# GENETIC STUDIES OF APHIDS (*Aphis craccivora* Koch) RESISTANCE IN COWPEA

**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MPHIL IN AGRONOMY (PLANT BREEDING)**

**AUGUSTINE BOAKYE BOATENG**

**AUGUST 2015**

# DECLARATION

I hereby declare that, I have under supervision undertaken the study and except for specific references which have been duly and appropriately acknowledged, this project is the result of my own research and has not been submitted either in part or whole for and other degree elsewhere.

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Augustine Boakye Boateng

(Candidate)

August, 2015

We declare that we have supervised the student in undertaking the study submitted herein and confirm that he has our permission to submit.

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Prof. Richard Akromah

August, 2015

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Dr. James Yaw Asibuo

August, 2015

Certified by: ……………………………………

Dr. Charles Kwoseh

(Head of Department)

## DEDICATION

This thesis is dedicated to my mother Esther Akosua Boatemaa for her prayers and the inspirational force behind my studies.

**ACKNOWLEDGEMENT**

I want to acknowledge the Almighty God as the source of my strength and inspiration for the successful completion of this project.

I am deeply appreciative of the constructive corrections, comments, suggestions, criticism and rightful guidance of my supervisors, Prof. Richard Akromah and Dr. James .Y. Asibuo throughout this project work.

I am extremely grateful to all the Lecturers of the Department of Crop and Soil Sciences for their suggestions, contributions and directions particularly at seminars.

I would also like to express my earnest gratitude to Alliance for a Green Revolution in Africa (AGRA) for having sponsored this work with grant support, am very gratefully.

I would like to say a big thank you to staff of CSIR - CRI (Crop Research Institute, Fumesua, Legumes Department) for your support especially during my field work.

Sincere thanks also go to my family especially my mother, Esther Boatemaa for her encouragement, support and prayers during my academic pursuit. To my brother, Paul and sisters Dorcas and Abigail for your prayers, not to forget you Dad for your moral support.

Also my heartfelt gratitude goes to Dr. Robert Asiedu (IITA) and Mr. Akwasi Mensah Bonsu ( Former Deputy Finance Officer, K.N.U.S.T), I owe a debt of gratitude to you.

Finally, thanks to all faceless individuals who contributed in one way or the other to the successful completion of this work.

## ABSTRACT

Cowpea aphid (*Aphis craccivora*) is a serious pest in legume that causes significant grain yield losses. Chemical control measures are the most widely known form of control to this pest. However, breeding for resistance to this insect is very important because of its compatibility with other pest control methods; environment and eliminate dependence on environmentally toxic chemicals that resource poor subsistence farmers cannot afford. Breeding for cowpea aphid resistance was studied in crosses involving two resistant (Hewale and Asomdwee) and two susceptible (Asetenapa and Videza) genotypes. Crosses were made to generate direct and reciprocal $F_1$ generations and direct and reciprocal $F_2$ generations. All plants in each generation were evaluated for aphid resistance on the field using Randomized Complete Block Design. Each plant was infested with cowpea aphid two weeks after planting and the number of aphid colonies and damage scored between 0 – 5. The results showed most of the direct and reciprocal $F_1$ generations to be resistant to the cowpea aphid and the $F_2$ segregating generations were intermediate between the resistant and susceptible parents but were skewed towards the resistant parent. Results also showed that the resistance in Hewale and Asomdwee were controlled by a single gene and the $F_2$ generation conformed to the 3 resistant and 1 susceptible after a $\chi^2$ test. Reciprocal differences were not detected in the crosses suggesting the absence of maternal effect. Negative heterosis over mid-parent was observed for aphid resistance score. It is possible to improve cowpea aphid resistance in susceptible cowpea lines in a hybridization programme.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Cowpea (*Vigna unguiculata*) is an essential food legume and an important component of cropping systems in the drier regions of the tropics covering parts of Asia and Oceania, the Middle East, Southern Europe, Africa, southern USA, and Central and South America (Singh *et al*., 2002). It is an annual herb with great variation based on the variety, and prevailing soil and climatic conditions. There are also variations in growth habit and include the trailing, climbing and erect types.

It is one of the ancient grain legume crops cultivated in semi-arid West Africa where rainfall is characteristically low (mean annual range of 300-600 mm), variable in time and space and undependable (Fussell *et al*., 1991). It can be grown in regions with an average annual rainfall of 2.5 to 8 inches (Cook *et al.*, 2005). Being a fast growing crop, cowpea curbs erosion by covering the ground, fixes atmospheric nitrogen, and its decaying residues contribute to soil fertility. Cowpea grains contain between 17 and 32% protein on a dry weight basis and it is one of the cheapest sources of protein in the diets of peoples of West and Central Africa where cowpea is an important crop (Fatokun, 2002). The young fresh leaves, immature green pods and green seeds are used as vegetables; dry seeds are used in various food preparations while several snacks and main dishes are prepared from the grain and the haulms are fed to livestock as nutritious supplement to cereal fodder particularly during the dry season (Blade *et al*., 1997).

Cowpea feeds millions of people in the developing world with an annual worldwide production estimated around 4.5 million metric tons on 12 to 14 million ha (Diouf, 2011). Nigeria, Brazil, Niger and Burkina Faso (FAO, 2008) are among the major producers and account for over 70 % of the world crop. Cowpea production is constrained by many factors that are both biotic and abiotic (Hall *et al*., 1997). These factors include the use of unimproved varieties, poor soil conditions, inadequate management practices, poor cultural practices and heavy biotic stresses, particularly from insects, diseases and parasitic weeds which often attack in the field and weevils that destroy seeds in storage (Rachie, 1985).These factors are responsible for the generally low grain yield of cowpea across Sub-Saharan Africa in particular. Average grain yield has been reported to be about 324 kg/ha in the major cowpea growing countries of the world (Singh *et al*., 2002).

Every stage in the life cycle of cowpea has at least one major insect pest that can cause serious damage and impact yield negatively (Fatokun, 2002).

Cowpea aphid (CPA; *Aphis craccivora* Koch) is a serious pest in legume agriculture and has been reported on all continents except the Antarctic. It is considered as one of the important pests of cowpea in Africa, Asia and Latin America (Jackai and Daoust, 1986). Aphids primarily infest seedlings, large populations also infest flower buds, flowers and pods. They cause direct damage to cowpea seedlings by sucking the sap and indirect damage by transmission of aphid-borne mosaic viruses. They have been implicated as the main vectors of the non-persistent cowpea aphid-borne mosaic virus (Atiri *et al*., 1986). At least fourteen (14) legume viruses are transmitted by *Aphis craccivora* (Thottappilly *et al*., 1990). They can also cause leaf distortion, stunting and poor nodulation of root systems (Singh and Jackai, 1985) and in extreme cases, the plant is killed (Singh and van Emden, 1979).

A number of insecticides have been effective and most widely known form of control of aphids. The cost of insecticides and proper application equipment often are not accessible to the majority of resource-poor farmers who grow the crop, resulting in low yield and number of pods per plant (Singh and Allen 1980). Also insecticide application is harmful to human and environment and not compatible with other methods of pest control.

The use of resistant varieties appears to be the best option for the small-scale farmers of the semi-arid tropics owing to its low cost, compatibility with other control methods, and to the low income realized by farmers (Dent, 1991). Host plant resistance is one strategy that can be identified and deployed in important cultivars to manage aphids and offers the potential to reduce or eliminate dependence on environmentally toxic chemicals that resource poor subsistence farmers cannot afford and are not well equipped to handle (Jackai and Adalla, 1997). The importance of inheritance studies is to determine the proportion of phenotypic variation in a population that is attributable to genetic variation. Knowledge of genes and mode of inheritance of cowpea aphid resistance is required to accelerate breeding of resistant varieties.

The major objective of the study was to determine the inheritance of cowpea aphid (*Aphis craccivora)* resistance.

The specific objectives of the study were to;

1. Determine the number of genes controlling cowpea aphids (*Aphis craccivora*) resistance.

2. Estimate the heritability of resistance to cowpea aphid *(Aphis craccivora*).

3. Determine the contribution of maternal effects to cowpea aphid resistance.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Cowpea: Origin, Domestication and Distribution

Cowpea (*Vigna unguiculata* L. Walp.) (2n=2x=22) is a member of the Phaseoleae tribe of the Leguminosae family. It 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). The name cowpea probably originated from the fact that the plant was an important source of hay for cows in the southeastern United States and in other parts of the world (Timko *et al.*, 2007). Some important local names for cowpea around the world include "niebe," "wake," and "ewa" in much of West Africa and "caupi" in Brazil.

The precise origin of cultivated cowpea has been a matter of speculation and discussion for many years because of a lack of archaeological evidence. Some authorities feel that cowpeas originated either in the southern Sahel of north-central Africa or in Ethiopia, and then spread to Asia and the Mediterranean by way of Egypt (Alayande *et a*l., 2012). Another view is that they originated in India and were introduced into Africa some 2,000 to 3,500 years ago (Alayande *et* al., 2012). From West Africa, they made their way to the Caribbean and then to North America with the slave trade. Others also feel cowpea most certainly evolved in Africa, as wild cowpeas only exist in Africa and Madagascar (Steele, 1976). Carbon dating of cowpea (or wild cowpea remains from the Kintampo rock shelter in central Ghana) has been carried out (Flight, 1976), and is the oldest archaeological evidence of cowpea found in Africa.

The major center of diversity of cultivated cowpea is found in West Africa, in an area including the savanna region of Nigeria, southern Niger, part of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon (Ng and Marechal, 1985). Some evidence indicate that domestication occurred in northeastern Africa, based on studies of amplified fragment length polymorphism (AFLP) analysis, (Coulibaly *et al*., 2002). The wild cowpea *Vigna unguiculata* ssp. *Unguiculata* var. *spontanea* is the likely progenitor of cultivated cowpea (Pasquet, 1999).The wide geographical distribution of var. *dekindtiana* throughout sub-Sahara Africa suggests that the species could have been brought under cultivation in any part of the region. Today cowpea is grown throughout the tropic and subtropic areas around the whole world. It is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky *et al*., 2002; Tarawali *et al*., 2002; Sanginga *et al*., 2003).

### 2.2. Description, Classification and Importance

Cowpea is an annual herbaceous warm-season legume that is similar in appearance to common bean except that leaves are generally darker green, shinier, and less pubescent. Cowpeas also are generally more robust in appearance than common beans with better developed root systems and thicker stems and branches. Cowpea as an annual herb can reach more than 80 cm of height with strong taproot and many spreading lateral roots in the surface soil (Summerfield *et al*., 1974), Kay (1979) and Fox and Young (1982). Growth forms vary and include erect, trailing, climbing, or bushy depending on genotype although photoperiod and growing conditions can also affect plant stature. Most cowpea varieties have indeterminate stem and branch apices and others usually indeterminate growers under favorable conditions. Leaves are alternate and trifoliate with the first pair of leaves simple and opposite. Commonly the terminal leaflets are longer and larger than the lateral leaflets. Leaves exhibit considerable variation in size (6-16 x 4-11 cm) and shape (linear, lanceolate to ovate) and they are usually dark green. The leaf petiole is about 5 - 25 cm long. The stems are striate, smooth or slightly hairy and sometimes tinged with purple. The flowers are arranged in racemose inflorescence at the distal ends of 5-60 cm long peduncles. Flowers are borne in alternate pairs, with usually only two flowers per 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. Flowers open early in the morning and close at approximately midday. Anthesis takes place early in day between 6.30 and 9.00 a.m. Dehiscence of anthers is much earlier and it varies from 10.0 p.m. to 00.45 a.m. The safest time for emasculation is morning hours preceding day of anthesis.

Fruits are pods that vary in size, shape, colour and texture. Pods may be held erect, crescent shaped or coiled. They usually turn yellow when ripe, but may also be brown or purple in colour. There are usually 8-20 seeds per pod. Pods are usually cylindrical and may also be curved or straight. The seed coat can be either smooth or wrinkled and of various colors including white, cream, green, buff, red, brown, and black. Seed may also be speckled or patterned.

Emergence of cowpea is epigeal (similar to common bean and lupin), where the cotyledons emerge from the ground during germination. This type of emergence makes cowpea more susceptible to seedling injury, since the plant does not regenerate buds below the cotyledonary node.

Cowpea has many species however; cultivated cowpeas have been divided into five cultivar groups based mainly on pod and seed characteristics (Pursglove, 1968; Pasquet, 1999). Cultivar group *Unguiculata* is the largest and includes most medium- and large-seeded African grain and forage-type cowpeas while cultivar group *Textilis* is a rather rare form of cowpea with very long peduncles that were used in Africa as a source of fiber. Cultivar group *Melanophthalmus* includes “blackeyed pea” type cowpea with large, somewhat elongated seeds with wrinkled seed coats and fragile pods (Pasquet, 1998). Members of cultivar group *Sesquipedialis* (also known as “yardlong bean,” “long bean,” “Asparagus bean,” or “snake bean”) is widely grown in Asia for production of its very long (40 to 100 cm) green pods that are used as “snap” beans. Cultivar group *Biflora* (known as “catjang”) are common in India and characterized by their relatively small smooth seeds borne in short pods that are held erect until maturity. In spite of the striking differences in morphological characteristics among the cultivar groups, there are no practical barriers to hybridization or recombination between members of the different groups although outcrossing rate has been recorded around 5%. Members of the tribe Phaseoleae (of which cowpea is part) include many economically important warm season grain and oilseed legumes, such as soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and mungbean (*Vigna radiata*) (Timko *et al.*, 2007).

Cowpea has a wide variety of uses namely as a nutritious component in human diet as well as nutritious livestock feed (Langyintuo *et al.*, 2003). The nutritional content of cowpea grain is important because it is eaten in quantity by millions of p eople who otherwise have diets lacking in protein, minerals, and vitamins. The nutritional content of cowpea grain is similar to that of other annual legumes, with a relatively low fat content and a total protein content that is two to four times greater than cereal and tuber crops (Timko and Singh, 2008). However, it is deficient in methionine and cystine when compared to animal proteins. Cowpea grain is also a rich source of minerals and vitamins (Hall *et al.*, 2003) and it has one of the highest levels of any food of folic acid, a crucial B vitamin that helps prevent spinal tube defects in unborn children (http://www.cdc.gov/doc.do/id/0900 f3ec8000d558). It is usually harvested before the cereal crops are ready and therefore is referred to as "hungryseason crop". Cowpea can be used at all stages of growth as a vegetable crop. The young tender green leaves are an important food source in Africa and are prepared as a pot herb, like spinach. Immature snap pods are used in the same way as snap beans, often being mixed with other foods. The seed, or grain as it is sometimes referred to, is the most important part of the cowpea plant for human consumption. The seeds are most often harvested and dried for storage and consumption at a later time, either after cooking whole or after being milled like a flour product and used in various recipes (Nielsen *et al*., 1997; Ahenkora *et al*., 1998). Dry mature seeds are also suitable for boiling and canning. Also in many areas of the world, cowpea foliage is an important source of high-quality hay for livestock feed (Tarawali *et al*., 2002). Therefore, cowpea plays a critical role in the lives of millions of people in Africa and other parts of the developing world, and is a valuable and dependable commodity that produces income for farmers and traders (Singh, 2002; Langyintuo *et al.*, 2003).

Additionally, cowpea is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky *et al.*, 2002; Tarawali *et al.*, 2002; Sanginga *et al.*, 2003.

### 2.3. World Production

Cowpea is grown worldwide with an estimated cultivation area of about 12.5 million hectares annually and an annual worldwide production of over 3 million metric tons (Li *et al*., 2001). It is widely produced throughout the tropics, but Central and West Africa account for over 64% of the area (with about 8 million hectares, followed by about 2.4 million hectares in Central and Southern America, 1.3 million hectares in Asia, and about 0.8 million hectares in Eastern and Southern Africa). Nigeria is the largest producer and consumer of cowpea at estimated annual yields of 2 million metric tons (Singh *et al*., 2002;

Timko and Singh, 2008). Niger Republic is the next largest producer with 3 million ha and over 650,000 tons production. Other important production areas include lower elevation areas of Eastern and Southern Africa and in South America (particularly in northeastern Brazil and in Peru), parts of India, and the southeastern and southwestern regions of North America. Commercial trading of dry cowpea grain and hay are particularly important to the local and regional economies of West Africa (Singh, 2002, 2005; Langyintuo *et al*., 2003). A lot of the cowpea grain sold at large commercial markets in large urban centers of coastal West Africa is produced further inland where climates are drier and favorable to production of high-quality grain. As compared to other legumes, cowpea is highly adapted to high temperatures and resistance to drought stress (Hall *et al*., 2002; Hall, 2004). For example, Hall and Patel (1985) reported cowpea grain yields of as much as 1000 kg $ha^{-1}$ of dry grain in a Sahelian environment with low humidity and only 181mm of rainfall. Cowpea is also a valuable component of farming systems in areas where soil fertility is limiting and restores soil fertility for succeeding cereal crops (Carsky *et al*., 2002; Tarawali *et al*., 2002; Sanginga *et al*., 2003). This is because cowpea has a high rate of nitrogen fixation (Elawad and Hall, 1987), forms effective symbiosis with mycorrhizae (Kwapata and Hall, 1985), and has the ability to better tolerate a wide range of soil pH when compared to other grain legumes (Fery, 1990). Also, well-adapted, early maturing cowpea varieties capable of producing seed in as few as 55 days after planting often provide farmers with the first source of food from the current harvest sooner than any other crop (Hall *et al.*, 2003).

## 2.4. Cultural Practices

### 2.4.1. Choice of Cultivars, Planting Density and Spacing

The choice of cultivars of cowpea affects plant population because they respond differently to photoperiod. Cultivars with erect growth habits have a higher plant population than prostrate (creeping) or semi prostrate types, because the erect forms performs much better in narrow rows (Weber *et al.*, 1996). The use of early maturing cultivars helps farmers escape the effects of late season drought, but plants exposed to intermittent moisture stress during the vegetative or reproductive stages will perform very poorly. The environmental constrains of a land to be used, will determine the most favorable plant population for cowpea (Coetzee, 1995). Four or three seeds are planted at 20 cm along the ridge spaced 75 cm apart (20 cm x 75 cm) representing 133 000 plants/ha for erect/semi-erect varieties and (50 cm x 75 cm; 60,000 plants/ha) for the spreading types but later thinned to two seedlings per hill, one week after germination (www.daff.gov.za)

### 2.4.2. Fertilization

Fertilizer application in cowpea production depends on anticipated yield and soil fertility. The plant will perform well under low N conditions due to a high capacity for N fixation. A starter N rate of 27 $kg.ha^{-1}$ is sometimes required for early plant development on low-N soils (Rupela and Saxena, 1987; Bluementhal *et al.*, 1992). Cowpea like other legumes fixes its own nitrogen through a symbiotic relationship with a specific soil bacterium (*Rhizobium sp*.), which makes atmospheric nitrogen available to the plant via nitrogen fixation, and does not need nitrogen fertilizer. Although cowpea *Rhizobium* is widespread, seed inoculation with *Rhizobium* specific to cowpea would be beneficial in areas where it is not present and increase optimum nitrogen fixation. It is however, important to use

*Rhizobium* of the cowpea type to form effective nodules (Eaglesham *et al.*, 1977). Excess nitrogen (N) delays maturity, promotes lush vegetative growth, may reduce seed yield and suppress nitrogen fixation. Also application of a phosphate fertilizer is usually beneficial.

### 2.4.3 Weed Control

*Striga gesnerioides* and *Alectra spp.* are principal parasitic weeds that attack cowpeas, particularly in the semiarid regions (www.daff.gov.za). The most common *Striga* species that are pest to cowpea include *S. hermonthica, S. asiatica and S. gesnerioides.* Control of *Striga* is difficult and time consuming. Weedicides can be used to control *Striga* infested fields but currently, chemical control is not recommended, as the chemicals are expensive, handling them is very difficult, not environmentally friendly and no research results are available to support chemical treatment. Increase in soil fertility also affect *Striga* infestation; more fertile soils are less infested with *Striga***.** Mechanical control of the infested areas before *Striga* sets seeds is the most important control method. Annual grasses and other broadleaf weeds can be controlled by application of herbicide. Preplant tillage can reduce early weed pressure and the use of cover crops. Cowpea row cultivation may also be necessary, depending on the weed pressure, soil conditions, and rainfall.

## 2.5. Environmental Requirements of Cowpea

Patel and Hall (1990) indicated that cowpea can yield satisfactorily under greater diversity of climatic, soil, and cultural conditions than other leguminous crops. Cowpea is a warm season crop grown between $35^0$ N and $30^0$ S of the equator. The crop is much more tolerant to high temperature and extended drought periods than other Phaseolus beans, which are largely confined to higher elevations (Massey *et al.*, 1998). The optimum temperature required for successful cowpea production is $20^0$ – $35^0$C. It germinates rapidly at temperatures above $65^o$ C; temperatures below $20^0$C slow germination. Day length and temperature interact with genotype and other aspect of the environment to determine yield potential of seed legumes through their effects on duration of the vegetative and reproductive growth stages (Hadley *et al.*, 1983; Wien and Summerfield, 1984). Many cowpea genotypes exhibit heat-induced suppression of floral bud development, which results in a two-week delay in flowering when plants are grown in very hot field environments under long days (Warrag and Hall, 1984a, b; Patel and Hall, 1990), so developing improved cultivar for hot environments requires an understanding of genetic variation for these responses (Patel and Hall, 1990). Cowpeas are planted under both irrigated and unirrigated regimes (Davis *et al.*, 1991). Even though, cowpea can be grown in any soil, fertile loose soil rich in humus is required for large pods. Cowpea is well adapted to a wide range of soil conditions. It requires well-drained sandy loams or sandy soils which are less restrictive to root growth where the soil pH is in the range of 5.5 to 6.5 (Davis *et al.*, 1991).

## 2.6 Cowpea Production Constraints

### 2.6.1 Biotic Stress

#### 2.6.1.1 Diseases

Sufficient production of cowpea has been dwindled by several factors, but most importantly it is due to prevalence and persistence of pest and diseases. Cowpea is susceptible to a wide variety of pests and pathogens that attack the crop at all stages of growth (Allen, 1983). Cowpea is attacked by over 35 major diseases caused by viruses, bacteria, fungi, and nematodes (Lin and Rios, 1985; Patel, 1985). Symptoms of many diseases vary and include the rapid death of young succulent plants, discoloration of taproots, stunting, wilting and poor yields. Several viral diseases attack cowpea, more than 20 viruses have been identified which infect cowpea under field or experimental conditions worldwide (Thottappilly and Rossel, 1985; Mali and Thottappilly, 1986). Cowpea aphid-borne mosaic virus (CABMV) is considered an important constraint on cowpea crop in all agro-ecological zones, wherever it is grown (Emechebe and Lagoke, 2002; Bashir *et al*., 2002). Singh *et al*., (1984) reported that two bacterial diseases, bacterial pustule (*Xanthomonas spp*.) and bacterial blight (*Xanthomonas vignicola*), also cause severe damage to cowpea worldwide. *Cercospora* leafspot, brown blotch, *Septoria* leaf spot and scab are the most common fungal diseases (Abadassi *et al*., 1987). Cowpea Wilt caused by *Fusarium Oscysporium*, is also another important disease of cowpea. The best control of Fusarium wilt is by the use of a resistant cultivar. Most of these diseases cause severe losses and some can be as high as 90% (IITA, 2000). Most fungal and viral diseases can be reduced or controlled by planting certified seeds of resistant varieties, removing virus infected plants, treating high quality seeds coated with fungicides and controlling weeds.

**2.6.1.2. Insects**

Insect pests belong to the major biotic stresses in cowpea growing regions in both developing and developed countries (Dauost *et al*., 1985). Under severe infestation, 100% yield loss can be been observed (Singh and Allen, 1980). Some of the major insect pests of cowpea are aphids [*Aphis craccivora* Koch (Homoptera: Aphididae)], thrips (*Megalothrips sjostedti*), cowpea weevil (*Callosobruchus maculatus*), cowpea cuculus (*Chalcodermus sermus*), and the Southern cowpea weevil (*Mylabris quadrimaculatus*). In many cowpea growing areas, aphids are the major causing factor for significant yield losses. Early infestation, especially during seedling stage, often results in total crop failure. Their feeding especially on the fruiting stems reduce the amount of nutrients available for pod and seed development. Foliage infested with aphids turns yellow and die. Also due to thrips infestation, tremendous yield losses have been reported in Tanzania, Ghana, Cameroon and Nigeria (Ezueh, 1981; Price *et al*., 1983; Ta'Ama, 1983). Thrips (*Megalurothrips sjostedti*) are small, opportunistic and ubiquitous insects of often only a few millimeters length and generally yellow, brown or black in color (Morse and Hoddle, 2006). Flower abortion is of normal magnitude in plants that are infested with thrips. Flower damage by thrips is characterized by a distortion, malformation and discoloration of the floral parts. Thrips also feed on the terminal leaf bud and bracts/stipules and cause deformation (Ezueh, 1981). The most important post-harvest storage pest of cowpea is Cowpea weevil [*Callosobruchus maculatus* (Fabricius)]. The adult emerge after harvest in the store where real destruction happens due to re-infestations and easiness of larvae penetration into the seed because usually the seeds are stored after shelling (Booker, 1967).

### 2.6.2. Abiotic Stress

#### 2.6.2.1. Drought Stress

Linsley *et al.* (1959) defined drought as a sustained period of time without significant rainfall. Drought stress occurs when water uptake from soil cannot balance water loss through transpiration (Levitt, 1980). Agricultural drought occurs when there is not enough moisture available at the right time for the growth and development of crops. As a result, yields and/or absolute production decline (Glantz, 1987). Drought may start at any time, last indefinitely and attain many degrees of severity. It can occur in any region of the world, with an impact ranging from slight personal inconvenience to endangered nationhood

(Hounam *et al.*, 1975). Water stress is the most important environmental variable affecting plant growth and drought as one of the most important factors threatening the food security of the world (Baker, 1989). Leaf water potential reduces as transpiration occurs as a result of high temperature during drought periods. This reduced water potential is then carried down to the roots through the xylem. The soil water potential then decreases because of osmosis into the roots (Raven *et al.*, 1992; Eichhorn, 1992). Less water is absorbed which limits the vegetative growth resulting in low plant yields as a result of a smaller water potential gradient between the root and the soil. Drought does not only affect the yield, but also the quality of the grain and also the appearance of the plant. Hiler *et al.*, (1972) working on drought stress of cowpea found that the flowering stage is the most susceptible to severe imposed stress (-14 to -28 bars leaf water potential ), while Summerfield *et al.*, (1974) found that stress during the vegetative stage irreversibly reduced leaf area and caused significant yield decline.

#### 2.6.2.2. High Temperature

All plant metabolic processes are irreversibly damaged if high enough temperatures are imposed for sufficient time (Hall and Patel, 1985). Resistance to the stress caused by high temperatures requires that limiting plant processes are not irreversibly damaged. For cowpeas, considering the natural variation in temperatures that occur in the tropics and subtropics and studies on cowpea response to temperature (Warrag and Hall, 1984a, b), it can be concluded that high temperatures at night can be much more damaging to grain yield of cowpeas than high temperatures during the day. Also studies conducted by Warrag and Hall (1984b) in growth chamber and field demonstrated that the temperatures that commonly occur at night in the tropics can cause male sterility and substantially reduce grain yield by increasing floral abscission and decreasing the pods/m².These studies showed that increases in night temperature caused 4 – 14 % decreases in both pod set and grain yield for each degree celsius (ºC) above a threshold of 16 ºC. The main mechanism for these effects on cowpea is that high temperatures occurring in the late night during flowering can cause pollen sterility and indehiscence of anthers (Hall, 1992; 1993).

**2.7. Breeding for Insect Pest Resistance.**

Insect pest is a major problem in cowpea cultivation (Singh and van Emden 1979; Daoust *et al*., 1985). Due to the wide genetic variability of cowpea, much emphasis has been placed on the identification and development of insect-resistant cultivars (Singh and Jackai, 1985, Oghiakhe *et al*., 1992). Therefore, developing cultivars with sustainable resistance to insects is a key objective to many breeders worldwide.

The problem of insect infestation and damage is easily controlled by treatment with insecticides in the developed world while in many parts of 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 (Jackai and Adalla, 1997). Also, the use of insecticides is an environmental and human safety concern. Furthermore the imposition of new and significantly more stringent restrictions on the use of some popular insecticides is likely forthcoming and therefore alternative approaches to insect control are needed, especially for cowpea, where the number of registered products for use is low (Timko, 2008).

The development of insect-resistant cowpea cultivars would have a significant impact on yield and food availability and nutritional status in many regions. Pandey *et al*. (1995) have reported TVu 908 to be resistant to leaf beetles and Singh *et al*. (1996) also have reported several improved cowpea varieties with combined resistance to aphid, thrips, and bruchid. Achieving this goal will not be easy since cowpea is attacked by a large number and diversity of insect pests throughout its life-cycle and attack by any one of the major pests can be devastating. Resistance to multiple pests would therefore have to be developed to positively influence seed production/ yield without the use of insecticides. For example, if cultivars were developed with a high level of resistance to flower thrips, capable of protecting their floral buds from damage, any resulting flowers and pods on these plants would likely be destroyed by pod bugs and pod borers. However, resistance to individual pests can reduce the number of sprays needed to obtain optimal yields and would generally increase yields without insect protection.

### 2.7.1 Mechanism of Insect Pest Resistance

Maxwell and Jennings (1980) defined insect resistance as "those heritable characteristics possessed by the plant which influence the ultimate degree of damage done by insects". Resistance is relative and is measured by using susceptible cultivars of some species as controls. Additionally, host-plant resistance may be the result of a series of interactions between insects and plants which influence the selection of plants as hosts and the effects of plants on insect survival and multiplication. Host-plant resistance to insect pest damage is the most economically and environmentally sound method of pest management for both large scale and subsistence cowpea production. According to Hill and Walter (1982) resistance to pest attack is characterized by a lower pest population density or fewer damage symptoms on the resistant plants. There are three mechanisms within the context of plant resistance that influence the ability of a plant to grow productively in the presence of an insect. They include non-preference (or antixenosis), antibiosis and tolerance (Painter, 1958). However, sometimes it is not easy to make a clear distinction between antibiosis and antixenosis (Smith, 2005).

#### 2.7.1.1 Non-Preference or Antixenosis

Antixenosis is a physical or chemical property of a plant that makes it unpalatable such that it is largely protected from herbivore attack. It includes insect responses to plant characters that make a cultivar undesirable for use by insect as site for reproduction, food, shelter or any combination of the three. Kogan and Ortman (1978) proposed antixenosis to describe more accurately the term of non-preference of insects for a resistant plant. The plant characters that influence non-preference include colour, light reflection, type of pubescence, leaf angle, odour, taste, tough epidermis that do not provide the pest with a desirable feeding substrate and may also involve the presence of feeding repellents (or the absence of feeding attractants). Yellow-green varieties of pea are less palatable to the pea aphids than the blue-green ones (Painter, 1951).

The aphid of cabbage is attracted most to plants with leaves that reflect low intensities of light. Onion thrips are most prevalent on cultivars that have a small angle of separation between the leaves where the thrips live. Also Soybeans without pubescence can be extensively damaged by the potato hopper while those with pubescence seem to be unaffected. Resistance to grasshoppers in maize and sorghum seems to be related to taste (Fehr, 1987). Singh *et al*. (2002) suggested that cowpea varieties with pigmented calyx, petioles, pods and pod tips suffer less damage from *Maruca vitrata*.

#### 2.7.1.2 Antibiosis.

Antibiosis is when plants produce a wide variety of defensive compounds (allelochemicals) that protect the plant tissues from insects. These compounds may reduce growth, inhibit reproduction, alter physiology, delay or prolong maturation, or induce various physical or behavioral abnormalities in the insect (Painter, 1951). The defensive chemicals can also operate by a number of mechanisms; they can be toxins, antifeedants, or can prevent the insect from recognizing the plant tissue as a suitable food source or substrate for oviposition (Taiz and Zeiger, 1991; Gatehouse, 1991). Antibiosis effects are expressed in terms of weight and size of insects, sex ratio and proportion of insects entering into diapauses (Basandrai *et al*., 2011). By purposely selecting for plants with high levels of allelochemicals, or by breeding such plants with less resistant ones, it is often possible to develop new cultivars that resist pest injury yet retain desirable horticultural characteristics. Antibiosis is considered by some to be the only true form of insect resistance in plants. Two soybean varieties (Dowling and Jackson) were confirmed to have antibiosis as a category of resistance to *A. glycines* Li *et al.* (2004) and He *et al*. (1995) conducted studies of resistance to *A. glycines* in soybean fields and observed that resistant cultivars had much lower populations, were less preferred for feeding and habitat, and were more tolerant than susceptible varieties. Koona *et al*. (2002) also reported TVnu 151 to exhibit antibiosis resistance to nymphs of *Clavigralla tomentosicollis.*

#### 2.7.1.3 Tolerance

A tolerant cultivar is able to grow and reproduce in spite of supporting a population of insects similar to a population that would damage a nontolerant host. Some plant genotypes are simply able to "tolerate" injurious insects better than others. Tolerant cultivars may be exposed to the same pest populations as susceptible ones, but they do not suffer as much injury. Tolerance differs from non-preference and antibiosis in its mechanism: non-preference and antibiosis require an active insect response or lack of response.

However, tolerance is more subject to variation as a result of environmental conditions than non-preference and antibiosis. The age or size and general vigor of the plant and size of the insect-resistant population also strongly influence the degree of tolerance.

## 2.8 Artificial Hybridization

Artificial hybridization between parental genotypes is the first step to initiate segregating populations for breeding varieties. Cowpea is cleistogamous, producing viable pollens and receptive stigma before anthesis. This phenomenon imposes entirely self-pollination on the crop. The objective of hybridization is to combine desirable genes found in two or more different varieties and to produce pure-breeding progeny superior in many respects to the parental types. However, for genetic improvement purpose, hand or artificial pollination is necessary. The success of artificial pollination has been reported to be low ranging from 0.5 to 50% (Rachie *et al*., 1975) and varies with genetic and physiological factors as well as the care taken in handling floral parts during the process of emasculation. The wild and weedy subspecies of cowpea hybridize easily with the cultivated forms and produce viable hybrids (Baudoin and Maréchal, 1985; Ng, 1990). But according to Rawal *et al*. (1976), the wild form could only be used as the male parent and attempts to use it as the female parent were unsuccessful.

### 2.9 Heritability

Heritability is generally expressed as the proportion of the observed total variability that is genetic. That is selection of superior genotypes is proportional to the amount of genetic variability (Obilana and Fakorede, 1981). In other words, heritability serves as a guide to the reliability of phenotypic variability in any selection programme and hence determines its success (Hamdi, 1992). Heritability is often used in reference to the resemblance between parents and their offspring. In this context, high heritability implies a strong resemblance between parents and offspring with regard to a specific trait, while low heritability implies a low level of resemblance (Wray and Visscher, 2008).

In plant breeding, type of selection to be done and progress from selection for a particular character depends in part on the magnitude of heritability estimates. This is because the expected response under selection is a function of heritability, variation and selection intensity (Morakinyo, 1996).

The proportion of phenotypic differences due to all sources of genetic variance is termed broad sense heritability ($h_b^2$) whereas the proportion of phenotypic variance due solely to additive genetic variance is narrow sense heritability ($h_n^2$) (Plomin, 1990). Techniques for estimating heritability in crop plants fall into three main categories: parent-offspring regression, variance components from an analysis of variance and approximation of non-heritable variance from genetically uniform populations to estimate total genetic variance (Warner, 1952).

According to Mammud and Kramer (1951) heritability estimates based on regression were higher than those based on variance components. The method involves regressing the 20 mean values of characteristics in the progeny on the value for the same characteristics in the parent. However regression on mid-parent gives better precision than regression on one parent (Falconer, 1989).

### 2.10 Maternal Effect

Variation in an individual's phenotype may be determined not only by the genotype and environment of that individual but also by maternal effects, that is, the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent (Roach and Wulff, 1987). Maternal effect results in the production of difference between reciprocal crosses, which are shown between the offspring of both sexes in all the generations where they occur. Maternal effects are controlled by nuclear genes of the mother and are different from extra nuclear inheritance.

The importance of maternal effects has long been recognized by quantitative geneticists (Dickerson, 1947), although they have largely regarded them as non-genetic environmental sources of resemblance of relatives (Falconer and Mackay, 1996; Futuyma, 1998) and a nuisance that contaminates estimates of heritability (Wade, 1998). It may also result in the expression of previously unexpressed genes in the offspring that have significant phenotypic effects on their fitness (Maestripieri, 2005).

Non-genetic maternal effects provide a mechanism for cross-generational phenotypic plasticity and make a significant contribution to an organism’s fit with the environment (Bernardo, 1996; Mousseau and Fox, 1998a, b). By modifying the offspring’s phenotype or inducing the expression of new phenotypic traits, non-genetic maternal effects can also allow offspring to colonize new ecological niches and be exposed to new selective pressures.

## 2.11 Heterosis

Heterosis or hybrid vigor is referred to as the superiority of a hybrid over the mean of its two homozygous parents (Shull, 1908). Acquaah (2007) defined heterosis in two basic ways: better-parent heterosis and mid-parent heterosis. Better-parent heterosis is calculated as the degree by which the $F_1$ mean exceeds the better parent in the cross. Mid-parent heterosis is defined as the superiority of the $F_1$ over the means of the parents. Breeders utilize available genetic resources to modify varieties to meet the ever changing requirements. Heterosis in self- pollinated crops cannot be exploited directly and therefore hybrid vigor is used to identify superior hybrids as they offer more probability of developing better segregants (Sharif *et al.*, 2001).

Genetic explanation for heterosis has been made on the basis of three main hypotheses. The dominance hypothesis explains heterosis due to cumulative effect of favorable alleles with partial to complete dominance (Davenport, 1908; Bruce, 1910; Jones, 1917). However, pseudo-overdominance may occur due to repulsion phase linkages of such genes. The overdominance hypothesis attributes heterosis due to superiority of heterozygous genotypes over both parental homozygous genotypes (Hull, 1945; Crow, 1948). Two terms are routinely used in discussing models of heterosis (Birchler *et al*., 2010). The “dominance” model, in which recessive alleles at different loci are complemented in the hybrid, and the other is the “over-dominance” model, which posits that interactions between different alleles occur in the hybrid, leading to the increase in vigor.

## 2.12 Resistance to Cowpea Aphids (*Aphis craccivora* Koch)

The identification of sources of resistance to aphids that can be used in breeding programs to develop resistant cowpea lines is therefore necessary to ameliorate the situation.

Benchasri *et al.* (2007) reported that cowpea IT82E-16 displayed a high level of resistance after evaluating 24 yardlong bean and cowpea genotype for cowpea aphid resistance. Singh *et al*. (1996) reported several improved cowpea varieties with combined resistance to aphid, thrips, and bruchid. Nkansah and Hodgeson (1995) confirmed resistance of TVu 801 and TVu 3000 to the Nigerian aphid strain but found that the two lines were susceptible to aphids from the Philippines. Pathak (1988) studied the genetic resistance of cowpea aphid and reported that the cowpea aphid resistance was conferred by a single dominant gene, designated as $Rac_1$ and $Rac_2$. Ombakho *et al*., (1987) also studied in $F_1$ and $F_2$ generation of cowpea (TVU 310, ICV10 and ICV 11) and reported that resistant gene in TVU 310 and ICV 10 were designated by $Ac_1$ while resistant gene in ICV11 was $Ac_2$. However, plant reactions to insect attack may depend on plant genotype, insect biotypes and environmental factors.

### 2.13 Cowpea Aphids (*Aphis craccivora* Koch)

Cowpea aphid, *Aphis craccivora* Koch, commonly referred to as the black aphid, is an important pest of cowpea in most tropical areas where cowpea is grown (Obopile and Ositile, 2010). In West Africa, during the last decade, aphid populations have continuously increased, consequently causing major losses (Singh *et al.*, 1990). Several researchers have reported that aphid population dynamics are significantly influenced by environmental factors, such as temperature (Ruggle and Gutierrez, 1995; Diaz and Fereres, 2005). The adult aphid is relatively small (1.5 - 2.5 mm long) and usually shiny black, while nymphs are smoky gray and waxy. The adult may be winged (alate) or wingless (apterious) and when present, the wings are large and transparent, bearing few veins. Also, when viewed under magnification, the bottom half of the antennae and legs are light-colored or creamy white with blackish tips. Apterae and alate forms are always females that in asexual reproduction give birth to live young aphids and colonies entirely of females. Alate adult are produced whenever the aphids are subjected to stress, for example overcrowding, limited food supply and fluctuating temperature (Dixon, 1985; Obopile and Ositile, 2010; Whitworth and Ahmad, 2009). Eggs develop within the mother and nymphs are born alive. Within few days, nymph matures into reproductive adults and population density increase rapidly. Aphids primarily infest tender young seedlings, although large populations also infest succulent green stems, flower buds, flowers and pods. The damage is caused by both adults and nymphs and is either direct through depleting plants assimilates through sucking and through injection of its toxic saliva to the plant or through transmission of virus particles that in turn cause disease to the plant. They have been implicated as the main vectors of the nonpersistent cowpea aphid-borne mosaic virus (Atiri *et al.*, 1986). Small aphid populations have no major impact on cowpea production, but large populations can cause leaf distortion, stunting and poor nodulation of root systems (Singh and Jackai, 1985). Yield is reduced and, in extreme cases, the plant is killed (Singh and van Emden, 1979). An indirect and generally the most harmful effect, even at low population densities, is the transmission and spread of legume viruses, which severely reduce yield (Singh and van Emden, 1979). At least 14 legume viruses are transmitted by *A. craccivora* (Thottappilly *et al.*, 1990).

**2.14. Advances in Breeding for Cowpea Aphids Resistance**

Considerable progress has been made in the past years in developing cowpea varieties resistant to aphids. Singh *et al*. (1996) reported several improved cowpea varieties with combined resistance to aphid, thrips, and bruchid. Attempts to improve cowpea aphids resistance through conventional breeding programs have met with limited success because aphid resistance is a genetically complex trait. The use of molecular markers to identify and locate different genes and genomic regions possessing factors which influence resistance in cowpea will help to gain insight into the complex trait of aphid resistance. In addition, these markers can be used to select for multiple traits and combine genes underlying these traits in cultivars with improved aphid resistance. These properties and prospects have initiated an increased interest in the application of Marker-Assisted Selection (MAS) for improving pest resistance in many crops including cowpea.

### 2.15. Transgenic Cowpea

Traditional plant breeding has made only limited progress in breeding for resistance to the major insect pests of cowpea and "new genes" are apparently needed to protect cowpea. Until recently cowpea remained one of the last major grain legume species for which an efficient genetic transformation/regeneration system had not been developed (Van Le *et al.*, 2002; Avenido *et al.*, 2004; Popelka *et al.*, 2004), despite substantial efforts for more than ten years by several groups of researchers (Machuka, 2002a; Machuka *et al.*, 2002). Ikea *et al.* (2003) reported the successful genetic transformation of cowpea using the particle-gun bombardment of shoot meristems. They were able to isolate several plants in the T3 generation that showed strong expression of the transgene "bar" that confers resistance to the herbicide Basta, but these studies were inconclusive. An efficient and stable cowpea transformation/regeneration system has been developed recently (Popelka *et al.*, 2006), so that transgenic cowpea is now a reality.

Transgenic approaches should be undertaken to develop varieties of cowpeas with strong resistance to insect pests. Insect-resistant cowpeas would dramatically increase cowpea productivity in many developing countries and reduce costs, safety hazards, and environmental risks in virtually all cowpea producing countries.

## CHAPTER THREE

## 3.0 Materials and Methods

### 3.1. Study Site

The experiment was conducted at the research field of Crops Research Institute (CSIR-CRI), Fumesua, Ghana. Fumesua is located within latitude 6$^o$, 41 North and 1$^o$, 28 West. The area is characterized by a bimodal rainfall distribution with the major season rains around April to June and minor season rains also from August to November with annual rainfall of 1,345 mm per annum. The vegetation is that of humid forest with soil Ferric Acrisol Asuansi series type (Adu and Asiamah, 1992). The temperature is usually high throughout the year with annual mean temperature between 22$^o$C to 31$^o$C. The land was prepared by hand weeding, followed by application of glyphosate at the rate 900 ai per hectre.

### 3.2 Experimental Materials

Four cowpea genotypes were collected from Council for Scientific and Industrial Research (CSIR) – Crops Research Institute (CRI), Fumesua for the experiment. The genotypes comprised two cowpea aphids resistant genotypes; Hewale and Asomdwee and two cowpea aphids susceptible genotypes; Asetenapa and Videza. The four genotypes were used to generate four $F_1$ genotypes. The $F_1$ genotypes were selfed to generate $F_2$ genotypes. The aphids used for the experiment were reared or maintained on the susceptible varieties on the field at Crops Research Institute (CRI), Fumesua.

### 3.2.1 Genotype 1 - Asetenapa

Asetenapa is a medium maturing (65 – 70 days) variety with semi-erect growth habit, medium-size broad leaves, and long upright peduncles. It has medium-size seeds (16 g/100 seeds) with smooth white shiny testa and black hilum. It has a mean grain yield of about 1,023 kg/ha and a potential of 1.8 tons/ha. It has 29.75% protein and 1.91% oil. In spite of all the important attributes, Asetenapa has been observed to be susceptible to cowpea aphids at CRI, Fumesua in the Ashanti Region of Ghana. Farmers and consumers however like Asetenapa because of its shorter cooking time, and excellent taste.

### 3.2.2 Genotype 2 – Hewale

Hewale is a variety released by Crops Research Institute (CRI), Fumesua that shows some level of resistance to cowpea aphids (*Aphis craccivora*). It is an early maturing variety which flowers between 40 to 60 days and matures between 64 to 72 days after planting (DAP) with semi-erect growth habit and small-size seeds with brown testa. It also has a potential grain yield of 3130 kg/ha.

### 3.2.3 Genotype 3 – Videza

It is also an early maturing variety from Crops Research Institute (CRI), Fumesua. The preferred ecologies for the growth of this variety are the forest transition and savanna. It is an early maturing variety with 43 to 47 days to flower and matures within 65 to 72 days after planting. It has semi – determinate growth pattern and semi erect growth habit. It has a potential grain yield of 3043 kg/ha however Videza has been observed to be susceptible to cowpea aphids.

### 3.2.4 Genotype 4 – Asomdwee

It is a cowpea variety also released by Crop Research Institute (CRI), Fumesua. It has semi – erect growth habit and white flower colour. It has a semi - determinate growth pattern with 40 – 60 days to flowering and matures within 65 to 72 days. Asomdwee has been observed to be aphids resistant and has a potential grain yield of 2863 kg/ha.

## 3.3 Methodology

The experiment was conducted in three stages. The first and second stages involved the generation of the $F_1$ seeds and $F_2$ seeds. The two stages were carried out under full insecticide protection from May to November, 2014 on the field. The third stage involved the evaluation of the parents and the generated genotypes on the field under artificial infestation. The aphids for the study were reared on the susceptible varieties and introduced onto the experimental materials using camel's hair brush.

### 3.3.1 Stage 1

To prevent different flowering periods and synchronize the period of flowering, the planting dates of the parental genotypes were staggered. The late maturing genotypes Asetenapa and Asomdwee were planted seven (7) days before planting the other two genotypes Hewale and Videza. The four parental genotypes were grown and direct and reciprocal crosses made to produce $F_1$ seeds and their reciprocal $F_1$ seeds. i.e. $F_1$ (Asetenapa × Hewale), $F_1$ (Hewale × Asetenapa), $F_1$ (Videza × Asomdwee) and $F_1$ (Asomdwee × Videza). A planting distance of 20 cm within plants and 60 cm between rows were used. Four seeds were planted per hole and thinned to two plants per stand two weeks after planting. The plants were sprayed six times at 10 days after planting (DAP), 20 DAP, 30 DAP, 40 DAP, 50 DAP and 60 DAP to control aphids (*Aphis craccivora*), flower bud thrips (*Megalurothrips sjostedti*) and pod sucking bugs. The insecticides used were Lambda (600 ml/ha) and Sunpyrifos (1000 ml/ha) for pre- and post-flowering insect pests control. Weeds were controlled by weedicide application and hand weeding when necessary throughout the growing period of the plants.

### 3.3.2 Stage 2

In the second stage, the four $F_1$ populations (two direct $F_1$ seeds and two reciprocal $F_1$ seeds) were planted and allowed to self-pollinate to produce $F_2$ progenies. The seeds generated at the second stage were as follows:

1. $F_2$ (Asetenapa × Hewale)
2. $F_2$ (Hewale × Asetenapa)
3. $F_2$ (Videza × Asomdwee)
4. $F_2$ (Asomdwee × Videza)

### 3.3.3 Stage 3

In the third stage, the $F_1$ seeds, $F_2$ seeds and the parental seeds were planted in randomized complete blocks with three replications to evaluate for resistance to cowpea aphids (*Aphis craccivora)* on the field. The aphids for the study were maintained on the two susceptible varieties, Asetenapa and Videza. The aphids were transferred onto the experimental materials using a camel's hair brush two weeks after planting. Each replicate consisted of twelve treatments, one plot of each of the four parents, four $F_1$ genotypes (direct seeds and reciprocal $F_1$ seeds), four plots of direct $F_2$ seeds and reciprocal $F_2$ ($RF_2$) seeds. Each plot of the parents and $F_1$ genotypes were made up of a row, 2 meters long with 60 centimeters between rows and 20 centimeters within plants giving 10 plants per row. However, $F_2$ genotypes consisted of four rows, two meters long with 60 cm between rows and 20 cm within rows. Weeds were controlled by hand pulling and hoeing at 14 DAP, 32 DAP and 57 DAP.

**3.4 Crossing Procedure**

The four parental genotype, two resistant genotypes and two susceptible genotypes were planted in the field. The early maturing genotypes were planted seven (7) days before planting the other genotypes to synchronize the flowering period. The flowers of the un-opened buds were used as female parents and the fully opened flowers were also used as the male parent. Emasculation was done early in the morning between (6:00 am – 9:00 am) with sharply pointed forceps sterilized with alcohol to prevent contamination by unwanted pollen. The flowers that were used as a source of pollen were held between the thumb and the forefinger with the standard and wing folded back to expose the pollen. The pollen of the flowers was applied to the stigmatic surface of the emasculated flowers. Tags indicating the cross and date were affixed to the raceme beneath the pollinated bud to identify the cross and date of cross.

**3.5 Parameters Measured**

1. Days of 50% emergence
2. Plant vigour
3. Days to 50% flowering
4. Number of aphid colonies per plant
5. Damage Leaves scoring
6. Percentage (%) severity of aphid infestation

### 3.6 Aphids Population and Damage Rating

The number of aphids colonies per plant were estimated by using a scale of 0 – 5 and visual damage were also assessed for each treatment. Number of aphid colonies were scored two weeks after the artificial infestation to five weeks after planting. Extent of damage on leaves were also assessed between a range of 0 to 5. (Table 3.1)

**Table 3.1 Score of aphids colonies per each generation (Smith *et al*., 1994)**

| Score | Description |
|---|---|
| 0 | no aphids colonies |
| 1 | a few aphids colonies |
| 2 | few small individual colonies |
| 3 | several small colonies, |
| 4 | large individual colonies |
| 5 | large continuous colonies |

### 3.7 Heritability and Heterosis Estimates

Broad sense heritability was calculated using Allard (1960)'s formula of

$$H^2_b = \frac{V_P - V_E}{V_P}$$

Where $V_P$ = Phenotypic Variance and $V_E$ = Environmental Variance

$$V_E = \frac{(P1+P2+F1)}{3} \quad \text{and} \quad V_P = V_{F2}$$

Mid-parent heterosis was estimated as the percentage deviation of the mean $F_1$ value from the mid-parent value.

### 3.8 Statistical and Genetic Analyses

The statistical package used was Genstat discovery edition (version 12). Data for days to 50% germination, aphid population, plant vigour, damage rating, days to 50% flowering and percentage severity of aphids infestation were subjected to analysis of variance (ANOVA). Least Significant Difference (LSD) test at 5% was applied to separate difference in means. Restricted Maximum Likelihood (REML) variance components analysis which involves the use of mixed models approach to test the significance of week factor, generation factor and interaction between week and generation was used to analyze data for aphid colonies and damage rating taken weekly. Chi-square ($\chi^2$) test was performed to test the goodness of fit to a 3:1 ratio in the $F_2$ population. The segregation ratio of resistance to susceptible was calculated by the formula

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

Where O = observed values, E = expected values

Also relationships between traits were analysed using Pearson's product-moment correlation. The Pearson Correlation was used to measure relationship between number of aphid colonies, leaf damage and percentage (%) severity.

# CHAPTER FOUR

# 4.0 RESULTS

## 4.1 Days to 50% emergence and days to 50% flowering in all generations

From Table 4.1, days to 50% emergence ranged from five to six days. No significant ($p \geq 0.05$) differences were observed for days to 50% days of emergence among the parents and the $F_2$ generations. However, significant ($p \geq 0.05$) values were observed between the $F_1$ generations, parents and $F_2$ generations. There was no significant difference between the direct and reciprocal crosses of the $F_1$ generations, same as that of the direct and reciprocal crosses of the $F_2$ generations. All the $F_2$ generations reached days to 50% emergence within five days after planting and the $F_1$ generations with the exception of $F_1$ (Videza x Asomdwee) reached days to 50% emergence within six days after planting.

A significant ($p \geq 0.05$) difference was observed for days to 50% flowering between Videza and Hewale of the parental genotypes. $F_1$ (Asetenapa x Hewale) was the first genotype to reach days to 50% flowering with mean days of 45.67. There were also significant differences between the $F_1$ generations and the $F_2$ generations. There were significant ($p \geq 0.05$) differences between the reciprocal crosses of the $F_1$ generations, however no significant difference was observed between the direct and reciprocal crosses for the $F_2$ generations.

**Table 4.1 Mean values for days to 50% emergence and days to 50% flowering for all generations.**

| Generation | Days to 50% Emergence | Days to 50% Flowering |
|---|---|---|
| $P_1$ (Asetenapa) | 5.333 | 49.33 |
| $P_2$ (Hewale) | 5.000 | 50.00 |
| $P_3$ (Videza) | 5.333 | 48.33 |
| $P_4$ (Asomdwee) | 5.000 | 49.67 |
| $F_1$ (Asetenapa x Hewale) | 6.000 | 47.33 |
| $F_1$ (Hewale x Asetenapa) | 6.000 | 45.67 |
| $F_1$ (Videza x Asomdwee) | 5.667 | 47.67 |
| $F_1$ (Asomdwee x Videza) | 6.000 | 49.67 |
| $F_2$ (Asetenapa x Hewale) | 5.000 | 48.67 |
| $F_2$ (Hewale x Asetenapa) | 5.000 | 49.00 |
| $F_2$ (Videza x Asomdwee ) | 5.000 | 49.33 |
| $F_2$ (Asomdwee x Videza) | 5.000 | 49.67 |
| LSD (5%) | 0.4582 | 1.36 |
| C. V (%) | 5.0 | 1.7 |

Values are means of three replicates.

### 4.2 Damage score, percentage (%) severity and plant vigour of all generations

From Table 4.2, significant ($p \geq 0.05$) differences were observed among the generations in the damage score, percentage severity and plant vigour. There were no significant differences between the resistant parents and the direct and reciprocal $F_1$ generations for the three parameters with significant differences between the $F_1$ and $F_2$ generations. Damage score for all the generations were intermediate between the four parental genotypes. Hewale recorded the lowest damage score with a mean of 0.244 and the susceptible genotypes Asetenapa and Videza recorded the highest damage score with a mean of 0.389. The mean damage score of the $F_1$ generations were generally lower than the $F_2$ generations and skewed towards the resistant parents with low damage score. The susceptible parents recorded high percentages for aphid severity incidence with Asetenapa recording the highest percentage severity of 7.78. There were no significant difference between the susceptible parents and the $F_2$ generations for percentage severity score. The $F_1$ generations recorded low percentage score with no significant difference between the $F_1$ generations and the resistant parents. Plant vigour decreased slightly across all generations. However a significant difference was observed between the resistant parents and the susceptible parents.

**Table 4.2 Means for damage score, percentage (%) severity and plant vigour in twelve generations of parents, direct and reciprocal crosses**

| Generations | Damage score | % Severity | Plant vigour |
|---|---|---|---|
| $P_1$ (Asetenapa) | 0.389 | 7.78 | 4.767 |
| $P_2$ (Hewale) | 0.244 | 5.11 | 4.911 |
| $P_3$ (Videza) | 0.389 | 7.56 | 4.756 |
| $P_4$ (Asomdwee) | 0.256 | 5.56 | 4.9 |
| $F_1$ (Asetenapa x Hewale) | 0.256 | 5.33 | 4.9 |
| $F_1$ (Hewale x Asetenapa) | 0.278 | 5.56 | 4.878 |
| $F_1$ (Videza x Asomdwee) | 0.289 | 5.56 | 4.878 |
| $F_1$ (Asomdwee x Videza) | 0.256 | 5.33 | 4.878 |
| $F_2$ (Asetenapa x Hewale) | 0.378 | 6.89 | 4.789 |
| $F_2$ (Hewale x Asetenapa) | 0.344 | 7.11 | 4.756 |
| $F_2$ (Videza x Asomdwee) | 0.378 | 7.33 | 4.778 |
| $F_2$ (Asomdwee x Videza) | 0.344 | 7.56 | 4.789 |
| LSD (5%) | 0.094 | 1.89 | 0.081 |
| C.V(%) | 31.7 | 31.6 | 1.8 |

Values are means of three replicates.

**Table 4.3 Means and variances of aphid colonies scores in all generations of direct and reciprocal cowpea crosses**

| Generation | Mean | Variance |
|---|---|---|
| $P_1$ (Asetenapa) | 0.611 | 0.0311 |
| $P_2$ (Hewale) | 0.378 | 0.0194 |
| $P_3$ (Videza) | 0.656 | 0.0278 |
| $P_4$ (Asomdwee) | 0.389 | 0.0111 |
| $F_1$ (Asetenapa x Hewale) | 0.467 | 0.015 |
| $F_1$ (Hewale x Asetenapa) | 0.456 | 0.0153 |
| $F_1$ (Videza x Asomdwee) | 0.444 | 0.0103 |
| $F_1$ (Asomdwee x Videza) | 0.456 | 0.0103 |
| $F_2$ (Asetenapa x Hewale) | 0.567 | 0.0125 |
| $F_2$ (Hewale x Asetenapa) | 0.622 | 0.0269 |
| $F_2$ (Videza x Asomdwee ) | 0.656 | 0.0278 |
| $F_2$ (Asomdwee x Videza) | 0.644 | 0.0178 |

### 4.3 Responses of all generations to aphid infestation

The number of aphid colonies increased from the first week of artificial infestation to the fourth week. The build up of the aphid colonies on the resistant parents, Hewale and Asomdwee and the susceptible parents, Videza and Asetenapa were significant across the weeks. Videza recorded the highest score for aphid colonies across the four weeks with Hewale recording the least score for aphid colonies across the same weeks. The $F_1$ generations, both direct and reciprocal crosses recorded low scores for aphid colonies. The $F_2$ generations had a quarter of its progenies with large aphid colonies and had three quarters with small aphid colonies.

**Table 4.4 Means and standard errors of aphids score across the weeks for all generations**

| | Weeks after artificial infestation (Mean ± SE) | | |
|---|---|---|---|
| Generation | Week 2 | Week 3 | Week 4 |
| $P_1$ (Asetenapa) | 0.367 ± 0.03 | 0.6 ± 0.06 | 0.7 ± 0.06 |
| $P_2$ (Hewale) | 0.233 ± 0.03 | 0.4 ± 0.06 | 0.567 ± 0.03 |
| $P_3$ (Videza) | 0.467 ± 0.03 | 0.467±0.03 | 0.833 ± 0.03 |
| $P_4$ (Asomdwee) | 0.267± 0.03 | 0.433± 0.03 | 0.467± 0.03 |
| $F_1$ (Asetenapa x Hewale) | 0.333 ± 0.03 | 0.467±0.03 | 0.6 ± 0.0 |
| $F_1$ (Hewale x Asetenapa) | 0.333 ± 0.03 | 0.467 ± 0.07 | 0.6 ± 0.0 |
| $F_1$ (Videza x Asomdwee) | 0.333 ± 0.03 | 0.467 ± 0.03 | 0.533 ± 0.03 |
| $F_1$ (Asomdwee x Videza) | 0.367 ± 0.03 | 0.433 ± 0.03 | 0.567 ± 0.03 |
| $F_2$ (Asetenapa x Hewale) | 0.433 ± 0.03 | 0.6 ± 0.0 | 0.667 ± 0.03 |
| $F_2$ (Hewale x Asetenapa) | 0.433 ± 0.03 | 0.667 ±0.07 | 0.767± 0.03 |
| $F_2$ (Videza x Asomdwee) | 0.467 ± 0.09 | 0.633 ± 0.07 | 0.767 ± 0.03 |
| $F_2$ (Asomdwee x Videza) | 0.467 ± 0.09 | 0.667 ± 0.09 | 0.767 ± 0.03 |

## 4.4 Chi square ($\chi^2$) test

The phenotypic $F_2$ generations were tested by using the chi square for the hypothesis of 3: 1 resistant to susceptible ratio. The total of 240 $F_2$ generations were grouped into two categories 184 resistant and 56 susceptible, 176 resistant and 64 susceptible, 182 resistant and 58 susceptible and 178 resistant and 62 susceptible for $F_2$ (Asetenapa x Hewale), $F_2$ (Asomdwee x Videza), $F_2$ (Hewale x Asetenapa) and $F_2$ (Videza x Asomdwee) respectively. The probability for the expected 3resistant:1susceptible segregation in the $F_2$ generations was no significant. The results showed that the segregation $F_2$ generation fitted the 3:1 ratio, an indication that cowpea aphid resistance is controlled by a single dominant gene.

**Table 4.5 The chi-square values and the probabilities of goodness of fit for the expected ratio of 3 Resistant : 1 Susceptible cowpea aphids in the $F_2$ generations.**

| Generation | Resistance | Susceptible | Total | $\chi^2$ | P |
|---|---|---|---|---|---|
| $F_2$ (Asetenapa x Hewale) | 184 | 56 | 240 | 0.36 | 0.548 |
| $F_2$ (Asomdwee x Videza) | 176 | 64 | 240 | 0.35 | 0.554 |
| $F_2$ (Hewale x Asetenapa) | 182 | 58 | 240 | 0.09 | 0.765 |
| $F_2$ (Videza x Asomdwee) | 178 | 62 | 240 | 0.09 | 0.766 |

**4.5 Heterosis estimates**

Heterosis (based on mid-parent value) for aphid resistance are presented in Table 4.6. Negative heterosis values were observed for aphid colonies score in both direct and reciprocal generations. The aphid colonies score for the direct and reciprocal $F_1$ generations were lower than the mid parent score of aphid colonies.

**Table 4.6 Direct and reciprocal cross heterosis for aphid colonies score**

| Generation | Means | ($F_1$ – MP) | $\frac{(F1-MP)}{MP}$ | Heterosis (%) |
|---|---|---|---|---|
| $F_1$ (Asetenapa x Hewale) | 0.467 | -0.0275 | -0.0556 | -5.56 |
| $F_1$ (Videza x Asomdwee) | 0.444 | -0.0785 | -0.1272 | -15.03 |

| Generation | Means | ($RF_1$ – MP) | $\frac{(RF1-MP)}{MP}$ | Heterosis (%) |
|---|---|---|---|---|
| $F_1$ (Hewale x Astenapa) | 0.456 | -0.0385 | -0.0779 | -7.79 |
| $F_1$ (Asomdwee x Videza) | 0.456 | -0.0665 | -0.1273 | -12.73 |

$F_1$ = direct cross  $RF_1$ = reciprocal cross  MP = Mid parent

## 4.6 Broad sense Heritability estimates

Broad sense heritability for cowpea aphid resistance are presented in Table 4.7. (Asetenapa x Hewale) recorded a high heritability value of 74.4% and (Asomdwee x Videza) recorded a low heritability value of 7.87%.

**Table 4.7 Percentage heritability of aphid resistance in cowpea crosses**

| Generation | Heritability (%) |
|---|---|
| | Broad sense |
| Asetenapa x Hewale | 74.4 |
| Hewale x Asetenapa | 18.59 |
| Videza x Asomdwee | 41.0 |
| Asomdwee x Videza | 7.87 |

## 4.7 Correlation between aphid colonies score, damage score and percentage severity

Correlations between aphid colonies score and damage score and aphid colonies score and percentage severity were significant and positive across the weeks. The positive correlation between aphid colonies and damage score and aphid colonies score and percentage severity are indicative of the major effects aphid colonies have on damage score and percentage severity.

**Table 4.8 Correlation between aphid colonies score, damage score and percentage severity across the weeks.**

| | Damage score | Aphid score | Percentage severity |
|---|---|---|---|
| Damage score | - | | |
| Aphid score | 0.7834 | - | |
| Percentage severity | 0.9356 | 0.7669 | - |

# CHAPTER FIVE

## 5.0 DISCUSSION

From the study, no variation was observed between the parental genotypes and the $F_2$ generations for days to 50% emergence and this shows uniformity in seed viability among the populations. All the $F_2$ generations reached 50% emergence within five days after planting and the $F_1$ generations with the exception of $F_1$ (Videza x Asomdwee) reached days to 50% emergence within six days after planting.

$F_1$ (Asetenapa x Hewale) was the first generation to reach 50% flowering with mean days of 45.67. This shows a negative heterosis observed between $F_1$ (Asetenapa x Hewale) and the parents. The gain of four to five days by the hybrid $F_1$ (Asetenapa x Hewale) over the parents is interesting and beneficial as it could allow the hybrid to complete pod filling and escape terminal drought. There were also significant differences between the $F_1$ and the $F_2$ generations. The differences observed in days to 50% flowering between generations could also be a reflection of innate attributes that are associated with the different photoperiodic groups. Variations observed between the direct and reciprocal crosses of the four parental generations for days to 50% flowering is an indication of maternal effect observed between the progenies. The observation in the study conformed to work by Ishiyaku and Singh (2001) that there is a significant contributions of the maternal parent in the inheritance of traits.

Resistance and susceptibility in the study were based on the number of aphid colonies on each plant, damage score, percentage severity of aphid infestation and plant vigour. Differences were also observed among generations in damage score, percentage severity and plant vigour.

Variation in damage score was observed between the resistant and susceptible genotypes. Genotypic differences in damage score were probably related to the inherent resistance of Hewale and Asomdwee and susceptibility of Asetenapa and Videza. The two resistant genotypes recorded low damage scores with the susceptible parents recording high damage scores. All the $F_1$ generations were skewed toward the resistant genotypes with low damage scores. In the $F_1$ generations, damages were mild at first week and progressed slowly across the weeks, but never reached severity scores as high as their susceptible parents. No significant difference was observed between the direct and reciprocal $F_2$ generations for damage score. Asetenapa recorded the highest damage score among all the generations across the weeks with Hewale recording the lowest damage score across the same period. The potential to improve aphid resistance in the susceptible parents (Asetenapa and Videza) by repeated backcrossing method with the resistant parent (Hewale) exists for breeders and should be explored.

The leaves of the susceptible generations in the study turned yellow and became stunted across the weeks and this result was consistent with that of Bata *et al*. (1987).

A positive correlation was also observed between damage score and percentage severity. As the damage score increased across the weeks, the percentage severity across generations also increased across the weeks, with the susceptible parents recording the highest percentage severity infestation at the end of the fourth week.

Plant vigour decreased slightly across the weeks but a significant difference was observed among all generations. This shows that although plant vigour decreased slightly across all generations, the generations responded to plant vigour differently.

The results for number of aphid colonies score for all generations were significant. The progenies produced by artificial hybridization between the resistant parents and the susceptible parents were skewed toward the resistant parents with more than 90% having low number of aphid colonies. All $F_1$ progenies both direct and reciprocal crosses showed some level of resistance like that of the resistant parents. The build up of aphid colonies in all generations increased after the artificial infestation two weeks after planting. Hewale recorded the least number of aphid colonies score across the four weeks period with Videza recording the highest number of aphid colonies score. The experiment was consistent with the report of Salifu *et al.,* (1988b) which studied the resistance of bean flower thrips (*Megalurothrips sjostedti*) in cowpea in Nigeria. They found that the number of thrips increased rapidly and it was statistically significant between susceptible and resistant cultivars of thrips. The study was also consistent with that of Ofuya (1993) who also reported significant differences in number of aphids on susceptible and resistant varieties.

The high number of aphid colonies on Videza which had broad leaves and low aphid colonies on Hewale which also had narrow leaves conformed to that of Wuttiwong *et al.,* (2010) who reported that cowpea aphids tend to be attracted to plants with broad leaves than plants with narrow leaves.

Maternal effect, that is, the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent was absent in number of aphid colonies score. That is regardless of whether the resistant parent was a male or female, the $F_1$ progenies recorded low aphid colonies score as the resistant parents. The absence of significant difference between the direct and reciprocal crosses indicated that genes controlling cowpea aphid resistance were all nuclear genes and that the cytoplasmic genes of the mother had no effect on the inheritance of cowpea aphid resistance. Bata *et al*. (1987) and Pathak (1988) reported that two independent loci *Rac1* and *Rac 2* are involved in the expression of resistance to cowpea aphid.

In the study, the segregating $F_2$ generations fitted the ratio, 3 resistant to 1susceptible. That is three quarters of the $F_2$ generations were observed to be resistant and a quarter of the $F_2$ generations susceptible. This confirms that aphid resistance is controlled by a single allele of the dominant gene. The 3:1 ratio also confirmed Beta *et al.* (1987) report that gene resistance to cowpea aphid involved antibiosis and is conferred by a single dominant gene. The finding was also consistent with Klingler (2005) who reported that aphid resistance in crop plant is often qualitative rather than quantitative.

Even though the resistant generations did not show very strong aphid colonization they appeared to show some level of tolerance.

Mid-parent heterosis defined as the superiority of the $F_1$ over the mean of the parents was estimated. The mean aphid score of the $F_1$ generations were less than the mid-parent value and closer to the mean of the parents with lower aphid score. Negative heterosis over mid-parents was observed for aphid score indicating heterosis in the direction of the resistant parent (parent with low aphid score). There was no statistically significant difference observed between the direct and reciprocal crosses for heterosis. A slightly significant difference was observed for heterosis between crosses of (Asetenapa x Hewale) and (Videza x Asomdwee).

Broad sense heritabilities for cowpea aphid resistance were estimated for both direct and reciprocal crosses. A high heritability estimate was observed between (Asetenapa x Hewale) and (Videza x Asomdwee) indicating low effect of the environment on the trait and a strong resemblance between resistant parents and offspring with regard to aphid resistance. According to Ubi *et al*. (2001), heritability estimates along with genetic advance are more useful in predicting the resultant effect for the selection of the best individuals from a population. The results however, were consistent with Omo-Ikerodah *et al*. (2009) who found high broad sense heritability for flower bud thrips resistance.

# CHAPTER SIX

# 6.0 CONCLUSION AND RECOMMENDATION

## 6.1 Conclusion

The study was carried out to determine the inheritance of cowpea aphid resistance, the genetic control of the resistance, heritability of the resistance trait and whether maternal effects exist for the resistance. From the study the following conclusions can be drawn:

1. Statistically significant difference was observed between the parents for aphid score, damage score, plant vigour and percentage severity.
2. No significant differences were observed between resistant parents and all the $F_1$ generations. There was no significant difference between the direct and reciprocal crosses for all the parameters indicating the absence of maternal effects.
3. The segregating $F_2$ generations fitted the expected 3 resistant to 1 susceptible ratio.
4. The study showed that cowpea aphid resistance is controlled by monogenic inheritance with resistance dominant over susceptibility.
5. Negative heterosis over mid-parents was observed for aphid score indicating heterosis in the direction of the better parent (parent with low aphid score).
6. A high broad sense heritability estimate for aphid colonies was observed between (Asetenapa x Hewale) indicating low effects of the environment on the trait.
7. It is possible to improve aphid resistance in susceptible cowpea lines by backcross method.

## 6.2 Recommendation

Marker assisted selection to enhance the efficiency and effectiveness of cowpea aphid resistance breeding should be carried out. Also similar work should be carried out in the dry season to study the number of aphid colonies on both resistant and susceptible parents. Correlation between the trait for aphid resistance and yield should further be researched into. Caged experiments should be conducted to control aphid populations on plants and confirm reactions on resistant parents and the progenies.

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APPENDIX

**APPENDIX 1**

Analysis of variance

Variate: Days to 50% Plant Emergence.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 2 | 0.38889 | 0.19444 | 2.66 | |
| REP.*Units* stratum | | | | | |
| TREATMENT | 11 | 6.30556 | 0.57323 | 7.83 | <.001 |
| Residual | 22 | 1.61111 | 0.07323 | | |
| Total | 35 | 8.30556 | | | |

**APPENDIX 2**

Analysis of variance

Variate: Days to 50% flowering

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 2 | 2.3889 | 1.1944 | 1.84 | |
| REP.*Units* stratum | | | | | |
| TREATMENT | 11 | 52.9722 | 4.8157 | 7.42 | <.001 |
| Residual | 22 | 14.2778 | 0.6490 | | |
| Total | 35 | 69.6389 | | | |

**APPENDIX 3**

Analysis of variance

Variate: Percentage Severity

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 2 | 22.889 | 11.444 | 2.81 | |
| REP.*Units* stratum | | | | | |
| TREATMENT | 11 | 110.333 | 10.030 | 2.47 | 0.009 |
| Residual | 94 | 382.444 | 4.069 | | |
| Total | 107 | 515.667 | | | |

**APPENDIX 4**

Analysis of variance

Variate: Damage Score

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 2 | 0.02796 | 0.01398 | 1.37 | |
| REP.*Units* stratum | | | | | |
| TREATMENT | 11 | 0.35852 | 0.03259 | 3.20 | <.001 |
| Residual | 94 | 0.95648 | 0.01018 | | |
| Total | 107 | 1.34296 | | | |

**APPENDIX 5**

Analysis of variance

Variate: Plant Vigour

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 2 | 0.002407 | 0.001204 | 0.16 | |
| REP.*Units* stratum | | | | | |
| TREATMENT | 11 | 0.399630 | 0.036330 | 4.80 | <.001 |
| Residual | 94 | 0.710926 | 0.007563 | | |
| Total | 107 | 1.112963 | | | |