UNIVERSITY FOR DEVELOPMENT STUDIES

GENETICS OF COWPEA APHID RESISTANCE

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GENETICS OF COWPEA APHID RESISTANCE

BY

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Thesis Submitted to the Department of Biotechnology, Faculty of Agriculture, University for Development Studies, in Partial Fulfillment of the Requirements for the Award of Master of Philosophy Degree in Biotechnology

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CANDIDATE'S DECLARATION

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

Candidate's signature: _____ Date: _____

Name: Duah Agyeman Godfred

Supervisor's Declaration

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

Supervisor's signature: _____ Date: _____

Name: Dr. Nelson Opoku

Co-supervisor's signature: _____ Date: _____

Name: Dr. Francis Kusi

ABSTRACT

Cowpea is one of the most important dietary staples in tropical Africa. The cowpea aphid (*Aphis craccivora*) is a major pest of cowpea that causes damage from the seedling to pod bearing stage. The use of resistant varieties appears to be the best option for farmers in the tropics owing to its low cost. It has been observed that cowpea aphid resistant lines developed earlier show differential effects on aphid population from different geographical areas.

The legume Innovation lab project has put together eleven lines as sources of aphid resistance and susceptibility to be screened at selected locations in West Africa and California Riverside. In Ghana, the seedling screening at Savannah Agricultural Research Institute (SARI) Station at Manga showed that 5 lines, 58-77, IT9K556-6, KvX-295-2-124-99, SARC-1-57-2 and CB27 showed resistance to *A. cracivora*. A cross between IT9K556-6 and a known susceptible line (Apagbaala) from Ghana showed F_2 progeny segregating into the ratio 3 Resistant: 1 susceptible when infested with cowpea aphids and F_3 population segregating into 1 Resistant 2 segregating for resistance 1 susceptible confirming that the inheritance in IT9K556-6 was conferred by a single dominant gene.

The resistant lines IT9K556-6 and KvX-295-2-124-99 were also crossed to a known resistant line in Ghana (SARC-1-57-2) to determine allelic relationship of the resistant genes. F_2 generation of IT9K556-6 x SARC-1-57-2 segregated into 15:1 resistant/susceptible ratio, which was expected indicating that two different genes may be responsible for the expression of resistance. F_{2-3} population fitted into the ratio of 9:6:1 resistant/segregating/susceptible ratio. This ratio fits into dihybrid ratio for dominance at two loci.

The cross between KvX-295-2-124-99 and SARC-1-57-2 showed all to be resistant both in the F_2 and F_{2-3} populations indicating that it is the same gene causing resistance in both lines

Data from this study on the genetics of aphid resistance in these lines will help in accelerating the breeding program in future, including pyramiding of the different resistant genes in cowpea genotypes.

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DEDICATION

I dedicate this work to my ever loving parents Mr and Mrs Duah for their countless encouragement and support.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Cowpea (*Vigna unguiculata L.*) is the most economically important indigenous African legume crop (Langyntuo *et al*., 2003; Etana, 2013). It is one of the most important dietary staple in tropical Africa (FAO, 2012). About 66 % of the world's cowpea is produced in Africa, particularly in Nigeria and Niger (FAO, 2012). Outside Africa, the major production areas are Asia, Central America, and South America. Cowpeas thrive in relatively poor dry conditions, growing well in soils up to 85% sand (Craufurd *et al*., 1997). This makes them a particularly important crop in arid and semi-desert regions where not many other crops will grow well (Obatolu 2003). It is estimated that the annual cowpea grain production in the world is valued at approximately USD 1.53 billion (FAO, 2014). While it plays a key role in subsistence farming and livestock fodder, cowpea is also seen as a major cash crop by Central and West African farmers, with an estimated 200 million people consuming cowpea on a daily basis (Langyintuo *et al.*, 2003). Cowpea is referred to as the poor man's meat (Boukar *et al.,* 2010) because the grain is rich in protein up to around 30 % in some varieties and also contains micronutrients such as iron and zinc (AATF, 2012). All parts of the cowpea are used for food including the leaves, green pods and dry grains (Boukar *et al.*, 2010).

The production of cowpea is, however, greatly hampered by severe infestation and damage by insect pests and also factors such as poor soil fertility, drought, heat, soil acidity and stress due to intercropping with cereals (Singh and Tarawali, 1997; Singh and Ajeigbe, 2002; Ming and Moore 2008). Insect pest can be responsible for over

90% loss in yield (Jackai *et al.*, 1986). Every stage in the life cycle of the plant has at least one major insect pest that can cause serious damage and impact yield negatively (Fatokun, 2002). The major field pests of cowpea in Ghana 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). Aphids affect the plant during the seedling stage; flower thrips and Maruca are flowering pest, pod sucking bugs suck on the sap of the young pods whiles Bruchid weevils (*Callosobruchus maculatus*) cause serious damage after harvesting the seeds (AATF, 2012).

The cowpea aphid (*Aphis craccivora*) is a major pest of cowpea in Africa (Singh and Jackai, 1985). *Aphis craccivora* is polyphagous, but it prefers members of the bean family and is a serious threat to cowpea growers in Ghana (Kusi *et al.*, 2014; USDA 2015). Both nymphs and adults suck plant sap and cause serious damage right from the seedling to pod bearing stage. Although small populations of aphids have no major impact on cowpea production but in times of heavy infestation young seedlings succumb to death, whereas the older plants show symptoms such as stunting, crinkling, curling of leaves and delayed flowering, shriveling of pods and finally resulting in overall yield reduction (Singh and Jackai, 1985). The cowpea aphid also transmits numerous viral diseases (Dubey and Nene 1974).The most serious virus of cowpea transmitted by the aphid is the cowpea aphid-borne mosaic virus (CAMV) (Green and Kim, 1991). Aphids also cause damage through the secretion of honeydew which promotes growth of sooty moulds and other fungi on leaves, hence reducing photosynthetic efficiency of the plant (Annan *et al.*, 1996).

In Africa, *Aphis craccivora* populations tend to be spotty in numbers for cowpea sown early in the season, but if planting is delayed or made to coincide with drier periods, a heavy infestation generally occurs (Jackai and singh, 1988). Although there have been reports of a number of insecticides that work against the pest, these insecticides are often not accessible to small-scale farmers who produce most of these cowpea (Singh and Allen 1980; Sabo *et al.*, 2013). In the developing world, farmers have over-relied on chemical insecticides over the years to control aphids; this has resulted in the misuse and abuse of these chemicals. The use of insecticides increases production cost and environmental pollution. The continuous use of insecticides has led to resurgence of the aphids that are resistant to most insecticide (AATF 2012; Kusi 2014). According to Dent (1991), the use of resistant cowpea varieties appears to be the best option for small-scale farmers in the tropics because of its low cost for these farmers with low income .There have been many cowpea genotypes screened for resistance to various insects of economic importance. In most studies, resistance was ranked from moderate to high (Singh *et al.*, 1997; Kusi, 2014).

IITA has extensively studied the genetics of aphids. Three biotypes of *A. craccivora* have been identified, biotype A and biotype B occur in Nigeria and biotype K in Burkina Faso according to an IITA 1981 report. The expression of resistance was found to be controlled by two independent and non-allelic genes. However these studies have been limited to only few already identified resistance source (Pathak, 1988, Myers *et al*, 1996). The chemical basis for the resistance involves phenols and/ or flavonoids (Macfoy and Dabrowski, 1984) Aphid resistant lines that have been identified at IITA (Singh and Jackai, 1985) are been used in the breeding programmes to develop aphid resistant cultivars.

1.2 STATEMENT OF PROBLEM

Aphid resistant lines show differential response to aphid population from different geographical areas in Africa and California (Legume Innovation Lab Report 2015). Messina *et al.,* (1985) observed that some of the aphid resistant lines from IITA were susceptible to an aphid population in southern United States of America. Again IT 97K-499-35 line which is resistant to aphids in Nigeria was found to be susceptible to the aphids in Ghana (Kusi *et al*., 2010). Therefore, inherent aphid resistance in known cowpea germplasm alone may not suffice in combating the menace of *A. craccivora* in cowpea production. Knowledge on the genetics of aphid resistance in other sources of resistance would be beneficial in combating the pest. This study thus seeks to identify the genetic relatedness of cowpea lines found to be resistant to cowpea aphid in Ghana. The results of the study would help in accelerating breeding programs in future, including pyramiding of different resistant genes in cowpea genotypes.

1.3 OBJECTIVES

The primary aim of the study was to determine the genetic relatedness of different sources of cowpea aphid resistance.

The specific objectives were to determine;

1. The resistance of panel of aphid resistant cowpea lines to aphids in Ghana,
2. The mode of inheritance of the lines found to be resistant to cowpea aphid in Ghana,
3. Whether the same gene controls the resistance in those found to be resistant to aphids in Ghana and a known source of resistant line in Ghana.

CHAPTER TWO

LITERATURE REVIEW

2.1 ORIGIN AND DISTRIBUTION

Cowpea (*Vigna unguiculata (L.) Walp*) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times (Summerfield *et al*., 1974). A lack of archaeological evidence has resulted in contradicting and conflicting views supporting Africa, Asia and South America as origin. Some literature indicates that cowpea was introduced from Africa to the Indian subcontinent approximately 2000 to 3500 years ago (Alayande *et al*., 2012) at the same time as the introduction of sorghum and millet. Others state that before 300 BC, cowpeas had reached Europe and possibly North Africa from Asia (Summerfield *et al*., 1974; Tindall, 1983; Coetzee, 1995). The first written references to cowpea were in 300BC and the plant probably reached Central and North America during the slave trade through the 17th to early 19th centuries (Perrino *et al.*, 1994).

According to Ng and Maréchal (1985), Asia has been questioned as a center of origin due to the lack of wild ancestors. Most scientists believe cowpea most certainly evolved in Africa, as wild cowpeas only exist in Africa and Madagascar (Steele, 1976).According to Flight (1976) the oldest archaeological evidence of cowpea was found in Africa in the Kintampo rockshelter remains in Central Ghana dating about 1450–1000 BC adding to the point that it might have originated in Africa.

Even in Africa there is a debate about the origin of cowpea, some scientist believe it originated from West Africa, because of both wild and cultivated species that abound in that region (Ng and Marechal, 1985). Others hypothesized a southern Africa origin where the species moved northwards from the Transvaal to Mozambique and

Tanzania, where the subspecies *pubescence* evolved and owing to the presence of most primitive wild varieties in that region (Padulosi and Ng 1997).

However the name cowpea probably originated from the fact that the plant was an important source of hay for cows in the south-eastern United States and in other parts of the world (Timko *et al*., 2007).

2.2 CYTOLOGY

The cowpea plant according to Mukherjee (1968) is diploid with 2n = 2x = 22 chromosomes, one of which is short (19 µm), seven are medium length (26-36 µm) and three are long (41-45 µm).The genome size of cowpea is about 613 Mb (Arumuganathan and Earle, 1991).The chloroplast of the cowpea plant is maternally inherited (Corriveau and Coleman, 1988). Rachie and Roberts (1974) observed that some cowpea varieties and their closely wild relatives have 2n= 24 chromosome number.

2.3 TAXONOMY

Cowpea belongs to the order *Fabales*, family *Fabaceae*, subfamily *Faboideae*, tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna*, and section *Catiang* as indicated in Table 1 (Verdcourt 1970; Marechal *et al*. 1978). *Vigna* is a pantropical genus with several species, whose exact number varies according to authors: 184 (Phillips 1951), 170 (Faris 1965), between 170 and 150 (Summerfield and Roberts 1985), 150 (Verdcourt 1970), 154 (Steele, 1976), and about 84 (of which some 50 species are indigenous to Africa (Marechal *et al*., 1978).

All cultivated cowpeas are grouped under *V. unguiculata* subspecies *unguiculata*, which is subdivided into four cultigroups (Table 2), namely Unguiculata, Biflora, Sesquipedalis, and Textilis (Westphal 1974; Marechal *et al*., 1978; Ng and Marechal

1985). The classification of the wild relatives within *V. unguiculata* is more complicated, with over 20 different names having been used and between 3 and 10 subgroups described (Singh *et al.*, 1997).

Table 1 Classification of cowpea (*Vigna unguiculata (*L.) Walp)

TAXONOMIC PLACEMENT	SCIENTIFIC NAME
Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	*Fabales*
Family	*Fabaceae*
Sub-Family	*Faboideae*
Tribe	*Phaseoleae*
Sub-Tribe	*Phaseolinae*
Genus	*Vigna*
Section	*Catiang*
Species	*Unguiculata*
Botanical Varieties	*1.Vigna unguiculata var. unguiculata* *2.Vigna unguiculata unguiculata var. spontanea*

Verdcourt 1970; Marechal *et al.*, 1978

Table 2: The five cultivar groups of cultivated cowpea

Cultivar Group	Features
Unguiculata	Includes most African grain and forage types. More than 16 ovules/pod.
Melanophthalmus	Blackeye pea types. Less than 17 ovules/pod. Grown mostly in the Americas.
Biflora (Catiang)	Smooth seed in short erect pods. Common in India. Less than 17 ovules/pod.
Sesquipedalis	Asparagus or yard-long beans. Very long pods consumed fresh, especially in China.
Textilis	Rare form with very long peduncles once used for fibre in Africa.

Pasquet, 1999; 1998

2.4 MORPHOLOGY OF COWPEA

Cowpea is an annual herb with varying growth forms. It may be erect, trailing, climbing or bushy, usually indeterminate under favourable conditions. Canopy heights can be 30-60cm, depending on the variety (Department of Agriculture, Forestry and Fisheries, South Africa 2009). Structure of the mature plant varies depending on genotype, growth temperature, and the photoperiod in which the plant grows.

2.4.1 Leaves

The first pair of leaves is simple and opposite, sessile, and entire, while the rest are arranged in an alternate pattern and are trifoliate and petiolate. (Valenzuela, 2002). The trifoliate leaves are with oval leaflets, 6-15 cm long and 4-11 cm broad. The leaves are usually dark green in colour. (Feedipedia, 2015). The two lateral leaves are asymmetrical, and the terminal leaf is symmetrical (Figure 1). The plant also has extra floral nectaries, small pores on its leaves and stems of leaves that release nectar and attract beneficial insects (USDA, 2012).

Fig 1 Cowpea plant with trifoliate leaves

2.4.2 Inflorescence

Flowers are conspicuous, self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale blue or purple in colour (Department of Agriculture, Forestry and Fisheries, South Africa 2009). The inflorescence is also

axillary and formed on a peduncle 10 to 30 cm long, at the end of which, there is a rachis with each node bearing a pair of flowers and a cushion of extra floral nectaries that contribute to the attraction of insects (Fery, 1985). In cultivated forms, the flowers open at the end of the night and close in late morning, with the dehiscence of the anthers taking place several hours before the flower opens. Although considered autogamous, outcrossing rates as high as 5% have been observed, and therefore some precautions need to be taken to avoid outcrossing during the production of breeder and foundation seeds (Timko and Singh, 2008).

2.4.3 Fruit and Seeds

Pods vary in size, shape, colour and texture. They may be erect, crescent-shaped or coiled usually yellow when ripe, but may also be brown or purple in colour. Two or three pods per peduncle are common, and often four or more pods are carried on a single peduncle if growing conditions are very favourable (Figure 2). The presence of these long peduncles is a distinguishing feature of cowpea, and this characteristic also helps in hand harvesting (Timko and Singh, 2008). Seeds vary considerably in size, shape and colour. Usually the number of seeds per pod may vary from 8 to 20. The seeds are relatively large (2 to 12 mm long) and weigh 5 to 30 g/100 seeds. The testa may be smooth or wrinkled; white, green, buff, red, brown, black, speckled, blotched, eyed (hilum white, surrounded by a dark ring) or mottled in colour (Department of Agriculture, Forestry and Fisheries, South Africa 2009).

Fig 2 Cowpea pods on a peduncle

2.4.4 Stems

Cowpea grows rapidly, reaching a height of 19–24 inches (48–61 cm) when grown under favorable conditions. The upright stems are hollow and hairless, roughly 0.4 or 2/5 inch (1 cm) wide (Sustainable Agriculture Green Manure Crops Aug. 2002) The major plant growth habits are erect, semi-erect, prostrate (trailing), or climbing. Stems are striate, smooth or slightly hairy, sometimes tinged with purple (Aveling, T.1999).

2.4.5 Roots

It is an annual herb with a strong principal root and many spreading lateral roots in surface soil but in times of drought cowpea can grow a taproot (Figure 3) as long as 244cm to reach moisture deeper in the soil profile. (Sustainable Agriculture Green Manure Crops Aug. 2002).It has globular nodules which are smooth and spherical, about 5 mm in diameter. They are numerous on the main taproot and branches but are smaller on the smaller roots (Chaturvedi *et al.,* 2011).

Fig 3 Showing the roots of cowpea

2.5 ENVIRONMENTAL REQUIREMENTS

2.5.1 Temperature

The cowpea plant is a warm-season annual plant requiring temperatures of at least 18◦C throughout all stages of its development and having an optimal growing temperature of about 28◦C (Craufurd *et al.*, 1997).Varieties differ in their response to day length, some being insensitive and flowering within 30 days after sowing when grown at a temperature around 30°C. Even in early flowering varieties, the flowering period can be extended by warm and moist conditions, leading to asynchronous maturity (Department of Agriculture, Forestry and Fisheries, South Africa 2009). Higher temperatures can cause earlier flowering and flower abscission, resulting in poor pod set. High night temperatures (above ± 17 °C) can cause flower abscission in

some cultivars during flowering. According to Hall *et al.,* (2002) germination can occur quickly at temperatures above 19 ºC, but colder temperatures slow germination.

2.5.2 Rainfall

Cowpea tolerates drought more than many other crops. It can grow under rainfall ranging from 400 to 700 mm per annum. They also grow in rainfall environments up to about 2,000 mm per annum, but incidence of fungal disease increases (Cook *et al.*, 2005). Long taproot and mechanisms such as turning the leaves upwards to prevent overheating and closing the stomata are some mechanisms that confer tolerance to drought (Van Rij, 1999). This makes it the crop of choice for the Sahelian zone and the dry savannahs, though cultivars that flourish in the moist savannahs are available as well.

2.5.3 Soil requirements

Cowpea performs well on a wide variety of soils and soil conditions, but performs best on well-drained sandy loams or sandy soils. Sandy soils tend to be less restrictive for root growth (Hall 2002). It grows best in slightly acid to slightly alkaline soils (pH 5.5 – 8.3). It has little tolerance to salinity but is somewhat tolerant of soils high in aluminum but does not tolerate extended flooding or salinity. On heavy fertile soils, cowpea shows vigorous vegetative growth, but not necessarily a good grain yield. Cowpeas have been showed to be much less tolerant to cold soils than common beans (Cook *et al.*, 2005). Cowpea crop often responds favourably to added phosphorus, although there is no significant increase in cowpea grain yield up to Nitrogen application rate of 30 kg/ha (Agbenin *et al.*, 1990).

2.6 FERTILIZER APPLICATION

Cowpea forms symbiotic relation with a specific soil bacterium (*Rhizobium spp.)* and as such it fixes its own nitrogen, and may not need nitrogen fertilizers. In cowpea production, application of fertilizers normally depends on the soil fertility and expected yield (Davis *et al*., 1991). Sanchez *et al.*,(1997) suggested that methods for soil-fertility management range from recurring fertilizer applications to low external input of agriculture based organic sources of nutrients.

In tropical agriculture, fertilization can improve or increase production due to high weathered soils and limited reserves of nutrients (Stewart *et al.*, 2005). Although there have been an increase in fertilizer application throughout the world as a result of favourable policies (Bumb, 1989), sub-Saharan Africa has seen a reduction in the use of fertilizer application mainly due to the availability and the high cost of the fertilizers (Bumb and Baanante, 1996). About 1.38 million tons of fertilizer per year is applied in Africa resulting in an average fertilizer consumption of 8.3 kg ha^{-1}.This is low and represents only 2% of the worldwide demand and lowest in the world (Morris *et al.*, 2007). In Ghana, maize, sorghum/millet and rice receive a lot of attention in terms of application of fertilizers (Camara and Heinemann, 2006) with cowpea receiving very little or no attention from farmers when it comes to fertilizer application. Most farmers in Ghana prefer to apply fertilizers to cereals and rarely target grain legumes (Zingore *et al.*, 2008).These farmers believe that production of legumes do not require inorganic fertilizer application (Kan‟ankuk‟a, 1999)

Cowpea N requirements is improved by fertilization since it hardly satisfies its requirements (Chiezey *et al.,* 1990; Kan‟ankuk‟a, 1999; FAO, 2005). The cowpea plant will normally perform well under low N conditions. A starter N rate of 27kg ha^{-1} is required for soils with low N composition (Rupela and Saxena, 1987; Bluementhal

et al., 1992).In Ghana, a SARI report (2013) suggested that fertilizer application should be 20 kg N ha^{-1} on old land (continuously cropped land) where organic matter content may be as low as 1% and 40 kg P_2O_5 ha^{-1}.

2.7 WEEDS

Weeds are unwanted plants that normally compete for 1ight, nutrients and water and as a result causing a reduction in crop yields. Furthermore weeds also reduce the growth rate quantity and quality of grain yield as well as increase the cost of production (Akobundu, 1980; Ghanizadeh *et al.,* 2011). Weeds do not only reduce crop yields they also serve as host for insects, diseases and nematodes. Moody (1973) observed that when cowpeas were not weeded insect damage to the developing seed increased by 15.8 %.

An integrated weed management system has been developed which requires detailed information on weed: crop interactions, including the relative competitive ability of the crop during various phases off development on weed growth (Tollenaar *et al.,* 1994). Another part of the integrated weed management system is the use of competitive crops (Lemerle *et al.,* 1996).The innate ability of crops to suppress weed growth has become increasingly important and this comes from the fact the there is pressure to reduce the use of herbicide but then also to maintain cost effective weed control mechanism (Bilalis *et al.,*2009). Some factors that affect weed crop interaction are growth rates, shading ability (Lemerle *et al.,* 2001), tillering capacity, crop height (Korres and Froud-Williams, 2002), leaf area (Seavers and Wright, 1999), upright growth, long stem, high biomass (Ross *et al.,* 2001) and allelopathy (Khanh *et al.,* 2005; Seavers and Wright, 1999).Allelopathy crops can be helpful in reducing

noxious weeds when used as green manures or grown in rotational sequences (Ohno *et al.*, 2000; Ohno and Doolan, 2001).

2.7.1 Weed Control

The commonest forms of weed control in Ghana are hand and hoe weeding. This cultural methods for controlling the weeds is usually time consuming, energy sapping and costly. Hand weeding is mostly done within the rows of the crops where the hoe cannot be used. The hoe is the commonest and most widely used means to control weeds in the tropics. Though it is an effective means of weed control it is expensive, tedious and requires much labour. Hoe weeding is advantageous over the hand weeding because it is a much quicker operation and can be carried out at an earlier stage in the growth cycle of the crop.

Herbicides are considered to be an alternative to hoe weeding. This is because it gives rapid result, more convenient to the farmers, increase yield of crops and reduce labour costs (Melifonwu, 1992). Observations by Adigun and Lagoke (1994) suggested that herbicides is often applicable to large hectares of farm land where hand or hoe weeding may not be feasible due to labour and other logistic constraints. These chemicals are usually applied either before the crop and weed emergence or post emergence when the weeds start competing with the crop. Increasing labour cost and sometimes the unavailability of labour at critical times are making the use of herbicides more common in the tropics (Furtic, 1970; Akobundu, 1982).

Cowpea is more sensitive to herbicides than most other leguminous species. Studies done by Ugbe *et al*., (2016) found out that some herbicides were toxic to soyabean crops whiles the cowpea plant showed tolerance. According to Borget (1992) a mixture of trifluralin with Diphenamide or Linuron in weaker doses or 0.5kg/ha to

1.0kg/ha of trifluralin instead of the 3.0kg/ha recommended when the product is used on its own gives satisfactory results.

2.8 IMPORTANCE OF COWPEA

2.8.1 Nutritional Value

The nutritional value of cowpea is in the composition of its grain. The grain is rich in protein up to around 30 percent in some varieties (Table 3). Cowpea supplement cereals not only for protein, but also for minerals and vitamins; it also provide additional nicotinic acid and minerals. (Boukar *et al.*, 2010). Cowpea is rich in vitamin A and C and also has appreciable amount of thiamin, riboflavin, niacin, and pantothenic acid as well as small amount of foliate (IITA, 2009). Cowpeas are considered as poor man's meat because they have high protein content (18% – 35%) and carbohydrates contents (50% – 60%) together with amino acid. Comparing the nutritional value to that of cereal grains; however, makes cowpea a potentially important nutritional component in the human diet (Prinyawiwatkul *et al.*, 1996). Studies conducted profiled consumers in Northern Ghana and showed that women were greater consumers of cowpea than men (65% versus 35%). As home mangers, women regard cowpea as an important food to sustain the growth of children and to prevent iron deficiency; they perceive cowpea as a 'blood giving' plant (Abizaru *et al.*, 2013).

Table 3: Chemical composition of cowpea (%)

NUTRITIONAL COMPONENTS	SEEDS	HAY	LEAVES
Carbohydrates	56-66		8
Protein	30		4.7
Water	11	18	85
Crude fibre	5.9-7.3	9.6	2
Ash	3.4-3.9	23.3	
Fat	1.3-1.5	11.3	0.3
Phosphorous	0.146	2.6	0.063
Calcium	0.0104-0.076		0.256
Iron	0.005		0.005

Kay, 1979; Tindall, 1983; Quass, 1995

Table 4 Range of essential amino-acid content

Amino Acid	Percentage Total Protein	Average Percentage Total Protein
Lysine	5.7-9.6	6.6
Cystine	0.7-1.7	0.9
Methionine	0.7-1.6	0.9
Histidine	2.7-4.0	3.3
Threonine	3.4-5.3	4.1
Tryptophene	0.6-1.6	0.9

Rachie and Silvestre, 1977

Cowpeas are also consumed as boiled vegetables using fresh or rehydrated seeds or they are processed into flour to make other food products. Studies conducted shows leaves contain significant nutritional value (Nielson *et al*., 1993; Ahenkora *et al*., 1998). Like spinach, the young and tender leaves of cowpea can also be used to prepare pot herb (Mroso, 2003). Cowpea leaves are also cooked in stews and used as a weaning food or porridge. In Ghana, cowpea is generally prepared and eaten as a whole or as part of a meal. It is also used for preparing soup and stew (Appiah *et al*., 2011). Women in southern Africa particularly, value cowpea because its green pods and leaves are the earliest food available during the 'hunger months' prior to the main grain harvest and also because it plays an important role as a weaning food for infants (Quaye *et al*., 2009a). McWatters *et al*. (2003) prepared biscuits from cowpea composite flour, with very good sensory quality. The partial replacement of animal foods with cowpea also improves nutritional status (Guillion and Champ, 1966) due to lower cholesterol level in the plant food. In addition to human consumption, cowpea leaves and stems (stover) are also an important source of high-quality hay for livestock feed (Tarawali *et al*., 1997, 2002). Furthermore in the livestock industries, it serves as feed when mixed with cassava (Job *et al*., 1983). Cowpea fodder plays a particularly critical role in feeding animals during the dry season in many parts of West Africa (Singh and Tarawali 1997; Tarawali *et al*. 1997, 2002).

Medically, consumption of legumes has been related to many beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer (Simpson *et al*., 1981). Some other health benefits of cowpea include, toning the spleen, stomach and pancreas, helps induce urination and relieves damp conditions like leucorrhoea as researched and documented by Imrie (2004).

Despite its importance, cowpea's use as leafy vegetable in many African countries has been widely neglected in research and improvement programs (Barrett 1990; Schippers 2002), and it can, therefore, be considered as a neglected crop. Major limiting factors to the utilization of protein quality include poor digestibility, deficiency of sulphur amino acids and presence of anti-nutritional factors such as trypsin inhibitors, oligosaccharides and phenolic compounds. (Quintela 1997).

Soetan (2008) defined anti nutritional factors (Table 5) in cowpea as the plant's secondary metabolites which act to reduce food nutrient utilizations. These factors might affect susceptibility of grains to attacks by insect pests (Harborne, 1989). Anti-nutritional factors have been observed to show some pharmacological values, an example is tannin which has been observed to have anti-cancer and cytotoxic properties (Koratkar and Rao, 1997; Das and Mahato, 1983; Schopke and Hiller, 1990; Wakabayashi *et al.*, 1997).Also phytic acid is believed to prevent colon cancer and it does this by reducing the oxidative stress in the lumen of the intestinal tract (Vucenik and Shamsuddin, 2003; Jenab and Thompson, 2000). This chelating effect according to Klopfenstein *et al.*, (2002) may serve to prevent or even cure some cancers by depriving those cells of the minerals (especially iron) they need to reproduce. Although these factors have some important values, anti-nutritional have been observed to pose risk to human health for example phytic acid and Oxalic acid reduce mineral bioavailability that leads to various mineral deficiency diseases eg. anaemia (Gluthrie and Picciano, 1996). According to Ayedun and Sanni (2008) traditional processing techniques such as soaking, cooking, sprouting or roasting have limited effects on elimination of anti-nutritional factors, and sometimes could decreased protein quality and affect certain functional properties. Though there is a possibility of eliminating these anti nutritional factors by the use of genetic

modifications, but the other benefits derived from it means modifications could make food crops more nutritious without the capacity to improve other aspects of human health (Welch, 2004).

Table 5: Anti nutritional factors in grain of cowpea

Anti-Nutritional Factor	Cowpea
Phytic acid (mg/g)	14.0
Polyphenols (mg GA/g)	12.1
Oligosaccharides (mg/g)	31.7
Raffinose	10.3
Stachyose	17.8
Verbascose	3.6
Trypsin inhibitor activity (Units/g)	6981
Trypsin inhibitor activity	38.2

Sreerama *et al.,* 2012

2.8.2 Economic Value

Cowpea is the most economically important indigenous african legume. (Langyntuo *et al.,* 2003). It is estimated that the annual cowpea grain production in the world is valued at approximately USD 1.13-2.81 billion (AATF 2012). The global annual production of cowpea was about 3.6 million metric tons of which Africa accounts for about 64% (Mbene, 2000). Similarly, it was reported that Nigeria, being the largest producer of cowpea (Figure 4) in the world accounts for more than 2 million metric tons which represents about 50% of the total world cowpea production annually (Singh *et al.,* 2002). The average yield per hectare of cowpea in Nigeria is only 417

Kg per hectare, below an achievable yield of between 1500-3000Kg/ha and the grain yield per hectare of 2,666 Kg and 687 Kg obtained in Egypt and Malawi respectively in 2009 (Dzemo, 2010).

In Ghana, cowpea is the second most important food legume. It is second to groundnut in terms of area under cultivation and quantity produced and consumed annually (Egbadzor *et al.,* 2012). Cowpea is one of the widely cultivated legumes, mainly in the savanna and transitional zones (CRI, 2006). The yields of the crop in Ghana, however, are among the lowest in the world, averaging 310 kg/ha (Ofosu-Budu *et al*., 2007). Cowpea consumption is higher than its production in Ghana. In 2012 there was import of 3,380 MT of cowpea grains which supplemented the country's production of 219,300 MT in 2010 (Egbadzor *et al.,* 2012).

According FAO, as of 2012, the average cowpea yield in Western Africa was estimated to be 483 kg/ha (Figure 5), which is still 50% below the estimated potential production yield.

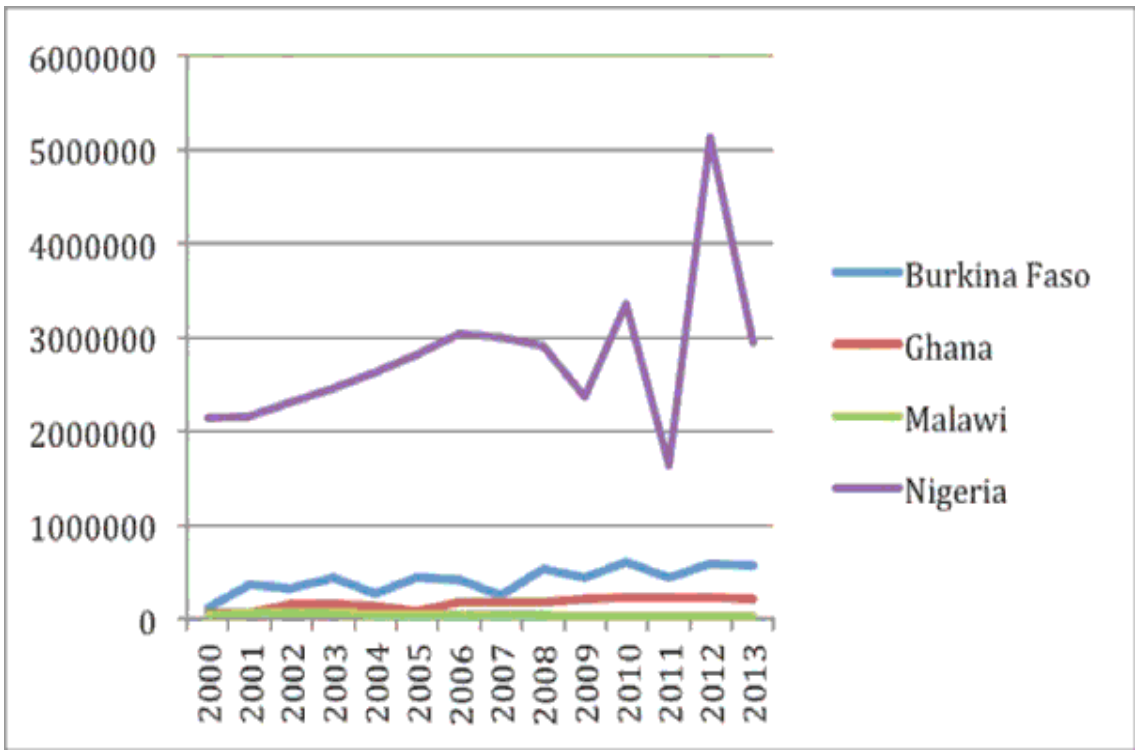

Fig 4: Production of Cowpea in Burkina Faso, Ghana, Malawi and Nigeria from 2000-2013. (Hectogram/hectare; 1 hectogram = 100 gm) (Sirinathsinghji 2015)

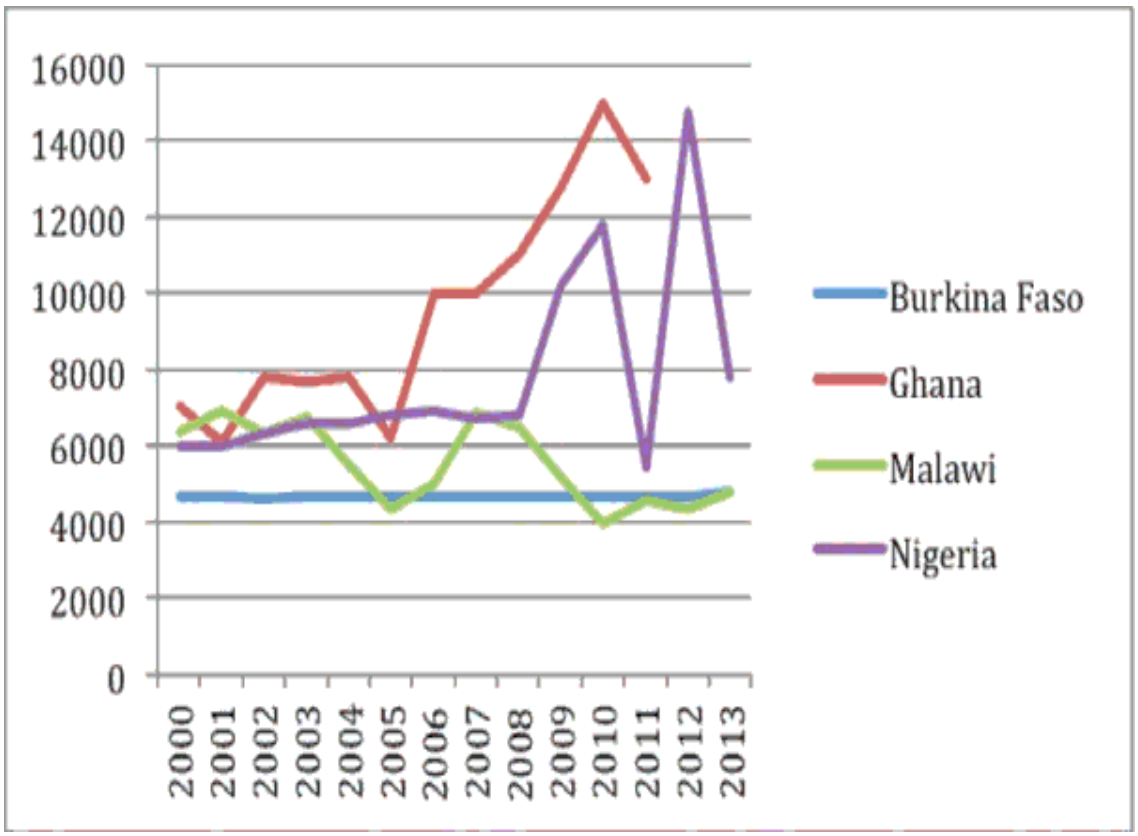

Fig 5: Cowpea yields (Hg/Ha) in four counties from 2000-2013 (Hectogram/hectare) (Sirinathsinghji, 2015)

2.8.3 Social Value

The cowpea value chain involves many people contributing to the development of the commodity in sub Saharan Africa (AATF 2012). Women play an important role in the cowpea value chain because cowpea offers a profitable and viable way to earn their livelihood, and is also an affordable source of protein (Modu *et al*., 2010; Osho and Dashiell, 1997). Improving agricultural production can increase rural incomes and purchasing power for large numbers of people, especially for women. Unfortunately, research and development programs rarely target rural women; as a result they are denied access to skills and new technologies (Satyavathi *et al*., 2010).

A lot of local farmers rely solely on the income for the up keep of their family. In most part of West Africa most rural farmers not only buy supplementary cereal grains, which are not grown in their locality but also buy fertilizer and other inputs for the coming season thereby safeguarding their food security (AATF, 2012).

2.9 COWPEA PRODUCTION CONSTRAINTS

2.9.0 Abiotic Factors

The major abiotic factors that hinder the production of cowpea include poor soil fertility, drought, heat, acidity and stress due to intercropping with cereals (Singh and Tarawali, 1997; Singh and Ajeigbe, 2002).

2.9.1 Drought

Drought is considered one of the most important constraints threatening the food security of the world (Barters and Nelson, 1994). In the Sudan and Sahelian semi-arid regions, the frequency and intensity of drought have increased over the last 30 years (Hall *et al*., 2003) due to climatic changes and human activities (Wittig *et al*. 2007).

Compared to other legumes; cowpea is known to have good adaptation to high temperatures and resistance to drought stress (Hall *et al.* 2002; Hall 2004). While cowpea is inherently more drought-tolerant than other crops, drought is still among the most significant abiotic constraints to its growth and yield (Timko and Singh 2008). Cowpea is grown mainly grown in the dry savanna and Sahel areas with no irrigation facilities, irregular rainfall patterns especially early in the season have adverse effects on the growth of the crop (Timko and Singh 2008). Drought affects various morphological and physiological traits associated with plant growth and development such as stomata closure, photosynthesis, respiration (Dulai *et al.,* 2006). It has been observed that early maturing varieties escape terminal drought (Singh 1987), but if exposed to intermittent moisture stress during the vegetative growth stage, they perform very poorly (Mai-Kodomi *et al.,* 1999a).

Drought tolerance is physiologically and genetically a complex trait whose expression depends on the magnitude and timing of stress in relation to plant-growth stage (Blum, 1996). Drought studies typically distinguish between early-, mid- and late-season drought stress, all of which present unique challenges to plant growth and productivity. Success in breeding for drought tolerance in cowpea has not been encouraging as with many other traits partly due to the lack of simple, cheap, and reliable screening methods to select drought tolerant plants and progenies from the segregating populations (Singh *et al*., 1997).

2.9.2 Low Soil Fertility

Soil fertility depletion has been described as one of the major biophysical root cause of declining per capita food production (Bationo *et al*. 2003a). According to Voortman and Brouwer (2003) even though much of West Africa has a semiarid

climate, several studies have concluded that low soil fertility is an even more important yield-limiting factor than rainfall .It is also one of the constraints to high productivity of cowpea, notably the low level of available phosphorus which is widely spread in the sahel region (Sanginga *et al*, 2000). The vast majority of farmers in the Sahel and savannah regions simply do not have access to fertilizers because of high cost and or lack of availability of these fertilizers (Trolove, *et al*., 2003), a situation that has not apparently changed for decades (Payne, 2006).

Research conducted has shown that cowpea is well adapted to the harsh growing conditions of the Sahel, including low soil fertility, high temperatures, and drought (Hiler *et al*., 1972 and Turk *et al*., 1980) provided there is sufficient soil P availability. Cowpea can fix nitrogen to improve soil fertility. The crop plays an important role in nitrogen fixation, by fixing considerable amounts of nitrogen (N) biologically ranging between 3-254 kg N ha-1 per year (Sanginga *et al.,* 2000). Despite many reports of strong crop response to P fertilizer, the addition of industrial forms of mineral-P fertilizer is often not seen as economically viable (Trolove *et al.,* 2003; Smalberger *et al*., 2006; Akhtar *et al*., 2007) because of high costs and low availability in rural areas.

2.9.3 Biotic Factors

2.9.4 Disease

Cowpea diseases caused by species of pathogens belonging to various pathogenic groups(Table 6) (fungi, bacteria, viruses, nematodes, and parasitic flowering plants) constitute one of the most important constraints to profitable cowpea production in all agro ecological zones where the crop is cultivated (Hampton *et al*. 1997).

Cowpea is infected by about 140 viruses worldwide (Hughes and Shoyinka, 2003), of which only nine had been reported to occur in Africa (Taiwo, 2003). According to

Kuhn (1990), numerous viruses are infectious to cowpea and are considered potential natural threat to cowpea production. In addition Emechebe and Florini (1997) suggested that the severity and yield loss vary from place to place, but some viral diseases occur and cause significant damage across the cowpea growing regions. However, Hampton *et al.,* (1997) reported that: blackeye cowpea mosaic potyvirus (BlCMV), cowpea aphid-borne mosaic potyvirus (CABMV), cucumber mosaic virus (CMV), cowpea mosaic comovirus (CPMV), cowpea severe mosaic comovirus (CPSMV), southern bean mosaic sobemovirus (SBMV), and cowpea mottle carmovirus (CPMoV) are viruses considered to be most dangerous to cowpea, cowpea golden mosaic geminivirus (CGMV) and cowpea chlorotic mottle bromovirus (CCMV) are two non-seed borne viruses that were also considered important by Hampton *et al.,* (1997)

Bacterial blight and bacterial pustule are some of the serious bacterial infections of the cowpea causing severe damage to cowpeas (Table 6) (Department of Agriculture, Forestry and Fisheries, South Africa, 2009).The disease incidence of cowpea bacterial blight is related to the seed borne nature of the pathogen (COPR, 1981). According to Okechukwu and Ekpo (2008) the bacterium can live up to about 6 months in the soil and even longer in debris. Some of its symptoms on leaves begin with small water-soaked spots that gradually coalesce into large, irregular, brown, necrotic lesions surrounded by yellow haloes (Cole *et al.,* 2011).

Some fungal species produces mycotoxins which are hazardous (Miller 1995). Some mycotoxins are stable chemical compounds that cannot be destroyed through food processing. Cercospora leaf spot, rust, brown blotch, *Septoria* leaf spot and fusarium wilt are all important common fungal disease that affect cowpea production (Abadassi *et al*, 1987).

Table 6: Diseases of Cowpea

PATHOGENIC GROUPS	EXAMPLES OF DISEASES
Virus	Cowpea aphid-borne mosaic virus (CABMV) Blackeye cowpea mosaic virus (BlCMV) Cowpea mosaic virus (CPMV) Cowpea severe mosaic virus (CSMV) Cowpea chlorotic mottle virus (CCMV)
Fungal and bacterial diseases	Septoria leaf spots Septoria. vignicola Scab (Elsinoë phaseoli) Cercospora leaf spot (Cercospora canescens) Fusarium wilt *(Fusarium sp)* Anthracnose *(Colletotrichum destructivum)* Powdery mildew *(Erysiphe polygoni)* Bacterial blight *(Xanthomonas campestris)* Bacterial pustule *(Xanthomonas axonopodis)*

OCED, 2015

2.9.5 Insects

Cowpea has been one of the major food crops in West Africa that has been attacked and damaged by insect's pests in all its stages of growth (Egho, 2011). These insects have remained the most important challenge to cowpea production, because each stage of the growth of cowpea attracts a number of these pests (Table 7). Based on the phase at which these insects attack the plant they can be grouped into three major groups: pre-flowering, flowering/post-flowering and storage (Dugje *et al.*, 2009).

Table 7: The cowpea growth stages and its major pests

GROWTH STAGES	INSECTS/MITES
0 stage,seeds	Ants,seedcom maggot
Seedling stage	Seedcom maggot,cutworm,Aphids, Leafhopper
Vegetative stage	Aphids,Leaf miner,Thrips
Reproductive Stage	Aphids,Bean fly,Bean pod borer,Leaf miner,Thrips
Maturation	Bean pod borer,Lygus bug,stink bug

Bissdorf, 2014

Pre-flowering pest

The cowpea aphid is one of the pre-flowering pest of cowpea that has adverse effect on the growth and production of the plant. It affects the crop by directly sucking its sap. Their feeding on cowpea causes cupping of the leaves, crinkling, defoliation and stunted growth (Pesticide Action Network, 2014). Another serious effect of the aphid is the ability to transmit the aphid borne mosaic virus. Affected plants show a green vein banding of the leaves (Dugje 2009).

Leafhopper is a common name applied to any species from the family *Cicadellidae*.These insects also attacks cowpea during the pre-flowering stage. They suck plant sap causing, the leaves discoloration of veins and margins and also cupping. Research has shown that severe attack can cause stunting and pre mature drying of the whole plant (Singh, 1998).

Cutworms are also another pre flowering pest that greatly affects cowpea production. These insects damage seedlings by cutting them off the ground level. Cutworms feed only at night time and are usually not found on the soil surface or plants during the day time. The young caterpillars eat feed on the leaves whiles the adult ones are capable of eating the entire plant (Bissdorf, 2014).

Fooliage beetle is another insect widely distributed in Africa. Adult beetle feed on the leaves of the seedling causing defoliation (Figure 6). The larvae feed on the roots of the cowpea. It also transmits a viral disease to the plant (Singh, 1998).

Fig 6 Cowpea plant with damage from foliage beetle attack (infonet-biovision.org)

Flowering/ post flowering pests

Blister beetles affect cowpea plants during the flowering period. They feed on the flowers causing considerable damage to the plant. A heavy infestation of these insects can cause a complete or total crop loss (Singh, 1998). Research has shown that

cowpea crops planted near or intercropped with maize often have the most serious effect in terms of damage (Dugje 2009).

Flower thrips also affect cowpea in the flowering stage. They feed on the flower and buds of the cowpea. When populations of these thrips are high the flowers are distorted and discoloured causing the flowers to fall off prematurely therefore preventing it from podding (Dugje 2009).

Bean fly's larvae feeds on the stem, leaves and taproot of the cowpea plant. The feeding of these parts causes wilting and eventually the death of the plant. The feeding of the leaves by the larvae causes a light yellow spots and larval mines with silvery curved stripes, these at a later stage becomes more visible as the holes and the larval mines turn dark brown (Bissdorf, 2014).The mature fly has a metallic blackish colour and it is about a quarter the size of a housefly.

Maruca pod borer lays its eggs on leaf buds, flower buds and in flowers. Its larvae feed on the tender parts of the plant like the stem, peduncles, flower buds, flowers and pods (Figure 7). This causes webbing of the flowers, pods, and leaves (Singh, 1998).It can also lead to the flowers becoming a mass of brownish-frass a day after infestation.

Fig 7 Pods damage caused by Maruca (Gianessi, 2013)

Lygus bugs are also another type of insects that attacks cowpea during the flowering stage. It feeds on the tender stems, flower buds, flowers and apical leaves of the plant. They cause the death of leaf aborted flowers and also distorted seeds (Bissdorf 2014).

Storage Pest

The major storage pest of the cowpea is the weevil (bruchid). It is a field-to storage pest. These weevils lay eggs either on the pods (Figure 8) or the grains and when the larvae hatches they enter the seeds and complete their life cycle. A characteristic of weevil infestation is the holes they make in the seeds (Singh *et al*., 1997).

Fig 8 Infestation of weevils in cowpea (Bissdorf 2014)

2.9.6 Parasitic weeds

The most prominent parasitic weeds that attack cowpeas, particularly in the semiarid regions are *Striga gesnerioides* and *Alectra spp* . According to Bagnall-Oakeley *et al.*, *(*1991) *Alectra vogelii* (Benth) is a hemiparasite that parasites a wide range of legumes in the West, East and South Africa. However work done by Aggarwal (1985) and Emechebe *et al.,* (1991) suggested that the species is more destructive in the Northern Guinea and Sudan agro ecologies. This they attributed to marginal nutrient status of the soils and unreliable rainfall patterns. Although damage caused by *Alectra* is less severe than that of *Striga*, total yield loss is possible in fields heavily infested by these parasites when susceptible varieties are planted (Emechebe *et al.,* 1983)

Striga hermonthica (Del.) Benth and *Striga asiatica* (L.) Kuntze have been reported to be the notorious *Striga* species whiles *S. gesnerioides* attack legumes the rest of the species affect or attack graminaceous crops. Conditions like low soil fertility, nitrogen deficiency, well-drained soils, and water stress accentuate the severity of Striga damage to the hosts'. Striga infestation is considered as the greatest single biotic constraint to food production in Africa (Bebawi and Farah, 1981; Lagoke *et al.,* 1991; Ejeta *et al.,* 1992).Weedicides generally can be used to control these parasitic plants, but that is not recommended since most are expensive and not environmentally friendly

2.10 PEST MANAGEMENT

One of the greatest risks to cowpea production is the incidence of pest infestation (Egho, 2011). Research has shown that aphids flower thrips, the Maruca pod borer and pod-sucking bugs are the most dangerous insects to cowpea (Gianessi, 2013). Although according to Gianessi (2013) it has been observed that 3 or more of these insect pests may affect the same plant at any particular time.

According to Allen and Singh (1980) a number of pesticides have proven effective against these pests of cowpea. In addition to that Nabirye (2003) suggested that cowpea production without insecticide application cannot be successful. Generally, 2-3 sprays with insecticides are required for a good crop of cowpea as stated by IITAs "Guide to Cowpea Production in West Africa" (Dugje, *et al.,* 2009). Flupyradifurone, Flonicamid and Malathion are common insecticides used in modern times against cowpea pest. Since the 1970s a lot of research has been conducted on the pests of cowpea by the International Institute of Tropical Agriculture (IITA) and they have come out with some varieties which are resistant to these pests (Adati *et al.*

2007).There are also biological and cultural methods of controlling these pests on cowpea, but research on these methods of controlling these pests have proved that it is not enough to suppress the growth of these pests by using the biological control method (Adati *et al*., 2007). Another research showed that combination of cultural practices and spraying once each at budding, flowering and podding stages was more effective and profitable than spraying cowpea weekly throughout the growing season (Gianessi, 2013).

Table 8: Some recommended insecticides for control of insect pests in cowpea

PRODUCT NAME	BRAND OR COMMON NAME	CONDITION OF USE
Lamda-cyhalothrine	Karate 2.5 EC, Karato 2.5 EC	Contact and ingestion. Apply at early infestation and against early stages of insects'life cycle.
Perfekthion	Dimethoate	Systemic action. Apply at early infestation and during early stages of insect life cycle.
Cypermertin plus dimethoate	Best action, Cyperdiforce, Superplus, Sherpaplus,	Contact and systemic action. Apply at early infestation and during early stages of insect life cycle
Diafuran 3G	Carbofuran	Contact, systemic and ingestion. Apply on the soil to control foliar pests through systemic action in plants
Actellic 25 EC	Actellic	Dry the grains properly and maintain proper hygiene to ensure protection from storage pests for at least 3 months
Apron Star 42WS	Apron star	Pre-plant seed coat treatment. Apply as slurry or dust and plant after treatment

Dugje *et al.*, 2009

2.11 COWPEA APHIDS (*Aphis craccivora*)

2.11.1 Morphology and Biology

Aphis. craccivora is a medium sized, shiny black insect whose biology is dependent on climate and soil conditions. When conditions are conducive for growth a generation may take only 13 days with adults living from 6-15 days and producing more than 100 progenies (Singh and Allen, 1980). Their eggs are laid in the buds, stems and the barks of the plant. The nymphs mature within 7-10 days and then they reproduce (Figure 9). The mature aphids or adults are about 3-4 mm long; they are also soft bodied with two projections on the rear end and two long antennae (Bissdorf, 2014).

Fig 9 Aphids on a plastic plate

Most female aphids can give birth to live nymphs as well as lay eggs; however for most species the primary means of reproduction is through asexual reproduction that

is the females hatching their eggs inside their bodies and giving birth to live ones (Schreiner, 2000). In the initial stages of infestation the aphids are wingless but as they become overcrowded or there is unfavourable climatic conditions subsequent generation develop winged forms and fly to other plants (Bissdorf, 2014). In the tropical regions these aphids reproduce asexually and their colonies consist entirely of females. Aphids spread readily and appear on cowpea soon after they are planted or during the seedling stage. Most of these insects are found on the plant tips, under the leaves flowers and developing bean pods, meaning they are primarily found on the growing points of the host plant (Schreiner, 2000).

2.11.2 Damage and Mode of Transmission

Nymphs and adults both feed on the plant sap. They are normally found on the under surface of the leaves on the young stem tissue and on the pods of the mature plants where they feed on. Aphids also inject some amount of toxins into the plant which normally reduces the plant vigour (Figure 11) and yield (Bissdorf, 2014).When in large quantities, they can cause the plants to become stunted and this is because they feed on the phloem and particularly damage the young growing areas (Singh and Allen, 1980).Also heavy removal of the sap by these aphids can cause wilting of the plant. Aphid damage can cause cupping and crinkling of the plants (Figure 10).

Fig 10 leaves of cowpea showing cupping and crinkling as a result of the aphids transmitting CAMV (infonet-biovision.org)

Fig 11.Plants from Apagbaala and IT82E-18 showing signs of damage from infestation from aphids

2.12 APHID MANAGEMENT

2.12.1Cultural control

Schalau (2004) defined cultural control methods as a broad range of normal management practices that can be modified or manipulated to manage one or more

pest problems. Some general cultural control methods includes crop rotation, tillage, timing of planting and harvesting, use of cover crops, choice of plant cultivar, competition, fertilizer or irrigation practices, sanitation, and soil solarisation.

According to Schalau (2004) it was observed that high levels of nitrogen fertilizer favour aphid reproduction and has been suggested that more nitrogen fertilizer usage than necessary be avoided. Also it is suggested that by controlling weeds you can reduce the aphid population in the field can be reduced. This is because ants take refuge in these weeds and they protect the aphids to harvest the honeydew. Another cultural method of controlling aphid is by varying the planting date of the crops. This works by creating asynchrony between the crop phenology and that of the aphid (Ferro 1987; Tobih, 2011). Furthermore practices such as species diversification (Omoloye *et al.*, 2000), intercropping and crop varieties have also been used to control the infestation of aphids (Okonman and Emosairue, 2005).

2.12.2 Biological and Physical Control

Generally, not much research has been conducted on biological control of aphids but according to Ofuya (1993), *A. craccivora* is attacked by many natural enemies and including parasitoids, pathogens and predators. Ladybugs, syrphids and lacewings are some of the predators for aphid (Spencer 2012).The potential of using biological control as a means of checking the growth and spread of the aphids is much higher in the tropical regions than in the temperate regions (Gullan and Cranston, 1994).Parasitoids that affect or attack the aphids include *Aphidius,Trioxys* and *Psyllaephagus* and the main pathogen that affect them are the entomophagous fungi (Ofuya 1993).

According to Singh and Jackai (1985) adverse climatic conditions generally limit the growth of *A. craccivora*. For example heavy rains wash away the aphids from the

plant during the rainy season. Persistent rainfall promotes the growth of entomophagous fungi which attack these aphids (Hill 1983).

2.12.3 Chemical Control

According to Hill (1983) most aphids are susceptible to insecticides. Flupyradifurone, Flonicamid and Malathion are common insecticides used in modern times against cowpea aphids. Although research by Gianessi (2013) showed that aphids are amenable to cultural control since both high plant density and early planting reduced their infestations and as a result insecticide application at the vegetative stage may not be essential. Synthetic insecticides have been mainly used in combating aphids on cowpea (Shen *et al.,* 2000). Arnason *et al.,* (1989) reported of insecticides of plant origin that can be used to combat these aphids, these insecticides do not have the associated problems with synthetic chemicals (Ofuya and Okuku, 1994) Organophosphates, organochlorines. Carbamates and pyrethroids are the 4 main classes of insecticides used in combating aphids (Oyewale, 2013).

2.13 CHALLENGES FACED USING CHEMICAL CONTROL ON APHIDS

In most developing countries, insecticides have been excessively and unwisely used creating a lot of challenges in the agricultural sector (Omongo *et al.,* 1997).Omongo *et al.,* (1997) reported that some farmers spray their crop 8 to 10 times during the growing season to combat these aphids. This phenomenon has led to serious challenges such as environmental pollution, toxicity to mammals, and destruction of beneficial organisms such as predators, parasites and parasitoids of the aphids (Alabi *et al.,* 2003). According to a report by African Agricultural Technology Foundation (2012), the over use of these chemicals to combat these aphids have led to the development of resistance to most insects to the insecticides. Furthermore, there have

been challenges associated with the cost of these chemicals and the equipment used in combating these aphids. This arises because most of these farmers are poor and cannot afford these chemicals (Afun *et al.,* 1991). Awareness is being created on the harmful effect of chemicals in the control of aphids. Although the application should be minimized, it should not be completely ignored since that will lead to reduction in crop productivity (Stern, 1973). Alternative control measures are being sought to reduce the application of these chemicals on the plant.

According to Dent (1991) using resistant varieties is one of the best option in reducing the application of these insecticides on the aphids and by so doing reducing the challenges faced by the use of the insecticides. He further argued that these small scale farmers could hardly afford the use of these insecticides because of their low income and the use of the resistant variety would be the best option for them.

2.13.1 Aphid Resistant Cowpea Lines

IITA has conducted a lot of research on screening of aphid resistant lines and has identified a lot of these resistant lines (Souleymane *et al.,* 2013). A lot of these lines have been screened against aphid populations from various locations in Africa and Asia (Chari *et al*, 1976: Dhanorkar and Daware, 1980: Macfoy and Dabrowski, 1984: Ofuya 1993, Souleymane *et al.,* 2013).These lines have the ability to survive without the application of insecticide against aphids.

Table 9. List of cowpea accessions that are resistant to aphids and their references.

LINES/ ACCESSIONS	REFERENCES
IT835-728-5, IT845-2246	Agele *et al.*,(2006); Githiri *et al* (1996)
V51	Laamari *et al* (2008)
ICV-12, IT82D-812	Firmpong (1988); Annan *et al.* (1995)
TVu1037, TVu2876, TVu 3000	Ofuya (1988); Nkansah-Poku and Hodgson, (1995)
ICV 10, IT82E-25, IT87S-1394, IT67S-1459	Githiri *et al*. (1996)
TVu310, TVu408-P2, TVu801	Ansari *et al*. (1992); Ombakho *et al*. (1987)
IT90K-76, IT90K-277-2, IT90K-59	Singh (2005)
TVu 9930, TVu36	Ofuya (1993)
Vs350, Vs438, Vs452	Joseph and Peter (2007)
ICV11	Ombakho *et al*. (1987); Githiri *et al.* (1996)
SARC 1-57-2 SARC 1-91-1	Kusi *et al* (2010)

Nualsri *et al.*, 2012

2.14 MECHANISMS OF PLANT RESISTANCE

Phloem feeding insects such as aphids are a widespread and serious constraint on plant production. These aphids have verily adapted and are successful in exploiting a broad range of vascular plants including cowpea (Klingler *et al.*, 2005). According to Maxwell and Jennings (1980) plant resistance are those heritable characteristics

possessed by the plant which influence the ultimate degree of damage done by insects. Hill and Walter (1982) further argued that resistance to pest attack is observed by a lower pest population or less or mild damage symptoms on the resistant plants. Kumar (1984) also suggested that it is the ability of the crop plant to prevent, retard or overcome pest infestation. Resistance can be considered relative and in some cases measured by using susceptible cultivars of some species as controls. Johnson and Law (1975) suggested a new term durable, to mean long lasting resistance to pest. Russell (1978) also suggested that durability does not necessarily mean resistance is effective against all variants of a pest, but rather that the resistance has merely given effective control for many years in environmental conditions favourable to the pest.

Immunity in terms of plant resistance can be defined as a variety that cannot be infested or injured at all by specific insect species under any condition and anything below this condition could be considered as resistance rather than immunity (Kusi, 2008).

According to Painter (1951) there three types of mechanism for plant resistance and these are non-preference or antixenosis, antibiosis and tolerance, though according to Smith (2005) it becomes a little difficult to distinguish between antixenosis and antibiosis types of resistance in plants. Tolerance is measured by different responses among infected plants to specific levels of infestation whiles antixenosis and antibiosis are in terms of the response of pests to the host plant (Hesler and Tharp, 2005).

2.14.1 Antixenosis or Non Preference Resistance

Antixenosis type of resistance basically prevents pest colonization of the host plant. The mechanism used can be morphological or chemical. Some factors that influence antixenosis include colour, light reflection, type of pubescence, leaf angle, odour,

taste, tough epidermis and the presence of feeding repellents or the absence of feeding attractants (Boateng, 2015). According to Kogan and Omar (1978) the type of cuticle wax and hairiness on plant stalks and leaves are some morphological characteristics that can affect and change the behaviour of the pest. Fehr (1987) observed that the resistance to grasshoppers by maize and sorghum was related to taste. It was also found out that cowpea varieties with pigmented calyx, petioles, pods and pod tips suffered less damage from aphids (Singh *et al*., 2002).

2.14.2 Antibiosis

It can be defined as a type of resistance in which insects feeding on the plant results in the mortality or disruption of growth, development and physiology in the aphids (Rector *et al.,* 2000). Plants produce some defensive compounds called allochemicals which protect the plants from these insects. According to Painter (1951) these compounds reduce growth, inhibit reproduction, alter physiology, delay or prolong maturation, or induce various physical or behavioral abnormalities. These effects may range from mild to severe. Furthermore these chemicals can operate on a number of mechanisms including them being toxic, antifeedants, or c preventing the insect from recognizing the plant tissue as a suitable food source (Gatehouse, 1991). In the prevention of viruses spread through arthropod; antibiosis is a favored resistance modality (Power and Gray, 1995). According to Dahms (1972) antibiotic effects of resistant plant on differential rate of aphid development were shown as, nymphs maturing in 5 days (susceptible variety), 10 days (intermediate antibiosis) and 20 days (high antibiosis).Wiseman (1999) argued that the effects of antibiosis include reduced food consumption, increased development time, low food reserves, death in pre-pupal or pupal stages and reduced fecundity. Basandrai *et al.,* (2011) further suggested that

antibiosis affects the weight and size of insects, sex ratio and proportion of insects entering diapauses.

2.14.3 Tolerance

Tolerance can be defined as the ability of the plant to grow and reproduce or the degree at which it can support an infestation in which a susceptible host would have been damaged. (Cuartera, et al.,1999). The concept of tolerance really means endurance of the plant against pests. According to Politowski (1978) a tolerant plant may be infected by a pest to the same extent as susceptible one, but there will be no reduction in yield both in quantity and quality.

2.15 ARTIFICIAL HYBRIDIZATION

The cowpea plant is cleistogamous, producing viable pollens and receptive stigma before anthesis (Asiwe, 2009).This process makes it a self-pollinating plant. According to Landeinde and Bliss (1977) there is no mechanical dispersion of pollen from the flowers of the cowpeas because the anthers release pollen during the first half of the night when the flowers are still closed. However for genetic improvement, artificial cross pollination is very necessary and its success has been reported to range from 0.5 to 50%, but these percentages may vary depending on the genetic and physiological factors as well as the process taken in handling floral parts during cross pollination (Rachie et al., 1975). It was reported that weedy subspecies of cowpea can easily hybridize with the cultivated forms and produce hybrids (Baudoin and Maréchal, 1985; Ng *et al.,* 1990). The first cross between wild relatives and cultivars to obtain disease resistant was reported by Rawal *et al.,* (1975).

2.16 PLANT BREEDING

Breeding can be defined as the science and technique of changing and improving the heredity of plants (Sleper and Poehlman 1995). The basic aim of cowpea breeding programs is to develop a range of high yielding cowpea varieties adapted to different agro ecological zones that possess regionally preferred traits for plant type (Pasquet and Baudoin, 2001), growth habit, days to maturity, disease resistance and seed type (Table 10). Higher grain yield and improved grain quality are the primary breeding objectives for nearly all breeding programs. Backcross, pedigree, or bulk breeding methods are used to handle segregation populations by most cowpea breeders because cowpea is a self-pollinating and varieties are pure lines (Timko *et al.,* 2007b).

Cowpeas are widely grown in a lot of areas but research efforts devoted to the crop have been limited compared to the staple cereal crop (Ehlers et al., 2002a). Earlier efforts to improve this crop have been restricted to the identification and control of insects and diseases, selection in limited collections of germplasm and hybridization among a small number of parents. International Institute of Tropical Agriculture (IITA) based in Nigeria has gotten the global mandate for improving cowpea cultivars. It develops and distributes various ranges of improved cowpea lines among 65 countries (Ehlers *et al.,* 2002a; Singh *et al.,* 2002; Hall *et al.,* 2003; Singh 2005; Timko *et al.,* 2007a). IITA has the largest germplasm of cowpea in the world with more than 14,000 accessions (Timko and Singh,2008).

Table 10 Major Breeding Objectives for Cowpea

BREEDING OBJECTIVE	SELECTION/IMPROVEMENT CRITERIA
High seed yield	Without inputs under intercropping conditions from 100 to 400 kg ha^{-1} With inputs under sole cropping conditions from 900 to 3000 kg ha^{-1}
Diverse types	Extra-early maturing (60–70 days) photo-insensitive grain type, for use as sole crop in multiple cropping systems and short rainy seasons Medium-maturing (75–90 days) photo-insensitive grain type, for use as a sole crop and intercrop Late-maturing (85–120 days) photo-insensitive dual-purpose (grain + leaf) types, for use as a sole crop and intercrop Photosensitive early-maturing (70–80 days) grain types, for Intercropping Photosensitive and photo-insensitive medium-maturing (75–90 days) dual purpose (grain + fodder) types, for intercropping Photosensitive late-maturing (85–120 days) fodder type, for Intercropping High-yielding, bush-type vegetable varieties
Resistance to biotic stresses	Insects: Aphid (*Aphis cracivorra*), Thrips ( ), leaf hoppers (*Empoasca sp*.), podborer (Maruca vitrata), *Clavigralla spp*., *Anoploenemis spp*., *Riptortus sp*., *Nezara viridula* Parasitic plants: *Striga gesnerioides* and *Alectra vogelii* Diseases: *Colletotrichum sp*., *Xanthomonas sp*., viral mosaics and mottling
Tolerance to abiotic stresses	Drought, high temperatures, low phosphorus, high BNF, and soil acidity; root architecture
Quality and acceptability of the seed	Size, color and texture of seed coat Protein content Mineral levels (Fe,Zn,Ca,K) Low cooking time

Pasquet and Baudoin (2001)

2.16.1 Breeding for Resistance to Biotic Stress

Effective screening methods have been developed for many bacterial, fungal and viral disease to allow researchers to identify cultivars with potential sources of resistance (Ehlers and Hall 1997). Improvements made through conventional breeding techniques has moved resistance of various bacterial, fungal, viral diseases, parasitic weeds (*S. gesnerioides* and *A. vogelii*), and root-knot nematodes into farmer-acceptable germplasm (Table 11). Timko and Singh (2008) suggested that resistance to these pathogens and parasites is usually governed by single genes that are often effective only in a restricted region due to pathogen/parasite variability and may be overcome in a relatively short period of time. Marker assisted selection can be used in assembling durable resistance by incorporating an array of resistance genes from other regions (Timko and Singh, 2008).

Insect pests are a major challenge in cowpea production therefore; developing cultivars with sustainable resistance to insects is a key objective of many breeding programs worldwide (Singh and van Emden, 1979). In the developed world the problem of insect infestation can easily be controlled by the use of insecticides (Singh and van Emden 1979; Daoust *et al.,* 1985) but in the developing world access to the insecticides themselves or the financial resources required to purchase the insecticides and the equipment required for proper application are not available (Timko and Singh, 2008). Using a combination of field and laboratory screening, a number of cowpea breeding lines have been developed with combined resistance to cowpea bacterial, fungal, viral disease and pest infestation (Van Boxtel *et al.,* 2000; Singh *et al.,* 2002; Lale and Kolo 2007).

Table 11 Improved Cowpea Varieties Released for Use in Africa, Asia and the Americas

Region	Variety/Breeding Line/Cultivar
Asia and Oceania	IT81D-897, IT82D-752, IT82D-789, IT82D-889, IT82E-18, IT93K-452-1,IT97K-1042-3, IT98K-1111-1, VITA-4, Victory, Breeze, Light,, Sky, Big Buff
East and Southern Africa	IT82E-16, IT82E-18, IT82D-889, IT85F-2020, IT86D-1010, IT87D-611-3,IT89KD-245,IT90K-59, IT90K-76, IT93K-2046-2,IT97K-568-18, IT97K-499, Hope, Pride, Gold from the Sand
West and Central Africa	TVx 3236, IT81D-985, IT81D-994, IT83S-818, IT83S-728-13, IT84S-2246-4, IT86D-719, IT86D-721, IT87D-453-2, IT89KD-245-1, IT89KD-288, IT88D-867-11, IT89KD-374-57, IT90K-76, IT90K-82-2, IT90K-277-2, IT90K-372-1-2,IT93K-452-1, IT97K-499-35, Melakh, Ein El Gazal, Mouride, Son of IITA, Korobalen, Ayiyti, Asontem, Bengpla, CRSP Niebe,Lori Niebe
North, Central, and South America	VITA-1, VITA-3, VITA-6, VITA-7, IT82E-18, IT82D-716, IT82D-789, IT82D-889, IT83D-442, IT83S-841, IT84D-449, IT84D-666, IT84S-2246-4, IT86D-314, IT86D-368, IT86D-782, IT86D-792,IT86D-1010,IT87D-697-2,IT87D-885, IT88S-574-3,TVx1836-01J,IT87D-1627,IT89KD-288, IT90K-284-2,IT91K-118-2,Titan,Cubinata,California Blackeye No.27, Bettergreen, Charleston Greenpack

Timko and Singh 2008

2.16.2 Genotypes developed for Tolerance to Abiotic Stress

Simple screening methods for heat, drought tolerance and root architecture have been identified and incorporated into improved lines (Matsui and Singh 2003). Maturity of the cowpea plant ranges from 60 to more than 90 days depending on the day length and temperature (Timko and Singh 2008). Varieties developed must be adapted to the length of the season and also to the coincidence of pod development with the end of the rainy season, thus ensuring good seed quality. This implies that where rainfall is restricted and uncertain, short duration types of cowpeas tolerant to drought are required. The best drought-tolerant varieties are IT89KD-374-57, IT88DM-867-11, IT98D-1399, IT98K-131-1, IT97K-568-19, IT98K-452-1, and IT98K-241-2, and the best heat-tolerant lines are IT93K-452-1, IT98K-1111-1, IT93K-693-2, IT97K-472-12, IT97K-472-25, IT97K-819-43 and IT97K-499-38 (Timko and Singh 2008).

2.16.3 Some Genotypes Developed for Improved Nutritional Quality

Most leguminous crops have higher Calcium content than most cereals and are also a good source of minerals (Osborn, 1977). Cowpea is usually used as a supplement for cereal flour to improve the Ca content. Cowpea also has high amount of K and Na which are essential for the development of strong bones and teeth (Khalid and Elharadallou, 2014). They have high nutritional importance due to their good quality protein content and significant amounts of iron and zinc which has made it receive attention as a crop for biofortification to improve its native iron as well as zinc concentration (Langyintuo *et al.,* 2004). Cowpea leaves form an important part of the diet in more than 18 countries in Africa including the northern part of Ghana and seven countries in Asia and the Pacific, there is therefore the need to breed or improve dual purpose cowpea varieties for higher leaf yields (Nielsen *et al* .,1997).

Under the Harvest Plus initiative funded by the Bill & Melinda Gates Foundation, a breeding program to develop cowpea with high levels of protein was started in 1993.The project has been to a great success with approximately 2,000 genotypes which have been evaluated revealing significant genetic variability in protein and micronutrient contents (Omueti and Singh 1987; Baker *et al.,* 1989; Nielsen *et al.,* 1993; Timko and Singh 2008). In developed countries soybean is been substituted for cowpea as consumers look to more traditional food sources that are low in fat and high in fiber and that have other health benefits. According to Nielson *et al.,* (1993) the fat content of cowpea ranges from 1.4 to 2.7%, while fiber content is about 6% (Bressani 1985).

Rangel *et al.,* (2004) observed that protein isolates from cowpea grains have good functional properties, including solubility and emulsifying and foaming activities and could be a substitute for soy protein isolates for persons with soy protein allergies. Cowpea-fortified baked goods, extruded snack foods, and weaning foods are the processed food products produced using dry cowpea grains (Phillips *et al.,* 2003).

2.16.4 Varieties with Regional Preference in Seed Type

Diverse regional preferences make the breeding objectives very challenging. Consumer preference is essential in cowpea production. Breeding against constraints without consumer acceptability considerations may result in the rejection of the improved varieties (Egbadzor 2013). In West and Central Africa white- and brown-seeded varieties with rough seed coats are preferred because of the ease of removing the seed coats for local food preparation whiles red or brown seeded varieties with smooth seed coats are preferred in East and Southern Africa and parts of Central and

South America this is because cowpea is used as boiled beans for which removal of the seed coat is not desirable (Timko and Singh 2008).

Synthesis of anthocyanins and other flavonoids is the cause of pigmentation in seeds and they are found in almost every plant (Holton and Cornish, 1995). It affects plants in response to abiotic factors such as drought (Chalker-Scott, 1999) as well as biotic (Makoi *et al.,* 2010; Sharma *et al.,* 2011) factors.

Varieties of persistent green grain has been developed by researchers in the USA that are a versatile product for frozen vegetable applications (Ehlers *et al.,* 2002a).They are green in colour when dry but when soaked resemble fresh shelled cowpea and they are used in frozen vegetable products to add color and variety.

2.16.5 Breeding for Cowpea Aphid Resistance

There have been various cowpea breeding programs across different geographical location across the globe with the International Institute of Tropical Agriculture (IITA) in Nigeria having the mandate for cowpea improvement by releasing varieties to meet regional preferences, specific seed types and adaptability to different environments (Singh 2012).

Since the early 1980s a lot of cowpea varieties mainly produced by IITA have been developed which are resistant to aphids. A lot of these lines have been screened against aphid populations from various locations in Africa and Asia (Chari *et al.,* 1976: Dhanorkar and Daware, 1980: Macfoy and Dabrowski, 1984: Ofuya 1993, Souleymane *et al*., 2013). According to Ansari (1984) most of these resistance is due to antibiosis.

Genetic studies conducted on these lines of resistance have all confirmed that the gene controlling the resistance is a single dominant gene (Singh and Ntare, 1985). Single gene inheritance suggests that the trait can be easily incorporated into a desired

adapter cultivar through backcrossing. Although it can be easily incorporated, Githiri (1995) argued that this pose a challenge since the aphids can develop fast biotypes which can overcome the resistant lines.

Some of the aphid resistant lines have shown differential effect on different aphid across various geographical locations. Messina (1985) observed that some of the aphid resistant lines from IITA were susceptible to an aphid population in southern United States of America. Emden (1991) also observed some of the resistant lines from IITA were susceptible to some of the aphids found in West Africa. These observations show that there are different types of biotypes in the species.

2.17 GENE PYRAMIDING

Josh and Nayak (2008) defined gene pyramiding as combining two or more genes which results in the expression of more than one gene in a variety to develop durable resistance. Gene pyramiding is an effective strategy to help overcome the problem with single gene resistance. Pyramiding genes can be a very difficult method due to the dominance and epistasis effects of governing disease resistance. With improvement in genetic engineering and biotechnology; plant breeding has reached a new dimension, molecular markers which are linked to the resistance genes makes identification of plants with more than two genes possible. These markers are of great importance to agronomic traits such as resistance to pathogens, insects and nematodes, tolerance to abiotic stresses and quality parameters which becomes a challenge to tag (Josh and Nayak, 2008).There has been a lot of important crops whose resistant genes to specific pests and diseases have undergone gene pyramiding (Table 12)

Table 12 Examples of gene pyramiding in some crops

CROP	TRAIT	PYRAMIDED GENES	REFERENCES
Rice	Blight resistance Blast resistance Gallmidge resistance	Xa4,xa5,xa13,Xa21 Pi(2)t,Piz5,Pi(t)a Gm1,Gm4	Huang *et al.*, 1997, Singh *et al.*, 2001, Narayanan *et al.*, 2002 Hittalmani *et al.*, 2000 Kumaravadivel *et al.*, 2006
Wheat	Leaf rust resistance Powderymildew resistance	Lr41, Lr42, Lr43 Pm-1, Pm-2	Cox *et al.*, 1994 Liu *et al.*, 2000
Cotton	Insect pest resistance	Cry 1Ac, Cry 2Ac	Jackson *et al.*, 2003, Gahan *et al.*, 2005
pea	Nodulation ability	Sym9, Sym10	Schneider *et al.*, 2002
Barley	Yellow mosaic virus resistance	rym4, rym5, rym9, rym11	Werner *et al.*, 2005
Soybean	Soybean mosaic virus resistance	Rsv1, Rsv3, Rsv4	Zhu *et al.*, 2006

Joshi and Nayak, 2008

2.18 TRANSGENIC COWPEA

There has been a lot of reliable genetic transformation and in vitro plant regeneration system for cowpea (Anand *et al.,* 2001; Van Le et al., 2002; Machuka *et al.*, 2002; Ikea et al. 2003; Avenido *et al.*, 2004). Genetic transformation of cowpea using the particle-gun bombardment of shoot meristems was done by Ikea *et al.*, (2003). Cowpea was first transformed by, obtaining kanamycin-resistant callus, however the research team were unable to achieve plant regeneration (Garcia *et al.,* 1986). Studies conducted by Muthukumar *et al.,* (1995) obtained four cowpea plants after co-cultivation of mature de-embryonated cotyledons and selection on hygromycin-containing media. The challenge from their studies was that the DNA gel blot analysis could demonstrate integration of the *hpt* marker gene in only one of the presumptive transgenic plants, and transference of the marker could not be shown in subsequent generation. Popelka *et al.,* (2006) developed an efficient and stable cowpea transformation or regeneration system

The traditional form of plant breeding has made only limited progress in breeding for resistance to the major insect pests of cowpea. Transgenic methods should be encouraged to develop varieties of cowpeas with strong resistance to insect pests. This will increase productivity in many growing areas of cowpea as well as reduce cost, safety hazards, and environmental risks.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Location of Experiment

The experiment consisted primarily of a screen house which was conducted at the Manga Research Station of the Savanna Agricultural Research Institute (SARI) in the Savanna zone of Ghana. This is located in the Upper East region of Ghana. The station is located between Latitude 11°-01° N and Longitude 00° -16° W with and elevation of 249 m above sea level (Sarpong, 2001).

The Sudan savanna ecological zone has a single rainy season from May to October and an average rainfall of 800 mm-1000 mm which is relatively less than most parts of the country (Sarpong, 2001)

Soil at manga ranges from sandy to sandy-loam. The soil is characterized by low fertility, low organic matter content, low pH and a slightly acidic upper layer that is easily prone to erosion (Mukhtaru, 2016).

All the crosses of the various lines used in the study were done in the screen house at manga (Figure 12). It is also the location where F_2 and F_3 plants were generated. The screen house had an average temperature of 30°C and a relative humidity of 74.1 %.

The insectary contained the aphid culture and it is the place where the screenings of the various lines were conducted (Figure 13). The insectary had temperature between 25°C and 33°C and a relative humidity of between 74.6% and 80 %.

Fig 12 Screen house at Manga Research Station

Fig 13 Insectary at Manga Research Station

3.2 Experimental Materials

Eleven cowpea genotypes were used as parents in the study. These genotypes were made up of two local genotypes and nine introductions. Table 13 shows the list of genotypes, their level of resistance and their origin. These lines were from the source of aphid resistance and susceptibility panel.

Table 13. The eleven sources of aphid resistance from the panel of aphid resistance and their susceptible checks

Name	Type	Origin
58-77	Aphid resistant source	ISRA
INIA19	Aphid resistant source	MOSU
IT97K-556-6	Aphid resistant source	IITA
KN1	Aphid resistant source	INERA
KvX-295-2-124-99	Aphid resistant source	INERA
SARC-1-57-2*	Aphid resistant source	SARI
APAGBAALA*	Aphid susceptible check	SARI
BAMBEY 21	Aphid susceptible check	ISRA
CB27	Aphid susceptible check	UCR
IT82E-18	Aphid susceptible check	IITA
VITA7	Aphid susceptible check	IITA

Panel of Aphid Resistance and Susceptibility; *- local genotypes

3.3 Testing For the Resistance of the Cowpea Lines

3.3.1 Aphid Culture

Aphid populations were taken alive from plants infested with aphids from the field and transported to the insectary. Four days old seedlings of the susceptible genotype, Apagbaala were infested with 15 aphids per plant from the field. The seedlings were watered to avoid moisture stress while the aphid populations built up. Care was taken not to wash away the aphid population on the plants whiles watering. Parasitoids and other natural predators of the aphids such as the mealybugs,whiteflies and ladybugs were checked regularly in the insectary in the mornings and if found were destroyed to prevent the destruction of the culture.

Susceptible genotypes destroyed by aphids were transferred to a healthy susceptible genotype to allow for the continuity of the culture.

3.3.2 Screening of panel of aphid resistant and susceptibility cowpea lines

The eleven lines from the panel of aphid resistance and susceptibility were planted in pots filled with top soil. Eight seeds of each line were planted per pot. The experimental setup was a completely randomized design with four replications. Each genotype was represented by one pot in each replication. The pots were watered regularly in the evenings to ensure the proper growth of the cowpea (Figure 14).

Fig. 14 Young seedlings of the eleven lines planted to screen for aphid resistance

3.3.3 Infestation of Cowpea Lines

Infestation of cowpea lines was done 3-4 days after the plants emerged. Live aphids were obtained from the aphid culture in the screen house. They were picked with a camel hair soft brush into white plastic petri dish. The aphids were then infested on the young seedling. Five, four-day old aphids were infested per seedling. The infestation was done in the morning. Counting was done in the evening to check the number of aphids per plant and this was done to ensure that uniform population of 5 per plant was maintained within 48 hours after infestation.

3.3.4 Observation of the cowpea lines

Seedlings were scored for visual aphid damage or symptoms from eleven to fifteen days after inoculation (Kusi *et al*, 2008). The scores were ranged from 1 to 5. The interpretations of the scores are presented in Table 14. The aphid population was allowed to build up until the susceptible check, Apagbaala died. Seedlings were then

grouped as either susceptible or resistant based on observations made in relation to the susceptible and resistant checks. The average temperature at the screen house was 29°C with a relative humidity of 74.1 %.

Table 14 Scale for scoring for aphid resistance in cowpea

SCORE	SYMPTOMS DESCRIPTION
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
5	Seedling with no aphids

Aphids Score (Kusi 2008)

3.4 Development of Breeding Populations

The experiment was conducted in two stages. The first and second stages involved the generation of the F_1 seeds and F_2 seeds respectively. The two stages were carried out in the screen house from August 2015 to January 2016.

3.4.1 Crossing procedure

All crosses were done in the screen house. The late maturing lines KVX-29 5-2-124-99 and IT97K556 were planted a week earlier before the early maturing ones SARC-1-57-2 and APAGBAALA. Opened flowers were picked in the morning between 6 am to 7:30 am and placed in a petri dish and stored in the fridge to be used as the pollen source for the pollination. In the evening between the hours of 4:30 and 5:30

pm the stored flowers were removed from the fridge and the outer cover of the tip of unopened flower buds were removed carefully with the aid of a blade. The exposed stamens were carefully removed leaving the pistil behind. Similar shape was cut from the tip of a flower from the fridge together with the petals and sepals and placed on the flower bud to allow for pollination. This method allowed the cut tip of a flower from the fridge together with the petals and sepals remain fitted to the pistil of the flower bud for three or more days. This ensured successful crossing since the pistil was completely covered with the replaced tip. The bud on the plant was tagged with a thread to indicate that it has been crossed

3.4.2 STAGE ONE

In the first stage, five parental genotypes were found to be resistant in the first screening namely SARC-1-57-2, CB27, KVX-295-2-124-99, 58-77, IT97K556.The genotypes **SARC-1-57-2,** a known resistant line in Ghana**, KVX-295-2-124-99, IT97K556** and **APAGBAALA,** a known susceptible line also in Ghana were planted in the screen house. Direct crosses were made between SARC-1-57-2 and KVX-295-2-124-99, SARC-1-57-2 and IT97K556-6 to generate the F_1 generation. Also direct crosses were made between IT97K556-6 and APAGBAALA to also generate the F_1 generation. Planting was staggered with the late maturing lines KVX-29 5-2-124-99 and IT97K556-6 planted a week earlier before the early maturing lines SARC-1-57-2 and APAGBAALA.

3.4.3 STAGE TWO

In the second stage, the F_1 populations were planted in the screen house and allowed to self to produce F_2 progenies. The populations generated at the second stage were as follows:

1. F_2 (SARC-1-57-2 × KVX-295-2-124-99)

2. F_2 (SARC-1-57-2 × IT97K556-6)

3. F_2 (IT97K556-6 × APAGBAALA)

3.5 EVALUATION OF F_2 POPULATION

Twenty six pots were used for F_2 (SARC-1-57-2 × KVX-295-2-124-99) with a total of 120 plants and F_2 (SARC-1-57-2 × IT97K556) with a total of 142 plants. Whiles 13 pots were used for F_2 (IT97K556 × APAGBAALA) with a total of 85 plants. In addition two pots of APAGBAALA and SARC-1-57-2 each also containing 4 seedlings were planted as the checks to the set up. 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). F_2s were evaluated using the Apagbaala and SARC-1-57-2 as susceptible and resistant checks. The plants were classified as resistant or susceptible based on the susceptible and resistant checks.

3.6 EVALUATION OF F_3 LINES

The resistant seedlings that survived were allowed to self to F_3 generation and seedlings which showed susceptibility were also rescued by spraying with lambda cyhalothrin insecticide. The seedlings for the resistant crosses (SARC1-57-2 x IT97K-556-6 and SAC1-57-2 x KVX-295-2-124-99) were also advanced to F_3 generation. Seeds from the individual F_2 plants were planted to a number of pots depending on the number of seeds and this was well labeled. Seeds from both resistant and susceptible $F_{2\text{-}3}$ were screened. Infestation was done 3-4 days after the plants emerged. Live aphids were obtained from plants infested with aphids from the aphid culture at the insectary. The aphids were infested on the young seedling. Five, four-day old aphids were infested per seedling. The infestation was done in the morning.

Counting was done in the evening to check the number of aphid and to ensure that uniform population of 5 per plant was maintained within 48 hours after infestation. Apagbaala and SARC-1-57-2 were used as checks. The populations were evaluated and grouped as resistant, susceptible and segregating populations (Figure 15).

Fig. 15 F_3 population of SARC-1-57-2 × IT97K556-6 tagged to ease identification after infestation

3.7 DATA COLLECTION AND ANALYSIS

3.7.1 Screening of panel of aphid resistant lines

Data collected on parental plants include a score of seedling vigour as response to aphids attack according to the scale of Kusi (2008) and the survival rate of seedlings. The following statistics were estimated using Statistix 9 software: analysis of variance to estimate the level of variability and significant differences between means among the lines and standard deviation calculation.

3.7.2 Screening of segregating population

For the segregating populations (F_2 and F_3) the count data sets were collected on number of resistant and number of susceptible seedlings. In order to know the gene frequency of the F_2 individuals, the F_3 counts were traced to their F_2 parents. F_3 pots that had both resistant and susceptible plants growing in them were counted as heterozygotes.

Chi-square ($\chi 2$) goodness of fit test was performed using Statistical Tool for Agricultural Research (STAR) version: 2.0.1 to test the goodness of fit of the observed phenotypic counts to classical Mendelian ratios 3:1, 1:2:1, 9:6:1 and 15:1.

CHAPTER FOUR

RESULTS

4.1 SCREENING FOR APHID RESISTANCE FROM THE PANEL OF SOURCES OF RESISTANCE

4.1.1 Degree of aphid resistance and survival rate in eleven cowpea lines

Degrees of aphid resistance were estimated by measuring the mean and variances for the eleven lines of cowpea from 10 to 15 days after infestation with aphids (Table 15). Among these lines, SARC-1-57-2 had a significantly higher ($p \leq 0.05$) mean resistance score of 4 whilst Apagbaala had the lowest score of 1.7. In general, 5 out of the eleven lines namely 58-77, IT9K556-6, KvX-295-2-124-99, SARC-1-57-2 and CB27 (Fig 16) were considered to be resistant with all these lines having a mean resistance score above 3.0, whiles INIA 19, KN1, Apagbaala, Bambey 21, IT82E-18 and VITA 7 had below 3.0 (Table 15).

Table 15 Mean scores of aphid resistance and variance for the eleven Lines

LINES	MEAN	VARIANCE	APHID RESISTANCE
58-77	3.5	0.33	RESISTANT
INIA 19	2.7	0.25	SUSCEPTIBLE
IT97K-556-6	3.7	0.25	RESISTANT
KN1	2.0	0.66	SUSCEPTIBLE
KvX-295-2-124-99	3.7	0.25	RESISTANT
SARC-1-57-2	4.0	0.00	RESISTANT
APAGBAALA	1.7	0.25	SUSCEPTIBLE
BAMBEY 21	2.3	0.25	SUSCEPTIBLE
CB27	3.7	0.50	RESISTANT
IT82E-18	2.5	0.33	SUSCEPTIBLE
VITA 7	2.7	0.25	SUSCEPTIBLE

Mean rating scale used to estimate aphid resistance: 3-5 = resistant (R), 1-2 susceptible (S).

Fig. 16 Infestation of two lines KvX-295-2-124-99 and KNI 14 days after infestation with aphids. With KvX-295-2-124-99 showing resistance and KNI being susceptible

Survival rate ranged from 12.5 % to 100 %. SARC-1-57-2 had the highest mean survival rate of 100% with IT97K556, CB27 and KVX-295-2-124-99 also having high mean survival rate of 97%, 95%, 90% respectively, 58-77. However, recorded a weak mean survival rate of 67.5% (Fig 17). Five of the lines, Apagbaala, Bambey, KNI 21, IT82E-18 and VITA 7 showed poor survival rate with values below 40 %. However, INIA 19 had an intermediate level of resistance with 57.5% survival rate.

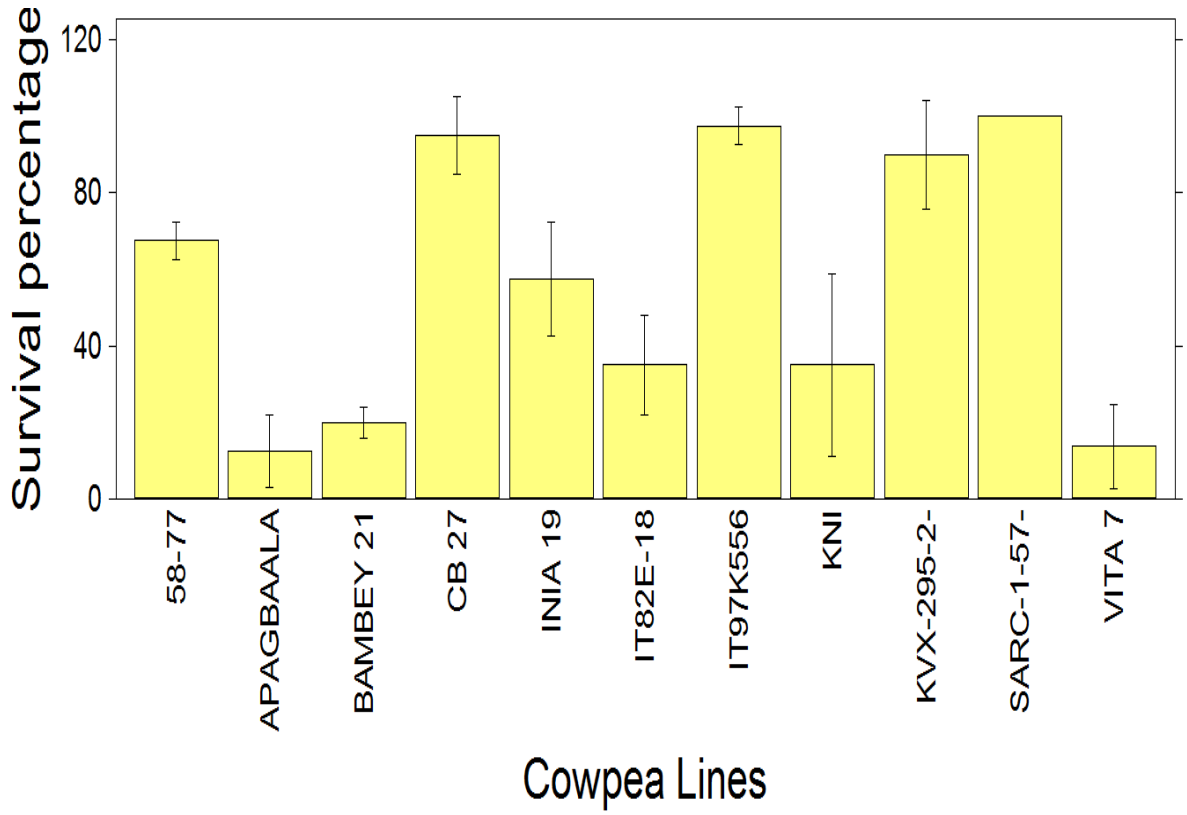

Fig 17 Mean percentage survival of cowpea lines 14 days after infestation

4.2 MODE OF INHERITANCE

Screening of the panel of resistance resulted in five out of the eleven lines being resistant against aphids in Ghana. These are SARC1-57-2, KvX-295-2-124-99, CB 27, IT97K-556-6 and 58-77. The study focused on KVX-295-2-124-99 and IT97K-556-6, SARC1-57-2 and Apagbaala to determine the mode of inheritance and allelism for the gene of resistance.

4.2.1 IT97K556-6(Resistant) x APAGBAALA (Susceptible)

Table 16 shows the genetic ratios and chi square values for aphid resistance in F_2 and F_{2-3} populations between IT97K556-6 and APAGBAALA crosses. The phenotypic F_2

generation was tested using the chi square for the hypothesis of 3: 1 resistant to susceptible ratio (Table 16). The F_2 generation of IT97K556-6 x APAGBAALA was grouped into two categories 59 resistant and 26 susceptible. Over 90% of the seedlings from the susceptible parent (Apagbaala) which was used as a check died from aphid infestation 15 days after infestation, the probability value for the expected 3 resistant: 1 susceptible segregation ratio in the F_2 generations showed no significant differences. Tracing the F_3 generation back to the F_2, the seedling segregated into 3 categories namely 27 resistant 32 heterozygotes and 26 susceptible. The numbers were not significantly different from the expected 1:2:1 ratio at 5 % significant level.

Table 16 Genetic ratios and chi square values for aphid resistance in F_2 and F_3 populations between IT97K556-6 and APAGBALA crosses

POPULATION	EXPECTED RATIO	RES	SEG	SUSC	CHI SQUARE	P VALUE
IT97K556-6	ALL R	ALL R				
APAGBALA	ALL S			ALL S		
IT97K556-6 X APAGBALA						
F_2	3:1	59	0	26	1.416	0.234
F_3	1:2:1	27	32	26	5.212	0.074

Res = All progeny resistant, Seg = Segregating into resistant and susceptible progeny, Susc = All progeny susceptible

4.3 ALLELISM OF APHID RESISTANCE

4.3.1 IT97K556-6 (Resistant) and SARC-1-57-2 (Resistant)

The phenotypic F_2 generations was tested using the chi square for the hypothesis of 15: 1 resistant to susceptible ratio (Table 17). The F_2 generation of IT97K556-6 and SARC-1-57-2 was grouped into two categories 128 resistant and 14 susceptible (Fig. 18). The probability for the expected 15 resistant: 1 susceptible segregation in the F_2 generations showed no significance ($\chi 2$ =3.149, P =0.076). The results showed that the segregation F_2 generation fitted the 15:1 ratio. The F_3 generation was also grouped into 3 categories 76 resistant 52 segregating 14 susceptible. The probability for the expected 9 resistant, 6 segregation and 1 susceptible in the F_3 generations showed no significance ($\chi 2$ =3.177, P =0.204).

Table 17 Genetic ratios and chi square values for aphid resistance in F_2 and F_3 populations between IT97K556-6 and SARC-1-57-2 crosses.

POPULATION	EXPECTED RATIO	RES	SEG	SUS	CHI SQUARE	P VALUE
IT97K556-6	ALL R	ALL R				
SARC-1-57-2	ALL R	ALL R				
IT97K556-6 X SARC-1-57-2						
F_2	15:1	128	0	14	3.149	0.076
F_3	9:6:1	76	52	14	3.177	0.204

R = All progeny resistant, Seg = Segregating into resistant and susceptible progeny, Sus = All progeny susceptible.

Fig 18 Susceptible F_2 plants from the cross between IT97K556-6 and SARC-1-57-2

4.3.2 KvX-295-2-124-99 (Resistant) and SARC-1-57-2 (Resistant)

Crosses between these resistant lines KvX-295-2-124-99 and SARC-1-57-2 were made to determine the allelic relationship between the dominant genes. Over 90% of the seedlings from the susceptible parent (Apagbaala) which was used as a check died from aphid infestation 15 days after infestation The phenotypic F_2 and F_3 generations all showed resistance (Table 18), thus no segregation was observed.

Table 18 Genetic ratios and chi square values for aphid resistance in F_2 and F_3 populations between KvX-295-2-124-99 and SARC-1-57-2 crosses

POPULATION	EXPECTED RATIO	RES	SEG	SUS	CHI SQUARE	P VALUE
KvX-295-2-124-99	ALL R	ALL R				
SARC-1-57-2	ALL R	ALL R				
KvX-295-2-124-99XSARC-1-57-2						
F_2	ALL R	120				
F_3	ALL R	120				

R = All progeny resistant, Seg = Segregating into resistant and susceptible progeny, Sus = All progeny susceptible

CHAPTER FIVE

DISCUSSION

5.1 SCREENING FOR APHID RESISTANCE FROM THE PANEL OF SOURCES OF RESISTANCE

The screening of the panel of resistance gave five of the lines resistant against aphids in Ghana. These were SARC1-57-2, KvX-295-2-124-99, CB27, IT97K-556-6 and 58-77. This was further confirmed with the high survival rating of these five lines fourteen days after infestation with the aphids.

SARC1-57-2 line is a source of resistance from SARI in Ghana and originated from a cross of Apagbaala/ UCR 01-11-52 (Kusi *et al* 2010).This line was used as a check of resistant and Apagbaala which was susceptible was used as a source of susceptibility check. BAMBEY 21 from ISRA, IT82E-18 and VITA7 both from IITA were susceptible to aphids in Ghana confirming earlier reports with their low mean survival rates of 20%, 35% and 13.75% respectively. Cowpea lines 58-77, IT97K-556-6 and KvX-295-2-124-9 which were all resistant from their sources (Legume Innovation Laboratory Report 2015) were also confirmed to be resistant to the aphids in Ghana. These lines had a high percentage of mean survival rates. Since these lines still maintained either their resistance or susceptibility status in their home country and Ghana, there could be the possibility of the same biotypes of aphids occurring in those regions and Ghana.

Among the lines known to be resistant in Ghana is CB27 known in California to be susceptible to the aphids. This observation is similar to findings by Messina (1985), who also found differential response to aphid population from different geographical areas in Africa and southern United States. In contrast some scientists have reported more aggressive aphid biotypes across West African countries (Martyn, 1991). The

differential response of CB27 could indicate different biotypes of aphids in Ghana and California. Furthermore two of the lines, INIA 19 and KN1, known in MOSU and Burkina Faso (INERA) respectively as resistant to aphids were found to be susceptible to aphids in Ghana. This is also consistent to the findings of Emden (1991) who also suggested differential response across some West African countries. Furthermore, Kusi *et al.,* (2010) observed similar findings when resistant lines from IITA in Nigeria were found to be susceptible to aphids in Ghana and attributed it to a more virulent biotype of aphids in northern Ghana than Nigeria. This differential response across West Africa indicates the presence of different biotypes of aphids even across the region. Although it is not so clear how these different biotypes have occurred, rare mutations chromosomal of rearrangement and mitotic recombination are possible causes of the variants (Hales *et al.,* 1997). Zaayman *et al.*,(2009) also argued that the occurrence of sexual reproduction in these species increases the risk of differential response or resistance-breaking for the cowpea lines.

Martyn (1991) reported of three distinct biotypes of the cowpea aphid that occur in Africa and Asia, and a distinct one occurring in United States. An earlier report by IITA (1981) identified and classified the ones found in West Africa as biotype A, biotype B and Biotype K. Biotypes A and B occur in Nigeria and Biotype K in Upper Volta or Burkina Faso. The differential responses observed in this study can be due to the differences in biotypes from these geographical locations. However, the presence of different biotypes might also suggest that these aphids may also require different resistant genes to control them.

5.2 MODE OF INHERITANCE OF APHID RESISTANCE

5.2.1 F_2 AND F_3 POPULATION OF IT97K556-6 (Resistant) AND APAGBAALA (Susceptible)

The segregation ratio in the F_2 population between IT97K556-6 (resistant) x Apagbaala (susceptible) fits into the 3:1 ratio for a single dominant gene ($\chi 2 = 3.26$, P = 0.0707). The F_2 were advanced to F_3 population and those also fit into the 1:2:1(1R:2H: 1S) genetic ratio still confirming that the gene that causes the resistance is a single dominant gene. This confirms earlier report by Bata *et al.,* (1987) who also found the resistance gene to be a single dominant gene. Furthermore, Pathaks (1988) showed that aphid resistance was governed by a single dominant gene and he proposed that the gene should be designated as *Rac*. Nualsri *et al* (2012) suggested that cowpea aphid resistance to IT82E-16 line was controlled by a single dominant and their study confirmed the findings of an earlier work by Benchasri *et al.,* (2007) and also of this present study. This monogenic inheritance nature of this aphid resistance can allow for easy incorporation into adapted susceptible genotypes through backcrossing but the challenge with single gene inheritance is that the aphids can easily develop fast biotypes which can overcome the resistant line (Githiri *et al.,* 1995). To solve these challenge different sources of resistance should be identified and once it is done, pyramiding of two or more resistance genes in a single line can be done. Duvick (1999) was of the view that resistance controlled by multiple genes is more durable than the resistance controlled by a single dominant gene. Joshi and Nayak (2008) also supported this notion when they observed that pyramided lines showed a wider spectrum and a higher level of resistance than lines with only a single gene. Work carried out by Sanchez, *et al.,* (2000) also confirmed this when they were

able to successfully transfer three bacterial blight resistance genes into three susceptible rice lines possessing desirable agronomic characteristics.

5.3 ALLELISM OF APHID RESISTANCE

5.3.1 F_2 AND F_3 POPULATION OF SARC-1-57-2(Resistant) x IT97K556-6 (Resistant)

Segregation in the F_2 population of the cross IT97K556-6 x SARC-1-57-2 lines gave good fit to the 15R:1S (Resistant: susceptible) phenotypic ratio ($\chi 2$ =3.149, P =0.076). This ratio indicates that two different genes may be responsible for the expression of resistance to the aphids in the F_2 population.

The segregation pattern of the F_3 progenies of this family when traced back to their F_2 parents gave a genotypic ratio 9:6:1, representing 9 non-segregating resistant genotypes, 6 segregating genotypes and 1 homozygous susceptible. This ratio fits into dihybrid ratio for dominance at two loci. This is similar to findings by Antoine *et al.*, (2016) when they worked on the allelic relations between KVX640 and KVX396-4-5-2 genotypes. They suggested that with a dihybrid ratio for dominance the resistance is determined by a dominant allele of each of the two loci that segregate independently. This indicates the presence of two different dominant genes controlling the trait. They also argued that from the ratio one can determine or be informed about the number of genes involved in the resistance as well as their eventual relationship.

It has also been deduced that these two loci have duplicate dominant epistasis in the population with one dominant gene present in each parent (Estakhr and Assad, 2002). Suryanto *et al.,* (2014) suggested that duplicate dominant epistasis occur when there is

complete dominance at both gene pairs, but either gene when dominant, epistatic to the other.

According to Acquah, (2007) the duplicate loci have cumulative effects with complete dominance at both loci and also the interactions between dominants at both loci also give a new phenotype.

In previous studies, SARC-1-57-2 was found to possess antibiosis-type of resistance to the aphid (Kusi *et al.,* 2010). Plants with antibiosis resistance negatively interfere with the reproduction of the aphid and thus control the insect effectively and this was similar to what was observed in this study. Wiseman (1999) argued that the effects of antibiosis may include reduced food consumption, increased development time, low food reserves, death in pre-pupal or pupal stages and reduced fecundity. Laamari *et al.,* (2008) found V23 and V51 lines to have antibiosis type of resistance and attributed their mechanism to certain substances produced during aphid's infestation. It was also observed that the gene that controls the resistance in SARC-1-57-2 is a single dominant gene which confirms Beta *et al.,* (1987) report that gene resistance to cowpea aphid involves antibiosis and is conferred by a single dominant gene.

IT97K556-6 line is an aphid resistant source from IITA in Nigeria. A survey conducted by Souleymane *et al.,* (2013), suggested that the resistance did not seem strong against aphids in Africa. However Bao-Lam *et al.,* (2014) observed a contrast in California where this line has been highly resistant against the aphids there. They attributed this to the different biotypes of aphids across these geographical locations.

From the present study it has been observed that a different single dominant gene controls the resistance in this line which is different from that of SARC-1-57-2.

Pathak (1988) identified a second dominant gene for aphid resistance and designated as *Rac-2* and attributed it to induced mutation in a susceptible cultivar.

Antibiosis type of resistance was also observed for IT97K556-6 as there was a reduction in fecundity as suggested by Basandrai (2011). According to Ansari (1984) antibiosis is the main mechanism responsible for aphid resistance in cowpea. His finding was further supported by later work done by Ofuya (1988b) and Laamari *et al,*. (2008) who found these lines TVu1037, TVu2876, TVu 3000 and V51 respectively to all have antibiosis as their main mechanism for resistance.

Although the gene conferring resistance is different in both lines, the mode of action which is antibiosis is same. However the present results indicating antibiosis as the type of resistance contradicts earlier report of Souleymane *et al.*,(2013). They suggested that the type of aphid resistance in IT97K556-6 line and TVNu 1158 was tolerance.

5.3.2 F_2 AND F_3 POPULATION OF SARC-1-57-2 AND KVX-295-2-124-99

All the F_2 population of the cross between the two resistant (SARC-1-57-2 and KVX-295-2-124-99) showed resistance in the phenotypic screening. From the F_2 population it could be deduced that the same gene that causes resistance in SARC-1-57-2 which is a known source of resistance in Ghana is the same gene that controls the resistance in KVX-295-2-124-99.

F_3 population was also screened and all the plants showed resistance to the aphids confirming the F_2 results and also confirming the fact that the gene causing resistance in SARC-1-57-2 is the same gene conferring resistance in KVX-295-2-124-99.This is in agreement with findings from Omwega (1990) and Antoine *et al.*,(2016) who observed similar results and stated that ,for allelic relationship, if the genes for

resistance from two resistant parents are the same, all the F_2 and F_3 progeny would be resistant and so there would be no susceptible plant .

KVX-295-2-124-99 is a source of aphid resistant line from INERA in Burkina Faso. From the current study it has been observed that the gene present in SARC-1-57-2 is the same gene present in KVX-295-2-124-99. Since it is the same gene that controls the resistance, KVX-295-2-124-99 also possesses antibiosis-type of resistance to the aphids. According to Kogan and Omar (1978) the results of antibiosis may include effect that influences fecundity, development time and body size through to acute direct effect resulting in death of the aphids. Generally fecundity was observed to be low for the aphids on this plant confirming the report by Kogan and Omar (1978).

CHAPTER SIX

6.1 CONCLUSION

From this study it was observed that out of the eleven lines from the panel of aphid resistance five of these lines were resistant against aphids in Ghana. These are SARC-1-57-2, KvX-295-2-124-99, CB27, IT97K-556-6 and 58-77.

The current confirmed the already known fact that suggests the resistance of IT97K-556-6 to aphids is controlled by a single dominant gene. It was also observed that the gene that controls resistance in this line is different from that of SARC1-57-2 which is a source of aphid resistance in Ghana. Antibiosis type of resistance was also observed for IT97K-556-6 line.

Identification of many sources of aphid resistance would be highly desirable to keep ahead of different biotype development in the aphids (Pathak *et al.,* 2007). Once new sources of resistance is found as observed in this present study, pyramiding of the different resistant genes in these cowpea genotypes to a single cultivar can be effected to combat the menace of aphid infestation .

Furthermore, genetic evidence also suggests that the gene causing resistance in KvX-295-2-124-99 is the same as the one found in SARC-1-57-2. This means that the gene controlling aphid resistance in both genotypes occupy the same loci and hence could not interact. Also the mechanism of resistance observed was due to antibiosis. In addition it has also been confirmed that the aphid biotype found in northern Ghana is the same in Burkina Faso.

Lines KvX-295-2-124-99 and SARC-1-57-2 from our study are important sources of breeding materials for the development of aphid resistant lines. The trait causing

resistance in these genotypes can be incorporated into high yielding genotypes both in Burkina Faso and Ghana where they come from respectively. International cowpea breeding centers such as International Institute of Tropical Agriculture (IITA) and International Centre for Insect Physiology and Ecology (ICIPE) could also adapt these lines in their breeding programs since these lines showed stronger resistance to the aphids.

The sources of aphid resistant panel have already been genotyped on the SNP platform and polymorphic markers have been identified, the phenotypic data generated from the current study should therefore facilitate genetic mapping of the aphid resistance gene and its deployment in marker assisted selection

6.2 Recommendations and Further Studies

It is highly recommended that a cross of KvX-295-2-124-99 and Apagbaala should be carried out to determine the mode of inheritance in the KvX-295-2-124-99 line. Also it is suggested that a cross of KvX-295-2-124-99 and IT97K-556-6 should also be done to serve as a check to the results from this study. In addition the other lines found to be resistant namely CB27 and 58-77 should further be crossed with Apagbaala to determine the mode of inheritance and then to SARC1-57-2 to determine the allelism of the resistant gene.

It is also suggested that further trials be conducted in other ecological zones to investigate the existence and classification of different biotypes of aphids.

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APPENDIX

Appendix 1

Statistix 9.1 1/1/2013, 11:09:16 AM

Descriptive Statistics for COWPEA = 58-77

	FAIL	observed	SURV
N	4	4	4
Mean	32.500	3.5000	67.500
SD	5.0000	0.5774	5.0000
Variance	25.000	0.3333	25.000
SE Mean	2.5000	0.2887	2.5000
C.V.	15.385	16.496	7.4074
Minimum	30.000	3.0000	60.000
Median	30.000	3.5000	70.000
Maximum	40.000	4.0000	70.000
Skew	1.1547	0.0000	-1.1547

Descriptive Statistics for COWPEA = APAGBAALA

FAIL	observed	SURV
N	4	4	4
Mean	87.500	1.7500	12.500
SD	9.5743	0.5000	9.5743
Variance	91.667	0.2500	91.667
SE Mean	4.7871	0.2500	4.7871
C.V.	10.942	28.571	76.594
Minimum	80.000	1.0000	0.0000
Median	85.000	2.0000	15.000
Maximum	100.00	2.0000	20.000
Skew	0.4934	-1.1547	-0.4934

Descriptive Statistics for COWPEA = BAMBEY 21

FAIL	observed	SURV
N	4	4	4
Mean	80.000	2.2500	20.000
SD	4.0825	0.5000	4.0825
Variance	16.667	0.2500	16.667
SE Mean	2.0412	0.2500	2.0412
C.V.	5.1031	22.222	20.412
Minimum	75.000	2.0000	15.000
Median	80.000	2.0000	20.000
Maximum	85.000	3.0000	25.000
Skew	0.0000	1.1547	0.0000

Descriptive Statistics for COWPEA = CB 27

FAIL	observed	SURV
N	4	4	4
Mean	5.0000	3.7500	95.000
SD	10.000	0.5000	10.000
Variance	100.00	0.2500	100.00
SE Mean	5.0000	0.2500	5.0000
C.V.	200.00	13.333	10.526
Minimum	0.0000	3.0000	80.000
Median	0.0000	4.0000	100.00
Maximum	20.000	4.0000	100.00
Skew	1.1547	-1.1547	-1.1547

Descriptive Statistics for COWPEA = INIA 19

FAIL	observed	SURV
N	4	4	4
Mean	42.500	2.7500	57.500
SD	15.000	0.5000	15.000
Variance	225.00	0.2500	225.00
SE Mean	7.5000	0.2500	7.5000
C.V.	35.294	18.182	26.087
Minimum	30.000	2.0000	40.000
Median	40.000	3.0000	60.000
Maximum	60.000	3.0000	70.000
Skew	0.2138	-1.1547	-0.2138

Descriptive Statistics for COWPEA = IT82E-18

FAIL	observed	SURV
N	4	4	4
Mean	65.000	2.5000	35.000
SD	12.910	0.5774	12.910
Variance	166.67	0.3333	166.67
SE Mean	6.4550	0.2887	6.4550
C.V.	19.861	23.094	36.886
Minimum	50.000	2.0000	20.000
Median	65.000	2.5000	35.000
Maximum	80.000	3.0000	50.000
Skew	0.0000	0.0000	0.0000

Descriptive Statistics for COWPEA = IT97K556

FAIL	observed	SURV
N	4	4	4
Mean	2.5000	3.7500	97.500
SD	5.0000	0.5000	5.0000
Variance	25.000	0.2500	25.000
SE Mean	2.5000	0.2500	2.5000
C.V.	200.00	13.333	5.1282
Minimum	0.0000	3.0000	90.000
Median	0.0000	4.0000	100.00
Maximum	10.000	4.0000	100.00
Skew	1.1547	-1.1547	-1.1547

Descriptive Statistics for COWPEA = KNI

FAIL	observed	SURV
N	4	4	4
Mean	65.000	2.0000	35.000
SD	23.805	0.8165	23.805
Variance	566.67	0.6667	566.67
SE Mean	11.902	0.4082	11.902
C.V.	36.623	40.825	68.014
Minimum	40.000	1.0000	10.000
Median	65.000	2.0000	35.000
Maximum	90.000	3.0000	60.000
Skew	0.0000	0.0000	0.0000

Descriptive Statistics for COWPEA = KVX-295-2-

FAIL	observed	SURV
N	4	4	4
Mean	10.000	3.7500	90.000
SD	14.142	0.5000	14.142
Variance	200.00	0.2500	200.00
SE Mean	7.0711	0.2500	7.0711
C.V.	141.42	13.333	15.713
Minimum	0.0000	3.0000	70.000
Median	5.0000	4.0000	95.000
Maximum	30.000	4.0000	100.00
Skew	0.8165	-1.1547	-0.8165

Descriptive Statistics for COWPEA = SARC-1-57-

FAIL	observed	SURV
N	4	4	4
Mean	0.0000	4.0000	100.00
SD	0.0000	0.0000	0.0000
Variance	0.0000	0.0000	0.0000
SE Mean	0.0000	0.0000	0.0000
C.V.	M	0.0000	0.0000
Minimum	0.0000	4.0000	100.00
Median	0.0000	4.0000	100.00
Maximum	0.0000	4.0000	100.00
Skew	M	M	M

Descriptive Statistics for COWPEA = VITA 7

FAIL	observed	SURV
N	4	4	4
Mean	86.250	2.7500	13.750
SD	11.087	0.5000	11.087
Variance	122.92	0.2500	122.92
SE Mean	5.5434	0.2500	5.5434
C.V.	12.854	18.182	80.631
Minimum	75.000	2.0000	0.0000
Median	85.000	3.0000	15.000
Maximum	100.00	3.0000	25.000
Skew	0.2780	-1.1547	-0.2780

Appendix 2

LSD All-Pairwise Comparisons Test of FAIL by COWPEA

COWPEA Mean Homogeneous Groups

APAGBAALA 87.500 A

VITA 7 86.250 A

BAMBEY 21 80.000 AB

IT82E-18 65.000 B

KNI 65.000 B

INIA 19 42.500 C

58-77 32.500 C

KVX-295-2- 10.000 D

CB 27 5.0000 D

IT97K556 2.5000 D

SARC-1-57- 0.0000 D

Alpha 0.05 Standard Error for Comparison 8.3655

Critical T Value 2.035 Critical Value for Comparison 17.020

There are 4 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of observed by COWPEA

COWPEA Mean Homogeneous Groups

SARC-1-57- 4.0000 A

CB 27 3.7500 A

IT97K556 3.7500 A

KVX-295-2- 3.7500 A

58-77 3.5000 AB

INIA 19 2.7500 BC

VITA 7 2.7500 BC

IT82E-18 2.5000 CD

BAMBEY 21 2.2500 CD

KNI 2.0000 CD

APAGBAALA 1.7500 D

Alpha 0.05 Standard Error for Comparison 0.3744

Critical T Value 2.035 Critical Value for Comparison 0.7617

There are 4 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pairwise Comparisons Test of SURV by COWPEA

COWPEA Mean Homogeneous Groups

SARC-1-57- 100.00 A

IT97K556 97.500 A

CB 27 95.000 A

KVX-295-2- 90.000 A

58-77 67.500 B

INIA 19 57.500 B

IT82E-18 35.000 C

KNI 35.000 C

BAMBEY 21 20.000 CD

VITA 7 13.750 D

APAGBAALA 12.500 D

Alpha 0.05 Standard Error for Comparison 8.3655

Critical T Value 2.035 Critical Value for Comparison 17.020

There are 4 groups (A, B, etc.) in which the means

are not significantly different from one another.