly divided into three classes based on the method of their detection: (1) hybridization-based; (2) polymerase chain reaction (PCR)-based and (3) DNA sequence-based (Winter and Kahl, 1995; Jones *et al.*, 1997; Gupta *et al.*, 1999; Joshi *et al.*, 1999). Essentially, DNA markers may reveal genetic differences that can be visualised by using a technique called gel electrophoresis and staining with chemicals (ethidium bromide or silver) or detection with radioactive or colourimetric probes.

DNA markers are particularly useful if they reveal differences between individuals of the same or different species. These markers are called polymorphic markers, whereas markers that do not discriminate between genotypes are called monomorphic markers. Polymorphic markers may also be described as codominant or dominant. This description is based on whether markers can discriminate between homozygotes and heterozygotes. Codominant markers indicate differences in size whereas dominant markers are either present or absent. Strictly speaking, the different forms of a DNA marker (e.g. different sized bands on gels) are called marker ‘alleles’. Codominant markers may have many different alleles whereas a dominant marker has only two alleles.

2.5.2.1.2. Marker-Assisted Selection (MAS)

Selecting plants in a segregating progeny that contain appropriate combinations of genes is a critical component of plant breeding (Weeden *et al.*, 1994; Ribaut and Betran, 1999). Moreover, plant breeders typically work with hundreds or even thousands of populations, which often contain large numbers (Ribaut and Betran, 1999; Witcombe and Virk, 2001). 'Marker-assisted selection' (also 'marker-assisted breeding' or 'marker-aided selection') may greatly increase the efficiency and effectiveness in plant breeding compared to conventional breeding methods. Once markers that are tightly linked to genes or QTLs of interest have been identified prior to field evaluation of large numbers of plants, breeders may use specific DNA marker alleles as a diagnostic tool to identify plants carrying the genes or QTLs (Michelmore, 1995; Young, 1996; Ribaut *et al.*, 1997).

2.5.2.1.3. The Advantages of MAS

Some of the important advantages of MAS include the following:

- Time saving from the substitution of complex field trials (that need to be conducted at particular times of year or at specific locations, or are technically complicated) with molecular tests
- Elimination of unreliable phenotypic evaluation associated with field trials due to environmental effects
- Selection of genotypes at seedling stage
- Gene 'pyramiding' or combining multiple genes simultaneously
- Avoiding the transfer of undesirable or deleterious genes ('linkage drag; this is of particular relevance when the introgression of genes from wild species is involved)
- Selecting for traits with low heritability

- Testing for specific traits where phenotypic evaluation is not feasible (e.g. quarantine restrictions may prevent exotic pathogens to be used for screening)

2.5.2.1.4. Cost/Benefit Analysis of MAS

The cost of using ‘tools’ in breeding programs is a major consideration. The cost of using MAS compared to conventional plant breeding varies considerably between studies. Dreher *et al.* (2003) indicated that the cost effectiveness needs to be considered on a case by case basis. Factors that influence the cost of utilizing markers include: inheritance of the trait, method of phenotypic evaluation, field/glasshouse and labour costs, and the cost of resources.

In some cases, phenotypic screening is cheaper compared to marker-assisted selection (Bohn *et al.*, 2001; Dreher *et al.*, 2003). However, in other cases, phenotypic screening may require time-consuming and expensive assays, and the use of markers will then be preferable. Some studies involving markers for disease resistance have shown that once markers have been developed for MAS, it is cheaper than conventional methods (Yu *et al.*, 2000). In other situations, phenotypic evaluation may be time-consuming and/or difficult and therefore using markers may be cheaper and preferable (Young, 1999; Yu *et al.*, 2000; Dreher *et al.*, 2003). An important consideration for MAS, often not reported, is that while markers may be cheaper to use, there is a large initial cost in their development. An estimate for the cost to develop a single SSR marker was AUD$ 100,040 (Langridge *et al.*, 2001).

2.5.2.1.5. Marker-Assisted Backcrossing

Using conventional breeding methods, it typically takes 6–8 backcrosses to fully recover the recurrent parent genome. The theoretical proportion of the recurrent parent genome after n generations of backcrossing is given by: $(2n+1-1)/2n+1$ (where n = number of backcrosses; assuming an infinite population size). The percentages of recurrent parent recovery after each backcross generation are presented in Table 2.3. The percentages shown in Table 2.3 are only achieved with large populations; the percentages are usually lower in smaller population sizes that are typically used in actual plant breeding programmes

Table 2.3. Percentage of recurrent parent genome after backcrossing

Generation	Recurrent parent genome (%)
BC1	75.0
BC2	87.5
BC3	93.8
BC4	96.9
BC5	98.4
BC6	99.2

Although the average percentage of the recurrent parent genome is 75% for the entire BC1 population, some individuals possess more of the recurrent parent genome than others. Therefore, if tightly-linked markers flanking QTLs and evenly spaced markers from other chromosomes (i.e. unlinked to QTLs) of the recurrent parent are used for selection, the introgression of QTLs and recovery of the recurrent parent may be accelerated. This process is called marker-assisted backcrossing.

The use of additional markers to accelerate cultivar development is sometimes referred to as ‘full MAS’ or ‘complete line conversion’ (Ribaut *et al.*, 2002; Morris *et al.*, 2003). Simulation studies using PLABSIM (a computer program that simulates recombination during meiosis) indicate that efficiency of recurrent parent recovery using markers is far greater compared to conventional backcrossing (Frisch *et al.*, 1999; Frisch and Melchinger, 2000). Therefore, considerable time savings can be made by using markers compared to conventional backcrossing. Although the initial cost of marker-assisted backcrossing would be more expensive compared to conventional breeding in the short term, the time savings could lead to economic benefits. This is an important consideration for plant breeders because the accelerated release of an improved variety may translate into more rapid profits by the release of new cultivars in the medium to long-term (Morris *et al.*, 2003).

CHAPTER THREE

3.0. Introduction

Following the release of two early maturing varieties Apagbaala (ITX P48-2) and Marfo-Tuya (Sul 518-2) by the Savanna Agricultural Research Institute (Padi *et al*., 2004), several advanced breeding lines were generated from crosses between these adapted parents and exotic lines. These very popular varieties have been found to be susceptible to aphid infestation in the field. To further improve on the yield and agronomic characteristics of these cultivars, each genotype was crossed with an exotic line as Apagbaala × UCR 01-11-52 and UCR 01-15-127-2 × Marfo-Tuya. Ten advanced breeding lines (F_6) from the Apagbaala × UCR 01-11-52 population and six from the UCR 01-15-127-2 × Marfo-Tuya population were selected as lines with the highest yield potential in northern Ghana. Information on the reaction of these lines to field pests was, however, lacking. A study was therefore carried out to evaluate the reaction of the 16 advanced breeding lines and their adapted parents to infestation by the cowpea aphid. A local variety in northern Ghana, SARC-L02, and three varieties developed by the International Institute of Tropical Agriculture (IITA) were used as controls. At least the IT 97 K-499-35 variety from the IITA was known to have been developed with emphasis on resistance to the cowpea aphid (Singh 2004).

Progress has been achieved in identifying a source of resistance to the cowpea aphid, *Aphis craccivora* in an advanced breeding cowpea line and in identifying a marker linked to the resistance locus. The resistance gene imparts antibiosis to the cowpea plant such that the aphid's fecundity is significantly reduced when fed in a no-choice experiment on resistant plants (Kusi *et al*., 2010a). With the progress so far made, it is now possible to use this gene to improve upon the field resistance of existing cowpea cultivars in Ghana.

3.1. Materials and Methods

3.2. Selection of Resistant and Susceptible Progenies

The resistant genotype, SARC 1-57-2, was crossed to the susceptible genotype, Apagbaala. The susceptible parent served as the mother parent whilst the resistant genotypes served as the male in the cross. The F_1, F_2 and F_{2-3} seeds from the cross were developed in the screen house of the Council for Scientific and Industrial Research-Savannah Agricultural Research Institute (CSIR-SARI), Nyankpala. The seeds of parental F_1 and F_2 populations were sown in plastic buckets containing sandy loam soil from the experimental field of SARI and kept in the insectary. Three to four days after emergence, each seedling was infested with five, four-day old nymphs using camel hair brush (Bata *et al*., 1987; Githiri *et al*., 1996; Kusi *et al*., 2010a). On the eighth day after infestation, the genotypes were classified into various levels of resistance based on percentage of dead or heavily damaged seedlings (Bata *et al*., 1987; Githiri *et al*., 1996; Kusi *et al*., 2010a).

In order to get seeds from the non-segregating susceptible progenies, seedlings that had shown symptoms of susceptibility to the aphid attack were rescued by applying lambda cyhalothrin (Lambda Super®), a synthetic pyrethroid, at the rate of 20 g active ingredient ha^{-1} 6 and 7 days after infestation to control the aphids. The rescued susceptible seedlings were maintained to produce non-segregating susceptible seeds. The resistant seedlings that survived the progeny test of the F_2 population were maintained to generate F_3 seeds which were also screened using the same method described above. The screening of the F_3 seedlings which were generated from the resistant seedlings from the F_2 screening were further tested for their reaction to the aphid using procedures as outline above. Seedlings killed by factors other than aphid infestation such as disease infection were removed before the seedlings were classified into resistant and susceptible groups. Remnant seeds of the resistant F_3 segregants were kept for further analysis.

3.3. Identification of DNA Marker(s) Linked to the Cowpea Aphid Resistance Gene

A total of 50 DNA markers were screened and these are presented in Table 3.1.

Table 3.1. SSR primers used and their sequences

NAME	SEQUENCE	ANNEALING TEMPERATURE	NAME	SEQUENCE	ANNEALING TEMPERATURE
MS3F	GTTGGCTTCTGTTGTGGCAT	56	Y26F	CTAAATTATAATATTCGTCGGTC	52
MS3R	GTTACACCAATGCCAAAAAC		Y26R	GGTTAAGGAAAAGAGGGTAGG	
MS26F	TGCGGTTGAGATTTTGACGT	56	Y31F	CTATTGGAATCTTGCCGTTG	56
MS26R	CGTGAAGTTGAATGTGAAT		Y31R	CTTTACCTTTATGCAAACCAATTC	
SM29F	TTGATTAGTTGGCTCTTAGGGGC	56	Y45F	CGATTATCCTGGCTAACGATG	56
MS29R	GAGGACTTAATTAGAACAAACTTTG		Y45R	GGATCTGAGATAGTGTGAC	
MS31F	GTGACTACAATGGCGGAACT	56	CP115F	GGGAGTGCTCCGGAAAGT	56
MS31R	GGAGGTACCGAAAAGAAAG		CP116R	TTCCCTATGAACTGGGAGATCTAT	
Kad1F	TAGACCAGATGACATTGTAATTC	52	CP117F	GTGGAAGGAATGGGTCCAG	56
Kad1R	GTCGTAACTGGGCACAATAG		CP118R	AGGAAATTTGCATTCCCTTGT	
Kad26F	CCCAAAATCCTGACCCACTA	56	CP163F	CACTTTCTCCTAAGCACTTTTGC	56
Kad26R	TTTTCACGATGGAACAGTGC		CP164R	AAGTGAAGCATCATGTTAGCC	
Kod7F	CTAGAACGTTCCATCTTAATATTAC	55	CP171F	GTAGGGAGTTGGCCACGATA	56
Kod7R	TAACTATTTAAAGATGATTTC		CP172R	CAACCGATGAAAAAGTGGACA	
KOD17F	CTTATCGTGATAACATGTATTT	55	CP181F	GGGTGCTTTGCTCACATCTT	56
KOD17R	CAACAAGTATAAAATGAGTGTGAG		CP182R	TCCATGTGTTTATGACGCAAA	
Kod21F	GACTTTTACATTTATTACATG	52	CP197F	TGAATGGAGCAACTTCTTGGA	56
Kod21R	TAATCACCATACCCACTCTAC		CP198R	GTTGCACTGGTTGCCCTAT	
MS43F	CATTTTTTAATCCATTTTTATC	52	CP201F	GGTTTCCTAGTTGGGAAGGAA	56
MS43R	AAGTTTTTAGGGGCTATGGC		CP202R	ATTATGCCATGGAGGGTTCA	
MS50F	TTTAAAATGGTCCCTCCCGT	56	CP215F	CAGAAGCGGTGAAAATTGAAC	56
MS50R	CCTAAACGAATTCTACCTGG		CP216R	GCATGTTGCTTTGACAATGG	
MS52F	CCAGCAGTATATACATAAGA	50	CP239F	CACCCCCGTACACACACAC	56
MS52R	GCAAGCCAAGACAAAATAGTG		CP240R	CACTTAAATTTTCACCAGGCATT	
MS53F	GAAAGTATATGTTGTTAACTCT	50	CP333F	CAAAGGGTCATCAGGATTGG	56
MS53R	AAGAGTGACAAGAAAGATTT		CP334R	TTTAAGCAGCCAAGCAGTTGT	
MS98F	GATAAAGAGGAAAATAGACA	50	CP359F	TGAAAACAACGATATGCAGAAG	56
MS98R	AAAATGTGGCAGATAAGGAA		CP360R	TCAGTCTTAGAATTGATGGGGCTTCG	

Table 3.1 Continued

MS111F	TAATAAAGCAAAGATGGTCG	50	CP391F	TGCTATGCTTATGCCTGTG	56
MS111R	AATAACATAATAACGCGTGC		CP392R	GATGCCTGTTACTTGCCTTCT	
MS113F	GTTAAAGTTTTCTTCATCAT	50	CP393F	GCGGATGAAATTACGATAAAACA	56
MS113R	ATCTTGATCCAGAAAATGTTT		CP394R	GTGCAGAACAATGCAAAGGA	
MS120F	TTTCTAGGCAGTGAAGATAATCA	50	CP395F	GTTGTGAGCTTCCCCAGATG	56
MS120R	AAACAAAATACCAACTACCA		CP396R	AATTTTGAACCCACCACCAG	
MS121F	GGTTGTTCCGAAAAACTTATACG	50	CP397F	TCATGGGTTAAATTTGCTTCAA	56
MS121R	GATAGAAGTTTTAACATTACTC		CP398R	AAACCATGTGGTTGTTGCAC	
MS128F	CGTAATTTGTAATGTGTAGG	50	CP403F	TGCAATATGGACCAGAAGAAA	56
MS128R	AACCCTAAACAAACTTTTGGTAG		CP404R	ATGCCCCAACAACAACATTT	
MS138F	AACACATGGATAACAGAAAT	50	CP431F	CCTCAACACCTTTTGGAAGGA	56
MS138R	GATCTCGTCCACAAACAACA		CP432R	CAAATGCACCTCCTGTGCTA	
MS143F	ATGTTTCAGATCGGTTTAGA	52	CP433F	CAACTTCACAGCCCTCAA	56
MS143R	GAGCTGAAAAAATCGGTGTC		CP434R	TTGAAGGTATGGCCTTTTGTTT	
MS144F	GTGAGTTTAATGACATTTAC	50	CP435F	TGCTCATCGTGCTTTGTCTT	56
MS144R	GACTGCTATGTCATAATATT		CP436R	CACTTCAGACTTAGAGCGAAGAA	
Y16F	CACATTAACTCAAGTCCACACC	52	CP443F	GCTCGGATATGGTCCTGAAA	56
Y16R	CCAGTGAAATCATGTCAAAT		CP444R	TCAGTGTCAGCACCATCCC	
Y1F	GATATAGAATAGCATATTTAACATATTAG	52	CP573F	CAGAATCCTTGTGAACCTG	56
Y1R	GTTGAAAGTTTGATAGTAAAGTGG		CP574R	TTTCGCAATATGCCCTTTTC	
Y21F	GAGAACTTCACGCACAATAG	56	CP605F	AAAGAGATACACATGCCTAACA	56
Y21R	CGCGGTAGCATGATTGAATTTTG		CP606R	GACCAACAGCGACTTTGAGC	

3.3.1. FTA Protocol

The DNA from the samples used for the present study was extracted using fast technology for analysis (FTA) of nucleic acids card supplied by Kirkhouse Trust following the methodology described below:

The Leaf was cleaned with ethanol and then was placed over the marked circle (underside of the leaf facing down) on top of the FTA matrix card. The leaf was overlaid with parafilm and a small porcelain pestle was used to apply moderate pounding for 15 seconds over each sample circle area to burst the cell walls of the plant tissue. To visually verify that the plant material has transferred sufficiently into the FTA card matrix, the back of the FTA card matrix was checked to see if the plant tissue was being drawn through the matrix. When plant tissue transfer was complete, the FTA card was air dried for a minimum of one hour at room temperature. The FTA matrix card was placed on FTA sample mat and using 2.0 mm harries micro punch tool, a disc was removed from the centre of the dried sample area into1.5 ml microfuge tube. Care was taken when transferring the dry FTA discs into the microfuge tube because the static charge that could develop on some plastic laboratory ware could repel the discs. Alcohol was used to wipe the punch tip between samples. The disc in each tube was washed with 70 % ethanol for 5 minutes; the washing with ethanol was repeated until the disc turned white.

About 200 µl of FTA purification reagent was added to each tube, capped, inverted twice and incubated for 4-5 minutes at room temperature. After the incubation the FTA reagent was pipetted up and down twice, to ensure that the disc remained in the tube. A pipette was used to remove and discard as much of the reagent as possible. This was repeated for two FTA reagent washes. The discs were allowed to completely air dry for a minimum of one hour at room temperature. Whenever the PCR amplification could not be conducted within three hours of discs drying, the discs were stored at 4 °C or -12 °C. This was due to the fact that the DNA purification process removed the protective chemistry of the FTA technology.

3.3.2. PCR Amplification

A PCR amplification mix containing *Top* DNA Polymerase, dNTPs, reaction buffer, tracking dye, and patented stabilizer and was obtained from Bioneer. About 20 µl of the complete PCR amplification mix was added directly to the PCR tube containing the dried disc, assuming the DNA volume used was zero. Each of the 50 DNA markers was used to run the set of DNA samples extracted from the resistant parent (SARC1-57-2), susceptible parent (Apagbaala) and the resistant and susceptible progenies. PCR amplification was carried out in ABI 2720 thermal cycler (Applied Biosystems). PCR conditions consisted of denaturing at 94°C for 3 minutes, annealing at temperatures (Table 3.1.) for each primer for 30 seconds and extension at 72°C for 30 seconds. This cycle was repeated 35 times and final extension at 72°C for 10 minutes. The PCR products were further run on horizontal polyacrylamide gel electrophoresis (h-PAGE) (81-2325 by Galileo Biosciences, dimension of tank: 32 cm W x 37.5 cm L x 10.5 cm H; dimension of plate: 24.5 cm W x 27.5 cm L) to separate and resolve the bands with the protocol indicated below.

The 5% acrylamide gel was prepared as shown in Table 3.2.

Table 3.2. Preparation of 100 ml 5% acrylamide gel

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

3.3.3 Casting the Gel

Gel was cast in a tray (27.5 cm 24.5 cm) with barriers to retain acrylamide and a 50 well-forming combs were inserted to create wells. A lid was used to cover the tank with the gel to prevent oxygen inhibition of polymerisation. The 100 ml volume required to fill the tank was determined by weighing the tank before and after filling with water. The monomer and catalyst mix were prepared as shown in the Table 3.1. The mix was poured into the tank and distributed across the whole surface, removing bubbles. The comb was inserted, the lid was laid on the tank and lowered carefully and pressed gently against the comb. This was allowed to polymerise. The whole assembly was transferred into electrophoresis tank and the comb was removed when the assembly was submerged in buffer (3-5 mm above the lid). The PCR products were loaded into the wells. During loading care was taken to avoid the 'skirt.' of polyacrylamide that might fall into the well. The lid was left in place during loading and electrophoresis. The gel was run at to at least half way to the end of the glass and a spatula was used to prise off the lid after running the gel. The gel was stained with a solution of ethidium bromide, 0.5 µg/ml for 30 minutes using the same volume used to make the gel. The gel was photographed under Ultraviolet light. Polymorphic primer pairs were noted for further analysis.

3.4. Testing the Reliability of the Marker, CP 171F/172R, in F_2 Segregating Population

Upon identification of the marker CP 171F/172R as being polymorphic out of the 50 markers, the marker was tested for its effectiveness in selecting aphid resistant lines out of a large segregating F_2 population.

The resistant parent (SARC1- 57-2) was crossed to the susceptible parent (Apagbaala) to generate F_1 population. The F_1 population was advanced to F_2 population by selfing. A total of 169 F_2 plants were screened for reaction to aphid infestation. The 169 seeds were planted individually in plastic pots in the screen house and each plant was labeled. Leaf samples were taken from each plant after emergence for DNA extraction using FTA paper. Each plant was subsequently infested with five, four-day old aphids. After the infestation, the aphids were monitored within 24 hours to ensure that the five aphids settled on each plant.

The resistant and the susceptible parents were also planted with the F_2 population, and after every 10 individuals of F_2 lines, the resistant and the susceptible parents were planted in the experimental set up. The infested plants were observed until about 90% of the plants of the susceptible parent were killed by the aphids. The experiment was terminated and the numbers of dead and live seedlings of the F_2 lines were recorded. The ratio of dead seedlings (susceptible) to the live seedlings (resistant) was determined. Chi square test was carried out to determine the goodness of fit.

3.5. Genetic Analysis

The FTA cards with the leaf samples were sent to the Kirkhouse Trust Mobile Molecular Laboratory stationed at the Cocoa Research Institute of Ghana (CRIG) for genetic analysis. The experimental procedure described above was used to wash the FTA cards, run the PCR using the marker CP 171F/172R and the PCR product was run on h-PAGE as described above to separate and resolve the bands. The bands were photographed and classified in relation to the band pattern of the resistant and the susceptible parents.

3.6. Introgression of Aphid Resistance Locus into Ghanaian Cowpea Cultivars

3.6.1. Polymorphism Test

Polymorphism test was conducted to determine whether the marker CP 171F/172R could separate the resistant parent and four cowpea cultivars recommended for Northern Ghana. The cultivars were Padituya (SARC3-122-2), Zaayura (SARC4-75), Songotra (IT97K-499-35) and Bawutawuta (IT95K-193-2). DNA samples were taken from the leaves of the four (4) cultivars together with the resistant and susceptible parents. The samples were taken two weeks after planting using FTA card. The standard procedure detailed in Sub-sections 3.2.1-3.2.4 was used to wash the FTA cards and the PCR was run using the marker CP 171F/172R.

The PCR products were run on a non-denaturing h-PAGE II. The photographed bands of the parents, and the four cultivars were analysed to determine which of the cultivars is polymorphic with SARC 1-57-2 for the marker CP 171F/172R, to enable it to be improved for aphid resistance in a marker assisted backcrossing.

3.6.2. Marker Assisted Backcrossing

The following procedures were followed to introgress the cowpea aphid resistance locus into the cowpea cultivar Zaayura.

3.6.2.1. Development and Advance of Backcross Progenies

From a cross between SARC1-57-2 and Zaayura, the F_1 was backcrossed to Zaayura (the recurrent parent) to generate 20 BC_1 lines. All individuals were genotyped to select heterozygous plants. These plants were screened in the screen house to confirm their reaction to the aphid. Three resistant BC_1 plants were used for backcrossing to the recurrent parent to generate BC_2 individuals. This cycle of crossing, identification of heterozygous lines using SSR marker, CP 171F/172R, and screen house confirmation of resistance was followed till BC_4 plants were obtained. A heterozygous BC_4F_1 plant was selfed to generate 100 BC_4F_2 lines. These were genotyped with the CP 171F/172R to select plants that were homozygous dominant for the region of the resistance locus.

Twenty-five BC_4F_2 plants carrying homozygous resistant alleles based on marker CP 171F/172R were screened further with the aphid to confirm resistance. Five plants showing resistance to the aphid were selected for multiplication. This was bulked for larger scale field testing.

3.7. Determining the Stability of the Aphid Resistance Locus Across the Major Cowpea Belts in Ghana

The cowpea aphids were sampled from cowpea plants in farmers' fields in six cowpea growing regions in Ghana. These were Upper East, Upper West, Northern, Brong Ahafo, Central and Volta Regions (Table 3.2). Based on the seedling screening technique developed during the course of this research, the resistant lines SARC1-57-2 and SARC1-91-1, and IITA line IT 97K-499-35 together with known susceptible varieties (Apagbaala and Zaayura) were tested in each region with aphids in the region. Each of the cowpea lines were replicated 9 times in each experimental setup. The reaction of the test genotypes informed the possible existence of biotypes within *A. craccivora* in Ghana.

Table 3.3. Locations in the six regions where cowpea aphids were sampled

Region	Areas within the region	GPS Coordinates
Upper East	Bawku	11°03′N 0°14′W
	Navrongo	10°53′5″N 1°5′25″W
	Sakote	10° 44′ N 0°36′247″W
Upper West	Nandom	10°51′00″N 2°45′00″W
	Tumu	10°53′N 1°59′W
	Wa	10°04′N 02°30′W
Northern	Nyankpala	9° 24' 0"N 0° 59' 0"W
	Walewale	10° 21' 0"N 0° 48' 0"W
	Yendi	9°25′57″N 0°0′15″W
Volta	Kpeve	6°41′1″N 0°20′1″E
	Nkwanta	8°16′N 0°31′E
	Sogakope	6°00′N 0°36′E
Central	Asowanzi	5°53′0″N 1°13′0″W
	Mankesim	5°16′N 1°01′W
	UCC	5°06′N 1°15′W
Brong Ahafo	Kintampo	8°3′8″N 1°44′5″W
	Nkoranza	07°34′00″N 01°42′00″W
	Wenchi	07°34′38″N 01°55′45″W

A 5-point scale of score of seedling vigour was used where: 1 = dead seedling due to aphid damage, 2 = seedling with weak stem and leaves with symptoms of aphid damage, 3 = seedling showing symptoms of aphid damage, 4 = seedling with aphids without symptoms of damage and 5 = seedling with no aphid (as in control pots) (Kusi, 2008).

CHAPTER FOUR

4.0. Stability of the cowpea aphid resistant genotype across the major cowpea growing zones in Ghana

4.1. Introduction

Cowpea, *Vigna unguiculata* (L.) Walpers is cultivated mostly in tropical Africa and the edible seeds constitute a major source of protein in the human diet (Diouf and Hilu, 2005; Kamara *et al*., 2010). Cowpea is the most important in the genus *Vigna* in terms of planting area. Production area of cowpea is about 14 million hectares worldwide and annual global production of cowpea is approximately 3.3 million tons (CGIAR, 2011). West and Central Africa is the leading cowpea producing region in the world, producing about 64%. Nigeria is the largest producer and consumer, and accounts for 61% of production in Africa and 58% worldwide (Quin 1997; IITA, 2009). A major biological constraint to the production of cowpea in Africa is severe infestation and damage by various insect pests in the field and during storage (Kamara *et al*., 2007).

The cowpea aphid, *A. craccivora* Koch is considered to be an important field pest of cowpea in Africa, Asia and Latin America (Kusi *et al*., 2010a; Benchasri *et al*., 2012; Kamphuis *et al*., 2012). It is the most important worldwide pest of cowpea causing significant yield losses when either young seedlings or the pods of adult plants are attacked (Annan *et al*., 2000). Singh and Allen (1980) estimated yield losses of 20% to 40% in cowpea due to *A. craccivora* infestation in Asia and up to 35% in Africa. Cowpea aphid can be controlled by various methods including use of insecticides, cultural practices and biological control (Erbaugh *et al*., 1995; Jackai and Adalla, 1997; Omongo *et al*., 1997; Stoddard *et al*., 2010). Public concerns about the effects of pesticides have stimulated the search for more environmentally safe methods such as the use of host plant resistance to control the pest.

Host plant resistance is easy to apply, relatively inexpensive, needs no chemicals for pest control, and can be incorporated into integrated pest management programmes. When combined with biological control it gives a cumulative effect. The use of resistant cultivar minimises or eliminates adverse environmental effects caused by pesticides and is generally acceptable to the public.

Antibiosis has been shown as the main mechanism responsible for aphid resistance in cowpea (Ansari, 1984; Ofuya, 1988b; Singh, 1977; Laamari *et al.*, 2008) and is controlled by a single dominant gene (Bata *et al.*, 1987; Ombakho *et al.*, 1987; Singh, *et al.*, 1987; Pathak, 1988; Nualsri *et al.*, 2012). The dominance nature of aphid resistance genes in cowpea means that resistant progeny can easily be identified in segregating populations, thus making selection in a breeding process easy (Githiri *et al*., 1996). However, the major problem with single gene inheritance is that insects can develop biotypes very fast which could overcome the resistant cultivars (Githiri *et al.*, 1996). This problem can easily be encountered with aphids which have a very short life cycle and reproduce parthenogenetically (Githiri *et al.*, 1996). Three biotypes of *A. craccivora* have been reported from West Africa where cowpea is widely grown (IITA, 1981). Biotypes A and B occur in Nigeria and Biotype K in Upper Volta (Burkina Faso) (IITA, 1981).

Kusi *et al*. (2010a) found a cowpea line, IT97K-499-35 known to be resistant in Nigeria (Singh, 2004) to be susceptible to aphids in Ghana. They attributed this to the possible existence of a biotype of *A. craccivora* in Ghana or in the Guinea Savannah zone of Ghana that is more virulent than the biotype that existed in Nigeria where IT97K-499-35 was developed and evaluated earlier. Other researchers have reported the presence of more aggressive aphid biotypes in Burkina Faso and other West African countries than in Nigeria

(Martyn, 1991; van Emden, 1991; Ofuya, 1997a). The California types of cowpea aphids that are not controlled by the 'IITA' type of aphid resistance have also been reported (Messina *et al*., 1985). In related studies of Chari *et al*. (1976), Ombakho *et al*. (1987) and Martyn (1991), it has been reported that at least three distinct biotypes of the cowpea aphid may occur in Africa and Asia, and another biotype occurring in the United States and they all require different resistance genes to control them.

SARC 1-57-2 and SARC 1-91-1 were identified as resistant to *A. craccivora* among 22 cowpea genotypes evaluated with seedling screening method (Kusi *et al*., 2010a). SARC 1-57-2 significantly recorded the least percentage of seedlings killed by aphids among the 22 genotypes eight days after aphid infestation. SARC 1-57-2 was again evaluated among nine other genotypes to assess the rate of growth and reproduction of aphids on each of these genotypes. Significantly, SARC 1-57-2 recorded the least number of aphids per seedling at 3, 6 and 9 days after infestation (Kusi *et al*., 2010a).

The differential resistance response exhibited by cowpea lines developed with resistance to aphid in different growing eco-regions raised the concern for the existence of biotypes of the insect that may require different resistance genes. The objective of this study was therefore is to test for the stability of the aphid resistance gene in SARC 1-57-2 in all the major cowpea growing zones in Ghana. The reaction of the test genotypes would inform the possible existence of biotypes within *A. craccivora* in Ghana.

4.2. Materials and Methods

The experiments were carried out between July 2011 and January 2012. The experiments were conducted in six Regions in Ghana namely, Upper East, Upper West, Northern, Brong Ahafo, Central and Volta (Table 4.1).

Table 4.1. Characteristics of study areas in the six regions

Region	Areas within the region	GPS Coordinates	Agro-ecological Zone	Rainfall (mm/yr)	Length of growing Season (Days)	Dominant land use	Main food crops
Upper East	Bawku	11°03′N 0°14′W	Sudan Savannah	1000	150-160	Annual food crops	Sorghum , millet, cowpea , maize
	Navrongo	10°53′5″N 1°5′25″W					
	Sakote	10° 44′ N 0°36′247″W					
Upper West	Nandom	10°51′00″N 2°45′00″W	Guinea Savannah	1100	180-200	Annual food crops, cash crops and livestock	maize, yam, Groundnut sorghum, millet, cowpea
	Tumu	10°53′N 1°59′W					
	Wa	10°04′N 02°30′W					
Northern	Nyankpala	9° 24' 0"N 0° 59' 0"W	Guinea Savannah	1100	180-200	Annual food crops, cash crops and livestock	Ground nut maize, yam, Sorghum , millet, cowpea
	Walewale	10° 21' 0"N 0° 48' 0"W					
	Yendi	9°25′57″N 0°0′15″W					
Volta	Kpeve	6°41′1″N 0°20′1″E	Coastal Savannah/ Transitional	800-1300	Major season: 100-160 Minor season: 50- 90	Annual food crops, cash crops livestock	Maize, roots, cowpea
	Nkwanta	8°16′N 0°31′E					
	Sogakope	6°00′N 0°36′E					
Central	Asowanzi	5°53′0″N 1°13′0″W	Coastal Savannah	800	Major season: 100-110 Minor season: 50	Annual food and cash crops	Maize, roots and cowpea
	Mankesim	5°16′N 1°01′W					
	UCC	5°06′N 1°15′W					
Brong Ahafo	Kintampo	8°3′8″N 1°44′5″W	Transitional	1300	Major season: 150-160 Minor season: 90	Annual food and cash Crops	Maize, root and tuber, cowpea. Ground nut
	Nkoranza	07°34′00″N 01°42′00″W					
	Wenchi	07°34′38″N 01°55′45″W					

The cowpea genotypes used for the study were SARC1-57-2, SARC1-91-1, Apagbaala, IT97K-499-35 and Zaayura (Table 4.2).

Table 4.2. Description of the five genotypes of cowpea by parentage or source

Genotype	Description by pedigree or source
Apagbala (control)	Prima/TVu 4552/California Black eye No.5/7977. Cultivar, released in 2002 in Ghana. Largely of exotic background
SARC 1-57-2	Apagbaala/ UCR 01-11-52. Breeding line of SARI
SARC 1-91-1	Apagbaala/ UCR 01-11-52 Breeding line of SARI
IT97K-499-35	Breeding line from the IITA, Ibadan Nigeria. Cultivar, released in 2008 in Ghana as Songotra.
Zaayura	Marfo-Tuya/ UCR 01-15-127-2. Cultivar, released in 2008 in Ghana.

Each Region was zoned into 3 areas to provide 18 test locations. Aphid sampling and screening were carried out as described in (3.6). Screening was done in a fabricated mobile screen house (Fig. 4.1) which could easily be dismantled and conveyed from one location to the other.

Three mobile screen houses were constructed for the study so that the experiments in each of the three locations in a Region were conducted concurrently. The seedling screening method (Bata *et al.*, 1987; Githiri *et al.*, 1996; Kusi *et al.*, 2010a) was used to evaluate the cowpea genotypes. The aphids from the farmers' field were further sub-sampled with soft painter's brush to remove all predators that were sampled with the aphids from the field. The predator free sub-samples were used to infest the cowpea seedlings at 3-4 days after emergence.

The experimental design used was the completely randomised design with nine replications. The seedlings were raised in plastic containers measuring 20 cm deep and 20 cm wide. Each replication consisted of ten seedlings of the cowpea variety.

Mobile Screen House

Fig. 4.1. The Kirkhouse Trust Mobile Screen house

4.3. Data Collection and Analysis

Data collected included the percentage of seedlings killed and seedling vigour 10 days after infesting the seedlings with aphids. Seedling vigour was rated on a five-point scale (Kusi, 2008) where: 1 = dead seedling due to aphid damage, 2 = seedling with weak stem and leaves with symptoms of aphid damage, 3 = seedling showing symptoms of aphid damage, 4 = seedling with aphids without symptoms of damage and 5 = seedling with no aphid.

The data were subjected to analysis of variance (ANOVA) using Genstat statistical program (9th edition). Fisher's LSD was used to separate the means after ANOVA showed significant differences.

4.4. Results

There were no significant interactions between the cowpea varieties and the zones within the regions where the studies were conducted in terms of seedling mortality and plant vigour score. The zone main effect also did not show significant difference across the regions for seedling mortality and plant vigour score. Data were therefore presented for the cowpea variety main effects for seedling mortality and plant vigour.

4.4.1. Seedling Mortality

Significant differences were observed among the cowpea varieties for the percentage seedling mortality at each of the 18 locations (Table 4.3). Significantly ($P < 0.001$), SARC 1-57-2 recorded the least number of seedlings killed by the aphids across the regions and locations. On the other hand, Apagbaala, IT97K-499-35 and Zaayura recorded significantly higher number of seedlings killed by the aphids. At all the 18 locations, the three genotypes maintained their susceptibility to aphids. Apagbaala and IT97K-499-35 recorded as high as 96% seedlings death with Zaayura recording 85% mortality 10 days after infestation.

4.4.2. Seedling Vigour

Similar to the results on the mortality of seedlings, the vigour of the varieties 10 days following aphid infestation was highest in SARC 1-57-2 and least in Apagbaala and IT97K-499-35 (Table 4.3). SARC1-57-2 and SARC 1-91-1 maintained average vigour score of 3.8 and 3.4, respectively across the locations. The less vigorous genotypes were Apagbaala and IT97K-499-35 and Zaayura with average score of 1.16, 1.16 and 1.89, respectively. The general responses of the resistant and the susceptible lines due to the aphid infestation as manifested in the leaves colour and plant growth is represented in Fig. 4.2.

Table 4.3. Mean seedling mortality and plant vigour score following aphid infestation on five cowpea genotypes at 18 locations in Ghana

Variety	% Seedling killed	Seedling vigour score
Apagbaala	96.17	1.167
SARC1-57-2	3.70	3.802
SARC1-91-1	7.99	3.444
IT97K-499-35	96.54	1.167
Zaayura	85.56	1.870
Mean	**58.0**	**2.3**
s.e.d.	**0.413**	**0.043**
CV%	**0.7**	**4.6**

Fig. 4.2. Response of the susceptible lines (A and C), resistant lines (B) and D comparing leaf colour of resistant and susceptible lines due to aphid attack in the regions

4.5. Discussion

Insect species containing biotypes have been described by their ability to damage crops with host plant resistance genes (Puterka *et al.*, 1992; Gallun, 1977; Porter *et al.*, 1997). Insect biotypes are intra-specifically classified based on biological rather than morphological characteristics, and they are generally morphologically indistinguishable (Shufran *et al.*, 2007). The cowpea aphid infests seedlings of cowpea and causes direct damage on the crop by sucking plant sap, resulting in stunted plants and distorted leaves. The infested leaves are often cupped or distorted and become more or less yellow. In heavy infestation the plant dies, especially under water stress. Indirectly, cowpea aphid transmits aphid-borne cowpea mosaic viruses.

The performance of the genotypes in response to aphid infestation across the regions and locations was stable. The resistant genotypes (SARC 1-57-2 and SARC 1-91-1) and the susceptible genotypes (Apagbaala, Songotra and Zaayura) maintained their status across the regions and the locations within the regions. Between 10 to 15 days after aphid infestation, the growth and development of the aphids on the resistant genotypes were very slow. The seedlings of the resistant genotypes grew vigorously under aphids attack, they also maintained greenness in their leaves and eventually survived aphid attack. On the other hand, the susceptible genotypes showed heavy aphid population build-up, stunted growth and eventually resulted in the death of the seedlings. The number of aphids under artificial infestation is usually higher than that observed under natural field infestation so the mortality of some resistant seedlings is not expected to occur under natural field conditions.

The results did not show that biotypes of *A. craccivora* exist within the cowpea belt of Ghana distinguishable by differences in damage to cowpea genotypes. Even if there exist differences in the *A. craccivora* population in Ghana, they may be different in genetic composition but not in biological attribute that could make them overcome the resistant genotypes (Saxena and Barrion, 1987).

The current study has therefore confirmed that the aphid resistance gene in SARC1-57-2 is stable against *A. craccivora* in all the major cowpea growing belts in Ghana. SARC1-57-2 is thus a very important breeding material for cowpea breeders in Ghana aiming at breeding for cowpea aphid resistant varieties in any part of the country.

Similarly, SARC1-57-2 may be important to the international cowpea breeding centres such as IITA and University of California, Riverside. The cowpea aphid resistant genotype could feature prominently in their efforts to develop elite cowpea lines for evaluation and adoption in the National Agricultural Research Stations (NARS). Besides being resistant to *A. craccivora*, SARC1-57-2 is early maturing, has white seed coat colour (the most preferred seed coat colour in Ghana) and medium seed size. These additional attributes of SARC1-57-2 make it most suitable and easy to use breeding material for cowpea breeders compared to varieties with non-white coloured seed coats.

Introgression of the aphid resistance gene into most of the commercially important cowpea varieties in Ghana has therefore been identified as a follow up studies to the current study using marker-assisted backcrossing methodology. The marker-assisted backcrossing methodology is proposed to be used in order to get the improved varieties that can be released to the farmers as quickly as possible.

CHAPTER FIVE

5.0. Genetic Mapping and Inheritance of the Aphid Resistance Locus in Cowpea

5.1. Introduction

In cowpea cultivation, attack by insect pests represents an important constraint to obtaining economic yields (Blade *et al*., 1997; Montimore *et al*., 1997). In the savanna regions of West Africa where the bulk of the crop is produced, *A. craccivora* is the most important insect pest during the vegetative phase of the crop (Singh *et al.*, 1990; Obeng-Ofori, 2007). The pest primarily infests the seedlings of cowpea and causes direct damage on the crop by sucking plant sap, resulting in stunted plants and distorted leaves. *Aphis craccivora* also causes indirect damage by transmitting aphid-borne cowpea mosaic viruses (Singh and Jackai, 1985).

Research at the International Institute of Tropical Agriculture (IITA) identified resistant sources in cowpea against *A. craccivora*, with antibiosis as the main basis for resistance (Ansari, 1984; Singh, 1977). Following this, a number of breeding lines with resistance to the pest were developed at IITA and distributed to cowpea breeding stations worldwide (Bata *et al*., 1987; Ofuya, 1997a; Singh, 2004). Field tests in many locations including Ghana had shown that the IITA type of resistance was not effective against local biotypes of the aphid in many locations (Messina *et al*., 1985; Kusi *et al*., 2010a). Resistance tests in Ghana with IT 97K-499-35, bred with the IITA source of resistance, for example, had been shown to be highly susceptible to *A. craccivora* (Kusi *et al*., 2010a).

On-going research at the CSIR-Savanna Agricultural Research Institute (CSIR-SARI) to identify resistance sources in cowpea to the cowpea aphid has uncovered a number of advanced breeding lines with high levels of resistance to the pest (Kusi *et al*., 2010a).

In these tests, lines with the IT 84S-2246 source of resistance that was identified at IITA were not more resistant than the susceptible Ghanaian cultivar, Apagbaala. The advanced breeding line SARC 1-57-2 was able to grow and yield successfully after manually infesting plants with *A. craccivora*.

On-going efforts at sequencing the cowpea genome (Timko, 2009), have provided the opportunity to obtain a large number of co-dominant PCR based markers for genome analysis. This also presents an opportunity to tag loci underlying key traits of agronomic importance with markers to facilitate marker-assisted breeding of the crop. Marker-based selection enhances the efficiency of breeding for simple inherited traits such as aphid resistance. Phenotypic screening for aphid resistance for instance is laborious, expensive and dependent on favourable environmental conditions. Availability of tightly linked markers will therefore facilitate early generation selection, reducing the effective size of breeding populations and enhancing the overall efficiency of cultivar development.

Knowledge of the inheritance of insect resistance is useful in the design of appropriate breeding procedures to develop resistant cultivars and for the identification of biotypes that may already exist or develop over time (Smith, 1989). This study sought to determine the mode of inheritance of aphid resistance in the line SARC 1-57-2 and to identify markers linked to the aphid resistance locus in cowpea to facilitate the use of this source in breeding cowpea for resistance to *A. craccivora*.

5.2. Materials and Methods

5.2.1. Plant Materials Used in the Study

The plant materials used were progenies of the cross between the Ghanaian cowpea cultivar, Apagbaala (Padi *et al*., 2004) and an advanced breeding line SARC 1-57-2. SARC 1-57-2 is an inbred line (F_8) selected from the cross between Apagbaala and a line with exotic pedigree, UCR 01-11-52 (Padi and Ehlers, 2008). SARC 1-57-2 was observed to be resistant to *A. craccivora* under both screen house and field conditions among a large number of test lines (Kusi *et al*., 2010a). One hundred and sixty-nine F_2 lines of the cross between Apagbaala and SARC 1-57-2 were tested for their reaction to aphid infestation in a screen house facility following standard protocols (Bata *et al*., 1987; Kusi *et al*., 2010a) as described in (3.3). As soon as individual lines could be unambiguously classified into resistant or susceptible classes, they were sprayed with lambda cyhalothrin (Lambda Super®) to control the aphids. Recovered plants were maintained to generate F_3 seeds for progeny testing. DNA was obtained from each of the field-grown lines and stored in a refrigerator until needed. Each F_2-derived F_3 family ($F_{2:3}$) was tested further for their reactions to the aphid using 20 seedlings per family.

5.2.2. Identification of Markers Linked to the Aphid Resistance Locus

Fifty simple sequence repeat (SSR) primer pairs randomly selected across the cowpea genome were provided by Professor Mike Timko of the University of Virginia. The primers were tested for their ability to generate reproducible banding patterns in the parents of the mapping population. The sub-set of primers that produced clear reproducible bands were tested on two groups of five resistant and five susceptible individuals based on classification of F_2 plants infested with the aphids. Primer pairs that showed polymorphism between the two sets of lines following denaturing polyacrylamide gel electrophoresis were tested further on all the 169 individuals.

5.3. Data Analyses

Chi-square tests were performed to test the goodness of fit of observed segregations among F_2 plants and $F_{2:3}$ families to that of a single dominant gene. Similarly, the segregation pattern of SSR markers was tested for their fit to that of a single locus. Segregation among $F_{2:3}$ families was analyzed after classifying each family as homozygous resistant (all plants showing same vigour as non-infested controls), homozygous susceptible (all plants dead by 10 days after infestation), and heterozygous (both resistant and susceptible plants were identified).

5.4. Results

5.4.1. Inheritance of Aphid Resistance in Line SARC 1-57-2

Only the parental phenotypes (Apagbaala, susceptible; SARC 1-57-2, resistant) were observed in the F_2 population of Apagbaala × SARC 1-57-2. On resistant plants, aphid colonies increased in numbers slowly on inoculated trifoliates within the first 10 days that it was easy to count the total numbers per plant. On susceptible individuals, infested plants were overcrowded with the insect and death of seedlings began after the 10th day of inoculation. The observed segregation ratio was 123 resistant plants to 46 susceptible plants which fits a 3:1 ratio ($\chi^2 = 0.44$; $P = 0.505$). After spraying the plants with insecticides to kill the aphids, only 128 plants could establish successfully in the field and produce the minimum of 20 seeds. This represented 108 resistant plants (88% recovery of plants) and 20 susceptible plants (43% recovery of plants). The $F_{2:3}$ families therefore did not accurately represent the F_2 population. The segregation ratio was 35 homozygous resistant families, 73 heterozygous families and 20 homozygous susceptible families. Because of the different recovery rates of F_2 plants in different resistance classes, only the ratio of resistant to segregating families was tested. The ratio of 35:73 resistant: segregating or heterozygous $F_{2:3}$ families significantly fit the 1:2 resistant/segregating (heterozygote) ratio expected for a monogenic dominant gene ($\chi^2 = 0.042$; $P = 0.838$)

5.4.2. Identification of Markers Linked to the Aphid Resistance Locus

Of the 50 SSR markers tested, only 31 amplified the cowpea lines in the two sets of five resistant and five susceptible classes, and produced reproducible banding patterns on denaturing PAGE. However, only four primer pairs (CP171F, CP172R; MS50F, MS50R; Y31F, Y31R and CP573F, CP573R) showed polymorphism between the two classes of resistant and susceptible lines. Out of these four, only CP 171F/172R (left sequence: 5’-TAGGGAGTTGGCCACGATA-3’; right sequence: 5’-CAACCGATGTAAAAGTGGACA-3’) displayed a segregation pattern consistent with the phenotypic scores obtained following aphid infestation of the 128 lines (Fig 5.1). The expected band size of 176 bp based on information in the cowpea genomics database (http://cowpeagenomics.med.virginia.edu/CGKB/) was observed following PAGE. To determine the degree of linkage between CP 171F/172R and the aphid resistance locus, the F_2 individuals were analyzed (Fig 5.2). CP 171F/172R was co-dominant and segregated in the expected 1:2:1 fashion following Chi square analysis ($\chi^2 = 0.25$; $P = 0.856$). Based on the SSR data, there were 10 misclassified plants that were possible single crossover events between marker CP 171F/172R and the resistance locus.

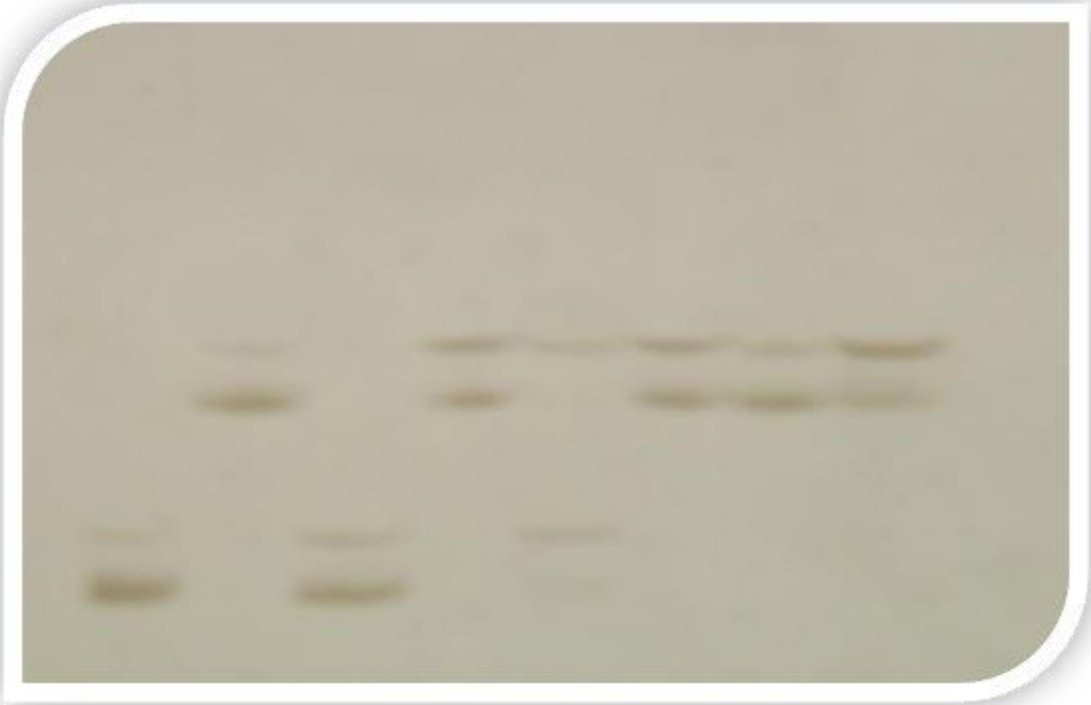

Fig 5.1. A silver stained PAGE showing the DNA banding patterns of resistant and susceptible parents and their progenies amplified by SSR marker CP 171F/172R. The faster migrating band is that of the susceptible parent (Apagbaala).

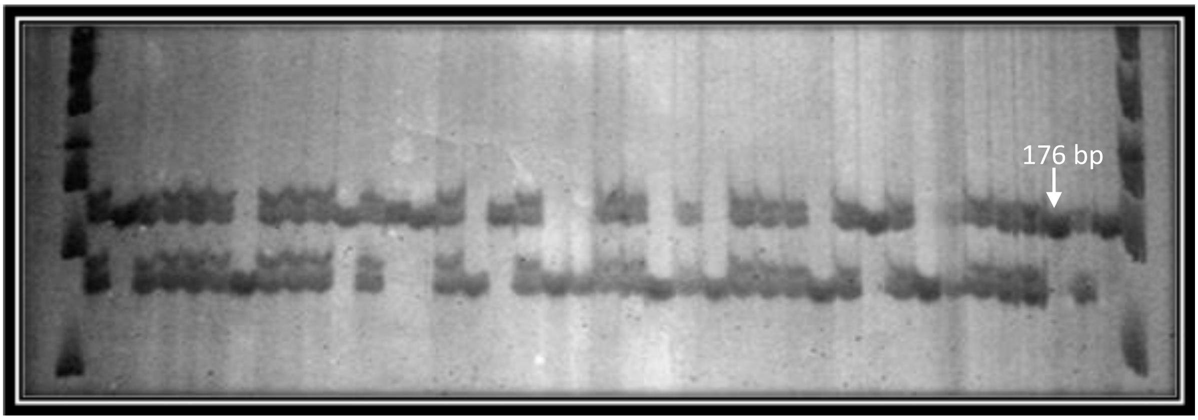

Fig. 5.2. A silver stained PAGE showing the DNA banding patterns of F2 plants derived from Apagbaala × SARC 1-57-2 amplified by SSR marker CP 171F/172R. The faster migrating band is that of Apagbaala.

5.5. Discussion

In peasant crop farming, as is cowpea production in West Africa, the use of insect resistant cultivars offers one of the simplest and most convenient methods of pest control (Dent, 1991). The use of resistant varieties appears to be the best option for the small scale farmers of the Semi-Arid Tropics (SAT) owing to its low cost, compatibility with other control methods, and to the low incomes realised by the farmers (Dent, 1991). The discovery of resistance in cowpea to the aphid in a local × exotic cross (Apagbaala × UCR 01-11-52, Kusi *et al*, 2010a) provided impetus to initiate breeding for resistance to the insect to improve existing cultivars. Previously reported sources of resistance were found ineffective in Ghana (Singh, 2004) and because Apagbaala is highly susceptible, the source of the resistance will be UCR 01-11-52.

Due to the simple inheritance of the gene found in SARC 1-57-2 and the ease of distinguishing resistant from susceptible plants in aphid resistance bioassays, cowpea breeders will be able to rapidly convert existing cowpea cultivars into aphid resistant cultivars using efficient backcross breeding procedures. In spite of the ease of distinguishing resistant and susceptible plants in phenotypic screen, conducting the entire resistance bioassay in large populations is tedious due to the need to maintain aphids on live plants, and use of nymphs of the same age. Moreover, relative humidity and temperature do influence the growth and survival of the aphids under screen-house conditions which may lead to inefficiencies in selection. Discovery of co-dominant SSR markers linked to the aphid resistance locus will facilitate marker-assisted selection (MAS) which is simpler than phenotypic screening, saves time, resources and effort (Akhtar *et al*., 2010). Moreover, homozygous and heterozygous resistant plants cannot be distinguished by the phenotypic screening, requiring further progeny testing to select desirable plants. Considering that the marker is some distance away from the gene locus, the most practical use of CP 171F/172R may be its application in reducing the size of plants in a population prior to phenotypic screening.

To my knowledge, this is the first account of linkage between a co-dominant SSR marker and aphid resistance locus with strong effect on *A. craccivora* infestation in cowpea. The map location of CP 171F/172R is the tip of linkage group 2 (Mike Timko, personal communication). With knowledge of its map location, markers within the vicinity will need to be tested to uncover others more closely linked to the resistance locus. The identification of this single resistance locus and a position on the cowpea genetic map will facilitate the deployment of resistance as a component of integrated management of *A. craccivora* in West Africa.

CHAPTER SIX

6.0. Introgression of Aphid Resistance Locus into Ghanaian Cowpea Cultivars

6.1. Introduction

For the introgression of qualitative traits such as pathotype-specific disease resistance, which are typically controlled by single dominant genes, backcross breeding (BC) has been used for a long time (Allard, 1960; Fabio *et al*., 2004). It allows the transfer of one or few genes from a donor genotype (typically with poor agronomic traits) into an elite recipient genotype, the recurrent parent (Welz and Geiger, 1999). Classical backcross breeding can be termed as phenotypic background selection (Welz and Geiger, 1999). In each BC generation, carriers of the target gene would be directly identified by a phenotype-based assay and the portion of unwanted donor genes would be halved. For the transfer of a single dominant gene, six BC generations would normally be conducted to recover 99% of the recurrent parent genome, a time-consuming procedure (Welz and Geiger, 1999).

The use of genetic and genomic analysis to help identify DNA regions tightly linked to agronomic traits in crops can improve the efficiency of breeding strategies for crop improvement. The use of molecular markers for indirect selection of improved crops speeds up the selection process by alleviating time-consuming approaches of direct screening under greenhouse and field conditions (Dita *et al*., 2006). Molecular markers are particularly useful when targeting characters controlled by several genes (Dita *et al*., 2006). The potential to map different Quantitative Trait Loci (QTL) contributing to a trait of agronomic importance and to identify linked molecular markers opens up the possibility to transfer simultaneously several QTLs and to pyramid QTLs for several important traits in one improved cultivar (Dita *et al*., 2006). However, Yu *et al*. (2004) outlined the following factors to be considered as prerequisites on studies to identify and validate potential markers: (a) level of polymorphism existing between parental lines, (b) unclear expression of some markers inherent to the marker class used, (c) false-positive markers, (d) discrepancy between the presence of the marker and target gene, which requires testing the gene with conventional screening and (e) presence of multiple genes scattered over several linkage groups.

The current study sought to deploy the co-dominant SSR marker, CP 171F/172R, linked to the aphid resistance locus to facilitate marker-assisted backcrossing to improve an elite cowpea variety, Zaayura for resistance to the aphid.

6.2. Materials and Methods

6.2.1. Polymorphism Test

Tests for polymorphism for marker CP 171F/172R were conducted with the methodology in (3.5.1.) on cowpea cultivars that had previously been shown to be susceptible to the cowpea aphid. The cultivars were developed and released for general cultivation in 2004 by the CSIR-Savanna Agricultural Research Institute (CSIR-SARI). The varieties were Padi Tuya, Zaayura, Songotra, and Bawutawuta.

6.2.2. Marker Assisted Backcrossing

Among the four cultivars tested, only Zaayura was polymorphic at the CP 171F/172R locus and was therefore selected as the recurrent parent for introgression of the aphid resistance locus. The methodologies described in (3.5.2.1) were used to introgress the cowpea aphid resistance locus into Zaayura.

6.3. Results

6.3.1. The Test for Polymorphism

The results of the polymorphism test between SARC 1-57-2 and the four cultivars, Padi Tuya, Zaayura, Songotra and Bawutawuta are presented in Fig. 6.1. Among the four cultivars that were tested, the marker CP 171F/172R showed polymorphism between Zaayura and the resistant cultivar, SARC 1-57-2.

Fig. 6.1. **DNA banding patterns for marker CP 171F/172R on four cowpea cultivars and susceptible and resistant checks.**

6.3.2. Determination of Plants from a Successful Cross (F_1 Plants)

The results from F_1 plants between SARC1-57-2 and Zaayura which were genotyped with CP 171F/172R prior to the backcrossing to the recurrent parent, Zaayura to determine which of the plants were from a successful cross is presented in Fig. 6.2. Four samples of the susceptible parent (Zaayura) and three samples of the resistant parents (SARC1-57-2) were included as checks. With the exception of the susceptible and resistant checks, all the other samples were heterozygous for the CP 171F/172R locus (F_1 plants).

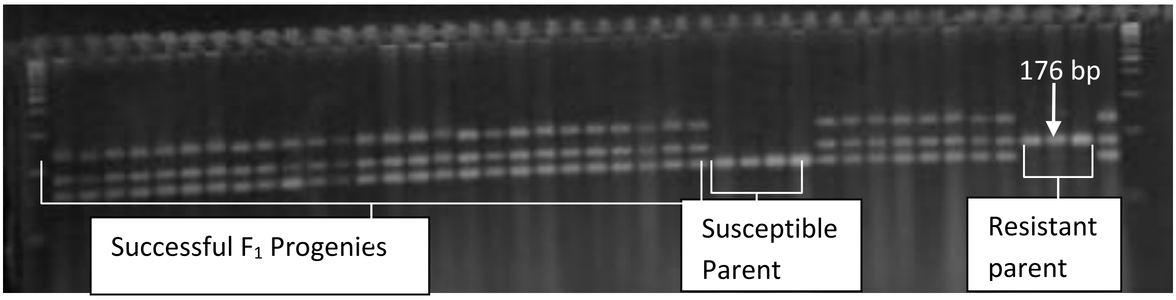

Fig. 6.2. DNA banding patterns of the F_1 plants from the crosses between SARC1-57-2 and Zaayura genotyped with CP 171F/172R.

6.3.3. Genotyping to Select Heterozygotes from the Backcross Populations

The results from individual plants from successive backcross populations (BC_1 to BC_4) genotyped for their banding pattern at the CP 171F/172R locus is presented in Fig. 6.3. Based on Chi-square tests for goodness of fit, the segregation ratios fit the expected 1:1 ratio for heterozygous and homozygous susceptible individuals ($\chi^2 = 0.138$; $P = 0.710$).

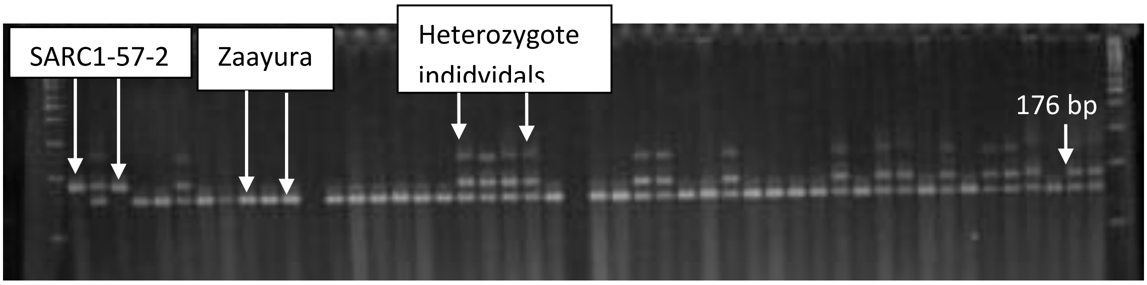

Fig.6.3. DNA banding patterns of successive backcross populations genotyped to select heterozygote individuals at the CP 171F/172R locus

6.3.4. Genotyping of BC_4F_2 to Select Homozygous lines

A BC_4F_1 individual was selfed to generate BC_4F_2 lines. BC_4F_2 were genotyped with the marker CP 171F/172R to select individual plants that are homozygous dominant for the marker locus (Fig. 6.4). Out of sixty BC_4F_2 plants screened, the segregation at the marker locus was 13:31:16 (homozygous resistant: heterozygous: homozygous susceptible) which fits the expected ratio of 1:2:1 for a single dominant gene using χ^2 tests ($\chi^2 = 0.37$; $P = 0.83$).

Fig. 6.4. DNA banding patterns of BC_4F_2 genotyped with the marker CP 171F/172R

6.4. Discussion

The tests for polymorphism between the donor of the resistace locus (SARC 1-57-2) and the cultivars determined that only the cultivar Zaayura was suitable as the recurrent parent for improvement. The size of the populations created and screened for resistance in the screenhouse studies in the various generations were reduced significantly in size by selecting only for segregants heterozygous for the marker locus. This reduced the total amount of time spent in selecting individuals that will serve as parents for a subsequent generation, and the overall effeciency of the backcross method of transfering the resistance locus.

Segregation distortion, though common in mapping populations (Lorieux *et al.*, 1995) was not observed in the current study. At each generation, the Chi square tests showed a good fit to the expected Mendelian ratio for a single locus.

At the BC_4F_1 where over 90% of the background of the recurrent parent is recovered and the aphid resistance gene is more or less fixed, the BC_4F_1 was selfed to generate BC_4F_2 individuals. The BC_4F_2 population when genotyped afford the opportunity to select individuals having the aphid resistance gene in a homozygous resistance state. These individuals were tagged and subjected to further phenotypic selection based on the feature of the recurrent parent (vegetative, podding and seed) and the seeds of the improved individuals were subsequently multiplied. In a field evaluation of the improved Zaayura it was realised that the mproved Zaayura has recovered all the physical features of the original Zaayura. This is an indication that the BC4 was adequate to retain the background of the recurrent parent. The multiplied seeds of the improved Zaayura have been presented for assessment and approval to be released as a variety.

Inspite of the limitation of the SSR marker CP 171F/172R, it has successfully been used in combination with intermittent aphid screening in a coordinated backcrossing programe to improve the field resistance of Zaayura. This was achieved within two years which could not have been possible under conventional approach. Conventional plant breeding is primarily based on phenotypic selection of superior individuals among segregating progenies resulting from hybridization. It is often time consuming as breeding a new variety in some crops takes between eight and twelve years and even then, the release of improved variety is not guaranteed (Ibitoye and Akin-Idowu, 2010). Hence, breeders are extremely interested in new technologies that could make this procedure more efficient. Molecular marker-assisted selection offers such a possibility by adopting a wide range of novel approaches to improving the selection strategies in crop breeding (Ibitoye and Akin-Idowu, 2010). Thus, molecular markers bring a systematic basis to traditional breeding, enhancing its precision and expediting the process (Kumar, 1999; Collard et al., 2005).

Unwanted self-pollination in field or during crossing programs is one of the major sources of impurity of hybrid seeds that interferes with trait improvement via conventional breeding programs or variety improvement via backcross scheme (Asadollah and Mehdi, 2010). Conventional characterization of hybrid seeds based on specific morphological and agronomic data is time-consuming, restricted to a few characteristics, and is influenced by environment. In contrast, DNA-based markers are highly heritable, available in high numbers, and exhibit enough polymorphism; hence they can be used to trace that alleles came from a given parent or to discriminate closely related genotypes of a plant (Yashitola *et al*., (2002); Wang *et al*., (2005); Asadollah and Mehdi (2010)).

This achievement underscores the importance of marker-assisted selection (MAS) in plant breeding in the national agricultural research system (NARS). The current efforts in capacity building in both human and infrastructure in most of the NARS in Ghana and Africa as a whole should therefore be given the necessary support by the state as a bold step towards achieving food security.

CHAPTER SEVEN

7.0. Yield Loss Assessment of Ten Cowpea Varieties

7.1. Introduction

Aphis craccivora Koch, a phloem feeding insect, is a major insect pest of cowpea with a worldwide distribution (Obeng-Ofori, 2007). They are economically important insect pests causing serious damage to several crop plants (Obeng-Ofori, 2007, Kusi *et al*., 2010a). They feed mainly by sucking plant sap from the succulent parts of the plant including leaves, stem, terminal shoots, petioles, flowers and pods (Asiwe *et al*., 2005). Damage is caused by both nymphs and adults sucking plant sap from seedling to pod bearing stage (Kusi, 2008). The harmful effects of cowpea aphid can be assessed either directly or indirectly. The direct damage on the crop due to the sucking of plant sap causes stunted growth and distorted leaves (Fatokun, 2002; Kusi, 2008; Souleymane *et al*., 2013). In a field study, Kusi *et al*. (2010b) observed delayed flowering and maturity in medium to late cowpea cultivars and attributed it to stunted growth as a result of the sucking of plant sap by *A. craccivora*.

Aphid feeding interferes with the amount of photosynthates translocated for leaf production, flower initiation and podding (Annan *et al.*, 1995; Giordanengo *et al.*, 2010). Ofuya (1993) indicated that, heavy feeding of aphids can kill young plants, distort leaves, delay flower initiation and reduce pod set in plants which survive aphid attack. Leaf distortion as a result of aphid infestation reduces the photosynthetic area of the leaves with consequent reduction in the photosynthetic rate of the plant resulting in stunted growth and low yield (Annan *et al.*, 1995; Giordanengo *et al.*, 2010).

Aphids have also been found to severely damage cowpea indirectly through the transmission of cowpea aphid-borne mosaic virus (Laamari *et al*., 2008). Cowpea aphid-borne mosaic virus is considered an important limitation in cowpea production. It is an economically important virus causing yield losses exceeding 87% under field conditions (Bashir and Hampton, 1996). The combined effects of cowpea aphid-borne mosaic virus and stunted growth has been reported to be the most damaging effect of cowpea aphid (Laamari *et al*., 2008). Blaney *et al*. (1990) indicated that the feeding action of aphids lower the yield, quality and marketability of cowpea by transmitting plant viruses.

The intensity of damage caused by insect pests varies greatly with the intensity of infestation, duration of occurrence and stage of growth of the plant (Dent 1991; Kusi *et al.*, 2010b). Dent (1991) indicated that the combination of these three factors in relation to the crop affect crop yield. Yield loss due to aphids is assessed by comparing the yield of cowpea from protected and unprotected fields. The yield from the protected field represents the attainable yield while the yield from the unprotected plot represents the actual yield with the difference between the two yields accounting for the yield reduction due to aphid infestation. The current study therefore assessed yield loss of 10 cowpea cultivars due to aphid infestation.

7.2. Materials and Methods

The assessment of the yield loss of the 10 genotypes was carried out at the Manga (Upper East Region) Station of CSIR-Savanna Agricultural Research Institute. The genotypes (Table 7.1) include advanced breeding lines from the IITA (SARC1-57-2, IT99K-573-3-2-1, SARC1-91-1 and IT99K-573-1-1) and cultivars (Apagbaala, Padituya, Songotra (IT97K499-35) and Zaayura) and two advanced breeding lines developed during the course of this study. The advanced backcross progeny were selected based on SSR marker scores at the aphid resistance locus either as resistant or susceptible. The treatments were replicated six times in a randomized complete block design. Each plot consisted of 2 rows of 4 m long. Plots were separated from each other at a distance of 1 m. Three seeds were sown per stand at 60 cm between rows and 20 cm within rows and were thinned to one plant per stand at 10 days after planting.

There were two trials, one trial serving as the control was sprayed on three occasions (seedling, flowering and podding phases) against insect pests with lambda cyhalothrin (Lambda Super®), a synthetic pyrethroid, at the rate of 20 g active ingredient ha^{-1}. The other trial was sprayed on two occasions (flowering and podding phases). The cowpea seedlings of the second trial were infested with five, four-day old aphids per seedling (Annan *et al*., 1995; Bosque-Perez and Schotzko, 2000; Kusi *et al*., 2010b; Benchasri *et al*., 2012) two weeks after planting. During the period of infestation, the seedlings were confined under insect proof net in order to limit the damage of the seedlings to only aphids and to prevent effects of predators and parasitoids on the aphids. The aphids were allowed to form colonies and fed on the seedlings until symptoms of damage were observed on the susceptible seedlings. When the susceptible seedlings became stunted with distorted and yellowing leaves at sixteen days after infestation (30 days after planting), the aphids were killed by spraying with lambda cyhalothrin (Lambda Super ®). At plant maturity (65 days after planting), the pods were harvested, dried, threshed and grain weight recorded using an electronic balance. Other agronomic data recorded include: days to 50% flowering, days to maturity, weight of pods, and weight of vegetative biomass at maturity.

Percentage grain yield reduction due to aphid infestation was calculated as:

$$\frac{\text{Yield in uninfested plot - Yield in infested plot}}{\text{Yield in uninfested plot}} X\,100$$

Table 7.1. Description of the 10 cultivars of cowpea by parentage or source

Variety	Description
APAGBALA	Prima/TVu 4552/California Blackeye No.5/7977. Cultivar, released in 2002 in Ghana. Largely of exotic background.
IT99K-573-1-1	Breeding line from the IITA, Ibadan Nigeria
PADITUYA	Apagbaala/ UCR 01-11-52 released in 2008 in Ghana
SARC1-91-1	Apagbaala/ UCR 01-11-52 Breeding line of SARI
BC_4F_3 (Zaayura //(Zaayura x SARC1-57-2) susceptible	Marfo-Tuya/ UCR 01-15-127-22/SARC1-57-2
ZAAYURA	Marfo-Tuya/ UCR 01-15-127-2 released in 2008 in Ghana
IT99K-573-3-2-1	Breeding line from the IITA, Ibadan Nigeria
IT97K499-35 (Songotra)	Breeding line from the IITA, Ibadan Nigeria. Cultivar, released in 2004 in Ghana as Songotra.
BC_4F_3 (Zaayura //(Zaayura x SARC1-57-2) resistant	Marfo-Tuya/ UCR 01-15-127-2/SARC1-57-2
SARC1-57-2	Apagbaala/ UCR 01-11-52 Breeding line of SARI

7.3. Data Analysis

GenStat statistical program (9th edition) was used to analyze the data. Fisher's LSD was used to separate the means after ANOVA showed significant differences.

7.4. Results

7.4.1. Grain Yield

Grain yield and percentage yield loss of 10 cowpea cultivars evaluated under aphid infestation and no infestation are presented in Table 7.2. The grain yield recorded under uninfested plots ranged between 775 and 1086 kg ha^{-1}. Padituya recorded significantly

(P<0.05) higher grain yield than six of the cultivars, however, there was no significant differences among Padituya, improved Zaayura, Apagbaala, SARC 1-57-2 and SARC 1-91-1. Apart from Padituya, there was no significant difference among the rest of the cowpea cultivars evaluated in the study under uninfested conditions. On the other hand, under infested conditions, Padituya recorded the highest grain yield whereas the susceptible progeny recorded the lowest grain yield. However, there were no significant differences among Padituya, Improved Zaayura, SARC 1-57-2, SARC 1-91-1 and IT97K-499-35. On the other hand, there were also no significant differences among susceptible progeny, Zaayura, IT99K-573-1-1, IT99K-573-3-2-1, Apagbaala, IT97K-499-35 and SARC1-91-1

Generally, all the cultivars yielded higher grains under uninfested conditions than under aphid infested conditions. The percentage yield loss ranged between 4.9% and 32.8%. The three cultivars that recorded the lowest yield loss due to aphid infestation were improved Zaayura, SARC 1-57-2 and SARC 1-91-1 which recorded 3.8%, 4.9% and 9.8%, respectively. On the other hand, the cultivars that recorded the highest yield loss were the susceptible progeny, IT99K-573-1-1 and Apagbaala with yield loss of 32.8%, 32.1% and 30.3%, respectively. The other cultivars that also suffered substantial yield loss due to aphid attack were Zaayura (26.6%) and IT99K573-3-2-1 (27.0%). Two cultivars, IT97K-499-35 (17.1%) and Padituya (16.1%) suffered moderate yield losses.

Table 7.2. Grain yield and percentage grain yield loss (kg) ha^{-1} of 10 cowpea cultivars evaluated under aphid infestation and no infestation

	Uninfested	**Infested**	
Variety	**Grain yield (Kg)/ha**	**Grain yield (Kg)/ha**	**% yield loss (Kg)/ha**
APAGBALA	897	625	30.3
IT99K-573-1-1	806	547	32.1
PADITUYA	1086	911	16.1
SARC1-91-1	850	767	9.8
BC_4F_3 (Zaayura //(Zaayura x SARC1-57-2) susceptible	806	542	32.8
ZAAYURA	775	569	26.6
IT99K-573-3-2-1	803	586	27.0
IT97K499-35 (Songotra)	814	675	17.1
BC_4F_3 (Zaayura //(Zaayura x SARC1-57-2) resistant	936	900	3.8
SARC1-57-2	850	808	4.9
Mean	**862.3**	**693.0**	
s.e.d	**120.9**	**126.0**	
CV%	**24.3**	**31.7**	

7.4.2. Biomass Production

The dry biomass per hectare and percentage vegetative biomass loss of the 10 cultivars evaluated under aphid infested and uninfested conditions are presented in Table 7.3. The improved Zaayura recorded the highest dry biomass yield; however, there were no significant differences (P>0.05) among the improved Zaayura and Padituya, susceptible progeny and Zaayura. The lowest dry biomass yield was recorded for Apagbaala which was not significantly different (P>0.05) from IT99K-573-3-2-1, IT99K-573-1-1, IT97K-499-35, SARC1-57-2 and SARC1-91-1.

Under the infested conditions, the improved Zaayura had the highest dry biomass yield, although it was not significantly different (P>0.05) from Padituya and Zayura. The cultivar IT99K-573-3-2-1 recorded the lowest dry biomass yield; however, it was not significantly different from IT99K-573-1-1, IT97K-499-35, SARC1-57-2 and SARC1-91-1, Apagbaala and SARC1-57-2.

The percentage dry biomass yield loss ranged between 3.3% and 14.3% (Table 7.3). The improved Zaayura recorded the least dry biomass yield loss while Zaayura suffered the highest yield loss. The cultivars that suffered dry biomass yield loss above 10% were Padituya (10.5%), the susceptible progeny (12.3%), Zaayura (14.1%) and IT99K-573-3-2-1 (14.3%). Four cultivars that suffered less than 10% dry biomass yield loss were SARC 1-57-2 (6.1%), IT99K-573-1-1 (8.9%), SARC 1-91-1 (9.4%) and IT97K-499-35 (9.5%).

Table 7.3. The dry biomass yield and percentage dry biomass yield loss (kg) ha^{-1} of the 10 cultivars evaluated under aphid infested and uninfested conditions

	Uninfested	**Infested**	
Variety	**Biomass (Kg)/ha**	**Biomass (Kg)/ha**	**% yield loss**
APAGBALA	3236	2942	9.1
IT99K-573-1-1	3528	3214	8.9
PADITUYA	4947	4428	10.5
SARC1-91-1	3439	3117	9.4
BC$_4$F$_3$ (Zaayura //(Zaayura x SARC1-57-2) susceptible	4528	3972	12.3
ZAAYURA	4900	4211	14.1
IT99K-573-3-2-1	3278	2808	14.3
IT97K499-35 (Songotra)	3719	3367	9.5
BC$_4$F$_3$ (Zaayura //(Zaayura x SARC1-57-2) resistant	5028	4864	3.3
SARC1-57-2	3667	3444	6.1
Mean	**4027**	**3637**	
s.e.d	**310.8**	**324.2**	
CV%	**13.4**	**15.4**	

7.4.3. Days to Flowering

The number of days to 50% flowering under both infested and uninfested conditions of the 10 cultivars is presented in Table 7.4. Significantly ($P<0.05$) SARC 1-57-2 and IT97K-499-

35 were early to flower although there was no significant difference between IT97K-499-35 and IT99K-573-3-2-1. On the other extreme, Zaayura, the susceptible progeny and Padituya significantly (P<0.05) flowered late among the cultivars. However, there were no significant differences between Padituya and the improved Zaayura. The rest of the cultivars fell between the two extremes which flowered after 40 to 42 days. Under uninfected conditions, SARC 1-57-2 and IT99K-573-3-2-1 significantly flowered earlier among the 10 cultivars; however, there were no significant differences between IT99K-573-3-2-1 and IT99K-573-1-1, SARC 1-91-1, IT97K-499-35 and Apagbaala. The cultivars that significantly (P<0.05) flowered late among the 10 genotypes were Zaayura, the susceptible progeny and Padituya.

Table 7.4. The number of days to 50% flowering under infested and uninfested conditions of the 10 cultivars

	Uninfested	**Infested**
Variety	**Days to 50% flowering**	**Days to 50% flowering**
APAGBALA	38	42
IT99K-573-1-1	38	40
PADITUYA	42	47
SARC1-91-1	37	40
BC_4F_3 (Zaayura //(Zaayura x SARC1-57-2) susceptible	42	48
ZAAYURA	42	48
IT99K-573-3-2-1	37	40
IT97K499-35 (Songotra)	38	39
BC_4F_3 (Zaayura //(Zaayura x SARC1-57-2) resistant	41	46
SARC1-57-2	36	38
Mean	**39**	**43**
s.e.d	**0.4**	**0.6**
CV%	**1.5**	**2.5**

7.4.4. Maturity period

The number of days to maturity under both infested and uninfested conditions of the 10 cultivars is presented in Table 7.5. Under the infested conditions, SARC 1-57-2, IT97K-499-35 and IT99K-573-1-1 significantly (P<0.05) matured earlier among the cultivars. However, IT97K-499-35 and IT99K-573-1-1 were not significantly different from IT99K-573-3-2-1. Similarly, IT99K-573-1-1, IT99K-573-3-2-1 and SARC 1-91-1 were not significantly different in terms of days to maturity. On the other hand, Zaayura significantly (P<0.05) recorded the longest days to maturity (70 days). The susceptible progeny and the improved Zaayura came next to Zaayura which also recorded significantly longer days to maturity than the remaining cultivars. Padituya and Apagbaala, although were significantly different, fell between the extremes with 64 and 68 days to maturity, respectively. Under the uninfested conditions, SARC 1-57-2, IT99K-573-3-2-1 and IT99K-573-1-1 significantly (P<0.05) matured earlier among the cultivars. Significant differences were not observed among IT99K-573-3-2-1, IT99K-573-1-1, IT97K-499-35, SARC 1-91-1 and Apagbaala. Padituya, the susceptible progeny, the improved Zaayura and Zaayura recorded the highest number of days to maturity ranging between 65 and 66.

Table 7.5. The number of days to maturity under infested and uninfested conditions of the 10 cultivars

	Uninfested	**Infested**
Variety	**Days to maturity**	**Days to maturity**
APAGBALA	61	64
IT99K-573-1-1	60	63
PADITUYA	65	68
SARC1-91-1	61	63
BC_4F_3 (Zaayura //(Zaayura x SARC1-57-2) susceptible	65	69
ZAAYURA	65	70
IT99K-573-3-2-1	60	63
IT97K499-35 (Songotra)	61	63
BC_4F_3 (Zaayura //(Zaayura x SARC1-57-2) resistant	65	69
SARC1-57-2	59	62
Mean	62	65
s.e.d	0.6	0.3
CV%	1.6	0.9

7.5. Discussion

Resistance to the cowpea aphid among advanced breeding lines selected from recurrent backcross selection using Zaayura as the recurrent parent has significantly been improved with the resistance from SARC 1-57-2. This was achieved within a period of two years using marker-assisted backcrossing methodology, which could otherwise be achieved in not less than five years using the conventional selection methods. The improved resistance of the selected lines was manifested in the reduced percentage of yield loss under no insecticide protection during the vegetative phase,

The extrapolation of this observation to field conditions will be improved stability of yield under typical farmers' production conditions. The resource poor farmers can hardly afford the high cost of insecticides and in some cases do not have access to the insecticides even when they have the money to buy. With the improvement of the field resistance of Zaayura, the farmers will not need to spray against aphids at the vegetative stage. Since aphid infestation is a major production constraint of cowpea at the vegetative stage (Singh and Jackai, 1985; Jackai and Adalla, 1997; Fatokun, 2002; Obeng-Ofori, 2007; Souleymane *et al*., 2013) the resource poor farmers could reduce up to 30% of the cost of insecticides and labour to spray, limiting insecticide protection to only the flowering and podding phases. The farmers will not only save cost on insecticide and labour, exposure to the harmful chemicals, contamination of water bodies, the environment, resurgence of insects and other hazards associated with the use of chemical insecticides will also be reduced.

In particular, the profitability of dry season production of cowpea on residual soil moisture or under irrigation is expected to increase considerably using the aphid resistant lines developed. In the major cowpea production belt in Northern Ghana for example, farmers move to the banks of the White Volta River between October and December after the rainy season farming activities to make use of the residual moisture in the soil to grow cowpea. Moreover, the bulk of the cowpea in the Upper East region is produced using the residual moisture as a result of the flooding due to the spillage from the Bagre dam in Burkina Faso. Under these conditions, lack of rainfall that is critical in the control of aphids worsens the pest problem and necessitates additional insecticide sprays to produce the crop.

The high dry biomass yield recorded against the improved Zaayura even under infested conditions is of great significance to the crop/livestock intensification production system in Northern Ghana. The northern savannah zone is a predominantly crop and livestock production area. However, feeding of the livestock during the long dry season of up to seven months in the Sudan and Guinea Savannah zones is a major constraint to livestock farming.

The nature of the livestock feeding during the dry season is both of inadequacy and imbalance of the feed. The animals are kept on free range to feed on limited crop residues of cereals, most of which are also destroyed by bush fire, with little or no source of leguminous crop residues.

The combination of good grain and biomass yield by the improved Zaayura even under aphid infestation indicates that the farmers will not only increase their income from cropping the improved Zaayura, but will also have cowpea haulm to improve the cereal crop residue to feed their animals during the long dry season. Improved feeding of livestock has been identified to increase growth, fecundity and productivity of livestock (Akbar *et al*., 2000; FAO, 2012). This will eventually lead to increased income and improved livelihood to the resource poor farmers as a result of improved crop/livestock intensification system.

The original Zaayura has been widely adopted by the farmers as a result of its bigger seed size, white seed coat, high yielding and early maturity. The marker-assisted backcrossing methodology used to develop the improved Zaayura has retained all these features in the improved cultivar. With the exception of its ability to withstand pressure of cowpea aphid infestation and ability to combine good grain yield and biomass, the farmers may not be able to identify any other distinguishing features between the original Zaayura and the improved Zaayura.

Therefore, very simple dissemination strategies such as on-farm demonstrations and field days could be enough to get the farmers adopt the improved Zaayura. It is also recommended that the field resistance of the improved Zaayura to *Striga gesnerioides* should be improved to further increase its yield stability.

CHAPTER EIGHT

8.0. General Discussion

Host-plant resistance is the relative proportions of heritable characteristics of a plant that influence the degree of damage produced by a pest (Painter, 1951; Cuartero *et al.,* 1999; Dent, 2000; Meyer, 2003). It has proved to be a successful tool against insects in many crops (Felkl *et al.*, 2005). Resistant crop varieties provide an inherent control that involves no environmental problems, and they are generally compatible with other insect-control methods (Kfir *et al.*, 2002; Kusi, 2008). The cultivation of resistant crop plants is not subject to the vagaries of weather as are chemical-control measures, and in certain circumstances it is the only effective means of control. Resistant varieties control even a low pest density, whereas insecticide use is justifiable only when the density reaches the economic injury level (Kfir *et al.,* 2002; Kusi, 2008). Identification of SARC 1-57-2 has therefore become an important breakthrough as an effective, affordable and sustainable management of cowpea aphid. The use of host plant resistance can be described as effective means of controlling pests because it addresses problems of chemical insecticide control such as sub dosal application of insecticides. This usually results in ineffective control of the insect pest which in most cases leads to the insects developing resistance to the insecticide and eventually leading to resurgence of insects. The use of host plant resistance is a sustainable means of pest control due to its compatibility with other pest control measures. It does not lead to resurgence of insect pests and it is environmentally friendly.

Identification of host plant resistance and its deployment in plant breeding or in insect management programme are two different things. In most cases, the protectionist identifies materials with various levels of resistance to major pests of crops and that ends it all. In most cases the genotypes found to be resistant to a major pest of a crop tend to trade off in terms of yield, growth habits and seed quality which limits their direct use in production. There is therefore the need for a strong collaboration between the protectionist and the plant breeder to deploy the resistant gene to improve the field resistance of elite genotypes that meet all qualities desired by the farmers. The current study identified the aphid resistant cowpea genotype (SARC 1-57-2) and introgressed the aphid resistance gene into one of the cowpea cultivars released by CSIR-SARI (Zaayura) using marker-assisted backcrossing methodology.

Marker-assisted selection (MAS) is time saving from the substitution of complex field trials (that need to be conducted at particular times of the year or at specific locations, or are technically complicated) with molecular tests. This leads to elimination of unreliable phenotypic evaluation associated with field trials due to environmental effects. Selection of genotypes is possible with MAS at seedling stage as well as gene ‘pyramiding’ or combining multiple genes simultaneously. Marker assisted-selection also limits transfer of undesirable or deleterious genes. Other advantages for using MAS include: selecting for traits with low heritability, testing for specific traits where phenotypic evaluation is not feasible (e.g. quarantine restrictions may prevent exotic pathogens to be used for screening).

The current study embarked on a search for a DNA marker that is tightly linked with the aphid resistance gene. The search led to the identification of CP 171F/172R which showed polymorphism between the aphid resistant genotype (SARC 1-57-2) and a susceptible genotype (Apagbaala). Zaayura was subsequently selected following a polymorphism study between SARC 1-57-2 and four elite varieties released by CSIR-SARI. The marker CP 171F/172R showed polymorphism between Zaayura and SARC 1-57-2. Marker-assisted backcrossing methodology was therefore used to improve the field resistance of Zaayura to cowpea aphid. The advanced breeding lines selected from the recurrent backcrossing of Zaayura are resistant to the cowpea aphid. In a replicated study, the new Zaayura maintained all the phenotyped features of the original Zaayura. These include high grain yield, early maturity, large seed size, white seed coat and brown eye colour. In addition, it combined both high grain yield and biomass yield even under no spray conditions. The improved Zaayura has been presented for assessment and release by the National Variety Release Committee.

8.1. Conclusion

To my knowledge, the current success in improving the field resistance of Zaayura to cowpea aphid using marker-assisted backcrossing is the first report of marker-assisted selection in cowpea leading to direct deployment in the field. This was achieved in the space of two years which would have taken a minimum of five years using the conventional breeding approach. Incorporation of marker-assisted breeding efforts into the breeding programmes is advocated on the strength of this study. Further investments in laboratory infrastructure are advocated to help improve the effectiveness of the breeding process, particularly for simply inherited traits.

8.2. Recommendations

Large-scale deployment of varieties with a single locus conditioning resistance may lead to breakdown of resistance, due to directional selection for virulent races that may be present at low frequencies within the pest population. A search for cowpea varieties that possess resistance conditioned by other loci is therefore recommended to reduce the chances of breakdown of the resistance. In this regard, it is also recommended that follow up studies should include the determination of the sources of resistance in SARC1-57-2 and SARC1-91-1 for possible pyramiding of genes if they are found to be different.

With the report of the existence of different biotypes of cowpea aphids in West Africa, it is recommended that future studies should aim at assembling the various sources of cowpea aphid resistant genotypes across West Africa. The similarity or otherwise of the genes controlling the resistance in the different genotypes should be determined for pyramiding of genes if they are found to be different genes.

It is also recommended that further search for DNA marker(s) closer than CP171F/172R to the aphid resistance gene in SARC 1-57-2 should be carried out to enhance efficiency in its deployment in marker-assisted selection. The biochemistry that underline the cowpea aphid resistance in SARC 1-57-2 is also very important to be investigated in follow up studies. This could help make clearer the mechanism of resistance and could lead to development of biochemical assays to rapidly identify other resistant sources in the cowpea germplasm.

The importance of resistant cultivar as a principal component of integrated pest management cannot be over emphasized, owing to its compatibility with other crop and pests management practices. It is therefore recommended that a search for sources of natural resistance in germplasm of field crops should be given further attention, and be more vigorously pursued in integrated pest management programmes.

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Appendices

The stability of the resistant genotype across cowpea growing zones in Ghana

Analysis of variance

Variate: Vigour score

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	8	8.1432	1.0179	6.80	
Rep.*Units* stratum					
Trt	4	1023.8642	255.9660	1710.87	<.001
Zone	17	2.3765	0.1398	0.93	0.533
Trt.Zone	68	11.9136	0.1752	1.17	0.172
Residual	712	106.5235	0.1496		
Total	809	1152.8210			

Variate: Percentage of seedlings killed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	8	111.91	13.99	1.01	
Rep.*Units* stratum					
Trt	4	1482422.84	370605.71	26791.14	<.001
Zone	17	167.19	9.83	0.71	0.793
Trt.Zone	68	1063.83	15.64	1.13	0.228
Residual	712	9849.20	13.83		
Total	809	1493614.97			

Yield loss assessment of ten cowpea varieties

Aphid infested treatment

Variate: Grain yield (kg/ha)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	131911.	26382.	0.55	
Rep.*Units* stratum					
Cultivar	9	1109644.	123294.	2.55	0.018
Residual	45	2174691.	48326.		
Total	59	3416246.			

Variate: Biomass weight (kg/ha)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	235276.	47055.	0.15	
Rep.*Units* stratum					
Cultivar	9	25815793.	2868421.	9.10	<.001
Residual	45	14186831.	315263.		
Total	59	40237900.			

Variate: Days to 50% flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	3.933	0.787	0.68	
Rep.*Units* stratum					
Cultivar	9	842.067	93.563	81.39	<.001
Residual	45	51.733	1.150		
Total	59	897.733			

Variate: Days to maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	4.2833	0.8567	2.71	
Rep.*Units* stratum					
Cultivar	9	544.0833	60.4537	191.35	<.001
Residual	45	14.2167	0.3159		
Total	59	562.5833			

No aphid infestation Treatment

Variate: Grain yield (kg/ha)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	185702.	37140.	0.85	
Rep.*Units* stratum					
Cultivar	9	462052.	51339.	1.17	0.336
Residual	45	1973262.	43850.		
Total	59	2621016.			

Variate: Biomass weight (kg/ha)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	560130.	112026.	0.39	
Rep.*Units* stratum					
Cultivar	9	29205604.	3245067.	11.20	<.001
Residual	45	13036752.	289706.		
Total	59	42802486.			

Variate: Days to 50% flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	3.9500	0.7900	2.19	
Rep.*Units* stratum					
Cultivar	9	355.4833	39.4981	109.60	<.001
Residual	45	16.2167	0.3604		
Total	59	375.6500			

Variate: Days to maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	19.883	3.977	3.87	
Rep.*Units* stratum					
Cultivar	9	332.017	36.891	35.87	<.001
Residual	45	46.283	1.029		
Total	59	398.183			