CHEMICAL RESISTANCE OF COWPEA GENOTYPES TO APHIDS (*Aphis craccivora* Koch) AND BRUCHIDS (*Callosobruchus maculatus* F.)

RESEARCH SUBMITTED

BY

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A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA SCHOOL OF AGRICULTURAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTER OF SCIENCE IN PLANT BREEDING AND SEED SYSTEMS

THE UNIVERSITY OF ZAMBIA
GREAT EAST ROAD CAMPUS
LUSAKA
2024

DECLARATION

I, declare that the dissertation hereby submitted to the University of Zambia, for the degree of Master of Science in Plant Breeding and Seed Systems has not previously been submitted by me for a degree at this or any other college or university; that, it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

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APPROVAL

This dissertation of Aaron Chimbelya Siyunda is approved by the University of Zambia as fulfilling the requirements for the award of the degree of Master of Science in Plant Breeding and Seed Systems.

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DEDICATION

This dissertation is dedicated to my sons David M. Siyunda and Aaron L. Siyunda, and the entire family, who have been with me every step of the way, encouraging, supporting, and most importantly, loving me unconditionally.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all those who rendered profound support and motivation to write this thesis. I express my sincere gratitude to my supervisors Dr. Mick Mwala, Dr. Natasha Mwila and Dr. Kalaluka Munyinda, for providing guidance during my research work and their profound efforts to see me present the research findings at the RUFORUM platform in Zimbabwe. The quick feedback they rendered to me was something that made me gain more experience and knowledge in this field of study.

I would also like to acknowledge and thank Dr. Kelvin Kamfwa and Mr. Gilson Chipabika for their profound interest and technical support throughout the study.

Lastly, I thank my course mates for their company, encouragement, and support.

ABSTRACT

Cowpea [*Vigna unguiculata* (L.) Walp] is an annual legume, considered important as a cash and nutritional security grain legume in the semi-arid regions of sub-Saharan Africa. Cowpea yields in the sub-Sahara African region are low partly due to several biotic stress factors, which includes several insects. One of the major insect pests at the developmental stage in cowpea production is the aphid species, *Aphis craccivora* Koch and at post-harvest stage, the bruchid (*Callosobruchus maculatus* F.). Therefore, this research seeks to contribute to knowledge on useful genetic materials and associated traits for cowpea resistance to aphids and bruchids in cowpea genotypes by firstly, identify cowpea genotypes that exhibit multiple resistance to aphid and bruchid infestation, secondly, by identify aphid and bruchid resistance metabolites linked to the genes associated with multiple insect resistance in cowpea and lastly, by determining the heritability of genes associated with production of metabolites linked to multiple insect resistance in cowpea. The germplasm used consisted of three parental lines namely; Musandile (MS-parent), Bubebe (BB-parent), and Lutembwe (LT-parent) obtained from Zambia Agricultural Research Institute (ZARI), one landrace variety namely; Namuseba and 101 advanced mutant lines (7th generation mutants) provided by the Department of Plant Sciences under the School of Agricultural Sciences, at the University of Zambia, and 5 purelines from the International Institute of Tropical Agriculture (IITA). The non-restricted aphid screening, was conducted in the field at the University of Zambia (UNZA) (Lat= -15.39058^{o}, Long = 28.32813^{o}), and the restricted aphid screening was conducted in the greenhouse at Natural Resources Development College (NRDC) ((Lat= -15.3833^{o}, Long= 28.3667^{o}),) in Lusaka Province, Zambia. Bruchid screening was conducted in the insectarium laboratory, Department of Plant Science, School of Agricultural Sciences at the University of Zambia. The metabolite analyses (qualitative analysis) were conducted in the chemistry laboratory at the University of Zambia, and by Food and Drugs laboratory (quantitative analysis). Significant differences at a 5% level of significance were observed among 110 genotypes under consideration with regard to aphid and bruchid resistance. Mutant lines were identified to possess different resistance traits MS1-8-2-6-8-1 (aphid resistance only), LT11-5-2-2-2 (bruchid resistance only), and BBBT1-11 (aphid and bruchid resistance). Phenolics were identified to be highly responsible for the multiple resistant trait expressed by BBBT1-11, under the control of three or more genes. BBBT1-11 can be utilized in breeding programs with a target of breeding multiple insect resistant

cowpea genotypes. Further research should also be conducted in order to genotype the BBBT1-11 and identify the QTLs responsible for the trait observed in BBBT1-11.

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ACRONYMS AND ABBREVIATION

AEP	Adult Emergence Percentage
ANOVA	Analysis of Variation
ARC	Agriculture Research Center
BB-PRT	Bubebe Parent
CABMV	Cowpea Aphid-Borne Mosaic Virus
CD	Critical Difference
CMV	Cucumber Mosaic Virus
CPSMV	Cowpea Severe Mosaic Virus
CRD	Completely Randomized Design
CSO	Central Statistics Officer
DAI	Days After Infestation
DF	Degree of Freedom
DP	Developmental Period
FP	Frass Produced
GI	Growth Index
HPR	Host Plant Resistance
IITA	International Institute of Tropical Agriculture
IPM	Integrated Pest Management
LT-PRT	Lutembwe Parent
MS-PRT	Msandile Parent
NRDC	Natural Resources Development College
QTL	Quantitative Trait Loci

RCBD	Randomized Complete Block Design
KG	Kilograms
PI	Percentage Infestation
SS	Sum of Square
MS	Mean Square
UNZA	University of Zambia
WLP	Weight Loss Percent
ZARI	Zambia Agriculture Research Institute

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Cowpea [*Vigna unguiculata* (L.) Walp.] is an annual legume, considered as an important cash and nutritional security grain legume in the semi-arid regions of sub-Saharan Africa (Ouedraogo et al., 2018). Cowpea is one of the major legume crops that is cultivated as a green manure crop, fodder crop, and pulse crop. Cowpea seeds contain approximately 60% carbohydrates, 1.8% fat, and 23.4% protein (Choudhary et al., 2015). It is also a rich source of iron and calcium (Khan, 2011). Pulse crops, including cowpea, have contributed to the improvement of agricultural economy in different countries, especially developing countries (Sarwar et al., 2003).

Cowpea plays a pivotal role in cropping systems of Semi-arid regions including part of the Middle East, Asia and Oceania, Africa, Southern Europe, and central and south America (Singh et al., 2002). In sub-Saharan Africa, the demand for cowpea is at 20,000 metric tons per year and was projected to grow by more than 40,000 metric tons over the next 10 years (Tembo et al., 2017). In Zambia, production of cowpea increased from 2,722 metric tons to 10,603 metric tons, on average, which represents a 74.3% annual increase, and the area planted also increased from 11,189 ha to 20,866 ha, on average, representing a 46% annual increase from 2009/2010 to 2015/2016 agricultural seasons (Mwila et al., 2022). This increase in cowpea production represents a proxy in the increased importance of cowpeas among smallholder farmers. The Southern Province of Zambia recorded the highest cowpea production in the 2011/2012 agricultural season, accounting for 33.6% followed by Western and Eastern Provinces with 23.1% and 10.2%, respectively, while the Copperbelt Province contributed the least at 0.7% (CSO, 2015). Regardless of the recorded increase in cowpea production, grain yield in cowpea production remains low, rarely exceeding 500 kg/ha in the traditional production system (Mwila et al., 2022).

Cowpea production is affected by several biotic stress factors, which includes several insect pests that damage the crop by infesting the crop at all developmental stages, in the field as well as at postharvest (Berberet et al., 2009). One of the major insect pests at the developmental stage that has remained a nuisance to cowpea production is the aphid species commonly called black aphids (*Aphis craccivora* Koch) as reported by Radha (2013) and also Huynth et al. (2015).

Cowpea aphid, is one of the main insect pests that affect cowpea production (Lourenco et al., 2018). Besides causing direct damage by sucking sap, cowpea aphids are also responsible for the transmission of a wide range of viruses i.e., cucumber mosaic virus (CMV) (Thottapilly et al., 1990), cowpea severe mosaic virus (CPSMV) (Blackman and Eastop, 2000), cowpea aphid-borne mosaic virus (CABMV) (Oliveira et al., 2012). At the post-harvest stage, the beetle commonly called a bruchid (*Callosobruchus maculatus* F.) presents the major pest (Kergoat et al., 2007). Heavy losses in cowpea from various insect pests are also experienced during storage. According to Semple et al (1992), three major pulse beetle species responsible for cowpea damage during storage are; *Callosobruchus chinensis*, *Callosobruchus analis, and Callosobruchus maculatus.* Cowpea beetle, is an important agricultural insect pest that is found throughout the tropical and subtropical regions (Langyintuo et al., 2003).

The most destructive stage of this beetle is the larval stage which feeds and develops on the seed of legumes (Fabaceae), while, the adults do not feed on the seed but spend their limited time (one to two weeks) mating and egg-laying (Sarwar, 2012). The beetle, *C. maculatus,* has the potential to cause 100% seed loss if left uncontrolled (Tembo et al., 2017).

Singh and Allen (1980) cited by Soleymane et al. (2013), reported several insecticides that have proved to be effective against aphids and bruchids, but insecticides are often expensive and not accessible to small-scale farmers who are mostly involved in cowpea production (Huynth et al., 2015). Besides the cost of the insecticides there is the safety and environmental concerns associated with use of chemicals in small scale agriculture as reported by Kebebe and Shimalis, (2018). The use of resistant genotypes in insect pest management is considered a good and viable proposition (Waghmare and Bantewad, 2020). This is because the resistant variety offers the best option to small-scale farmers owing to its low cost (Dent, 1991). Host Plant Resistance is an efficient, cheap, and environmentally friendly way of controlling *A. craccivora* and *C. maculatus* in an integrated pest control system. Different cowpea genotypes have been screened and different levels of resistance to aphids and bruchids have been identified as individual entities and not coexistingly (Aliyu and Ishiyaku, 2013). However, resistance to aphids and bruchids of most of the identified cowpea cultivars at International Institute of Tropical Agriculture (IITA) has broken down (Fatokun personal correspondence) as cited by Soleymane et al., (2013). Thus, there is a need to identify new sources of resistance to both *A. craccivora* and *C. maculatus* in a co-existing manner.

There has been a growing importance of host plant resistance, as a means to avoid usage of external factors to control pests, being environmentally friendly and acts as a preventive measure. However, there is inadequate information of dual resistance of aphid and bruchid in cowpea. The study of multi or dual resistance in cowpea to aphid and bruchid, helps to provide relevant information to inform the development of insect resistance genotypes, reduce quick crop resistance break down, while ensuring the yield potential of a crop can be maintained to enhance food security and livelihood of particularly the rural poor farmers.

1.2 Statement of the problem

Chemical control measures, although efficient in reducing insect pressure, pose a significant health hazard to cowpea consumers and are harmful to the environment if used (Tefera et al., 2019). Furthermore, pesticides are unaffordable to small-scale farmers in Africa and may result in the development of chemical resistance in insects, and the emergence of secondary pests. Besides, the application of insecticides in the field presents a threat to non-target organisms including natural enemies of insect pests (Devi, 2018). An alternative control measure is the host plant resistance (HPR), which is the inherent plant ability to limit insect damage through various defense mechanisms provided by its genetic make-up (Garcia-lara et al., 2010) and is fully compatible with all other integrated pest management strategies. Host plant resistance at its highest level can be exclusively applied to combat insect attacks without expensive and environmentally unfriendly interventions. Considering the myriad of insect species that either simultaneously or concurrently attack all cowpea parts, including leaves, stems, and seeds (Freitas et al., 2012) the development of HPR should target multiple insect resistance (Mihm, 1994). Instead of solving this problem using single insect pest approach which has been used in the past and is proving unsustainable due to the fact that it easily breaks down (Soleymane et al., 2013). The focus on multiple insect resistance approach implies lower cost on both the breeder and the farmer. Unfortunately, there is inadequate information on the multi-insect resistance in cowpea, with no studies conducted in Zambia. Consequently, the knowledge on genetic basis of cowpea multiple insect resistance to inform the process of developing multiple insect resistance in cowpeas is inadequate.

1.3 Justification

Mutational breeding has potential to create novel genetic variability for traits such as multiple resistance to aphids and bruchids. There is need to explore the resistance of developed mutants to aphids and bruchids. To exploit the associated metabolites with resistance to aphids and bruchids in breeding, there is need to understand their heritability. Knowledge of their heritability could provide insights into the likely genetic gains in breeding for resistance to aphids and bruchids using selection based on metabolites.

1.4. Objectives

1.4.1. Main Objective

The research seeks to contribute to knowledge on useful genetic materials and associated traits for cowpea resistance to aphids (*Aphis craccivora* Koch) and bruchids (*Callosobruchus maculatus*) in Zambian cowpea genotypes.

1.4.2. Specific Objectives

1. To identify cowpea genotypes that exhibit multiple resistance to aphid and bruchid infestation.
2. To identify aphid and bruchid resistance metabolites linked to the genes associated with multiple insect resistance in cowpea.
3. To determine the heritability of genes associated with production of metabolites linked to multiple insect resistance in cowpea.

1.5 Research Hypotheses

1. There are cowpea genotypes that exhibit multiple resistance to both aphids and bruchids
2. There are metabolites linked to the genes associated with resistance to both aphids and bruchids
3. Genes associated with metabolites conferring resistance to aphids and bruchids have high heritability.

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution and origin of cowpea

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most critical human foods and has been used as a crop plant since Neolithic times (DAFF, 2011). The production of cowpea spread to East and Central Africa, Asia, South and Central America (Saria, 2010). It originated in from the savannah region of west and central Africa (Jackai and Daoust, 1986). Some literature indicated that cowpea was introduced from Africa to the Indian subcontinent approximately 2 000 to 3 500 years ago, at the same time as the introduction of sorghum and millet, while others state that before 300 BC, cowpeas had reached Europe and possibly North Africa from Asia (DAFF, 2011). Cowpea is believed to have originated from West Africa by some workers, because both wild and cultivated species thrive in the region (Jackai and Daoust, 1986).

2.2 Importance and utilization of cowpea

Cowpea is an important crop grown worldwide (Singh et al., 1996). It is used as a vegetable using both the spreading types and grain types. Singh et al. (1996) revealed that utilization of cowpea grains as a vegetable provides an inexpensive source of protein in the human diet. Cowpea leaves are also an important component of the market, where their quality, taste, and color should be maintained (Mwila et al., 2022). Cowpea leaves are sold and consumed in most African countries, such as Zambia, South Africa, Ghana, Mali, Benin, Cameroon, Ethiopia, Uganda, Kenya, Tanzania, and Malawi DPP/ ARC (2011) as cited by Mwila et al. (2022).

The semi-spreading varieties are suitable for use as a vegetable. Fresh leaves and growth points are often picked and eaten the same way as Swiss Chard or bean leaves. Dried leaves are preserved and eaten as a meat substitute (ZAS, 2019). In many localities across the Zambian provinces, cowpea leaves are harvested fresh as a vegetable alone or for peanut soup preparation or cured for future use during the cold dry season when there is no rain to sustain the crop production (Mwila et al., 2022).

Apart from that, cowpea is also grown as a dual-purpose crop because the green pods are used as a vegetable and the haulms utilized for livestock fodder during the dry season when food is scarce (Asiwe, 2009). DAFF (2011) stressed that cowpea is a very palatable crop, highly nutritious and relatively free of metabolites. Cowpea meals are served with various popular maize meals and also complimenting custard, bread, and rice, in Zambia. Besides its importance and utilization, cowpea production in Zambia is low due to possible constraints that are to be outlined.

2.3 Constraints in cowpea production: pests and diseases

Production of cowpea in Zambia has recently experienced some major draw backs that caused major reduction in cowpea yield from farmers (Mwila et al., 2022). Research on production of cowpea, has been on a lower side in sub-Saharan countries in the last three decades (Tembo et al., 2017). This has been caused by lack of improved cowpea breeding lines, lack of knowledge on noble agronomic practices such as conservation agriculture, organic farming and integrated pest management. Insect pests are the most critical constraints to cowpea production because each phase attracts several insect pests (Singh et al., 1996). Cowpea is very attractive to insects (Dingha et al., 2021) due to the biochemical signals that the plant produces. The main pests during the growing season are pod sucking bugs (*Riptortus* spp., and *Nezara viridula*), aphids (*Aphis fabae, Aphis craccivora*), blister beetle (*Mylabris* spp.), and pod borer (*Maruca vitrata*) (Tembo et al., 2017). However, the bruchid (mainly *Callosobruchus maculatus*) is the leading pest during storage period (Devereau et al., 2002; Oluwafemi et al., 2013).

Apart from insect pests, the most important disease of cowpea is stem rot caused by *Phytophthora vignae* (Mwila et al., 2022). This disease frequently occurs in wetter areas and heavier soils that may become waterlogged (Mwila et al., 2022). Bacterial canker, bacterial blight (*Xanthomonas vignicola*) causes severe damage to cowpeas, while the most frequent virus encountered is the aphid-borne mosaic virus (CabMV) (Mwila et al., 2022). Fungal diseases, are Fusarium wilt, Cercospora leaf spot, rust and powdery mildew. Cowpea is susceptible to nematodes and should not be planted consecutively on the same land. No variety of cowpea in Zambia is tolerant to key pests and diseases (Mwila et al., 2022). Research is underway to identify promising tolerant genotypes of cowpea for insects and diseases at UNZA and ZARI. This is because chemical control is costly, particularly for small-scale farmers and is not sustainable (FAO and IIBC, 1992). There is still a need for continued breeding efforts for cowpea given the evolution of the pathogens that cause the major diseases of cowpea and also the possible emergence of new biotypes of the cowpea pests. These continued breeding efforts are also needed to develop varieties that are more resilient to climate change.

Parasitic weeds such as *Striga gesnerioides* (Wild.) and *Vatke vogelii* and *Alectra vogelii* affect cowpea production (Horn and Shimelis, 2020). Weeds have been reported as a dominant constraint to production by the farmers in Zambia (Chisanga et al., 2017). *Striga gesnerioides* and *Alectra* spp. are the principal parasitic weeds attacking cowpeas in Zambia (Chisanga et al., 2017). The most common *Striga* species pest to cowpea is *S. gesnerioides* (Jamil et al., 2021). There is limited research in this area. As a result, farmers are advised to improve soil fertility where this weed is a problem by using manure (Mwila et al., 2022). Striga finds an ideal environment for its proliferation with soils low in organic matter and soil biological activity (Jamil et al., 2021).

Other constraints that hamper the production of cowpea include no good quality seed for planting and poor marginal returns to farmers that has further worsened the limitations to cowpea production in Zambia (Nkhoma et al., 2020). The poor marginal returns are normally obtained because of crop failure. The major constraints to cowpea production have been discussed in several studies (Singh et al., 1996).

2.4 Major insect pests of cowpea

2.4.1 Aphids (Aphis craccivora Koch)

2.4.1.1 Biology and ecology of cowpea aphid

Cowpea aphids belong to the order Homoptera and family Aphididae. They are soft bodied plant lice half an inch in size and they range in colour; black, white, green and brown (Githiri et al., 1996). There are two types of aphids which attack cowpea and these types include; wingless and winged aphids (Jeff-Whitworth and Ahmad, 2009). The major effect of aphids on cowpea has been greatly studied. They are also known to be small sap-sucking insects because they consume the contents of the plant tissues, usually on the underside of the leaves where sap is found. Their mechanism of feeding is usually, phloem based (Singh et al., 1996). This results in curling of the leaves and yellowing due to the feeding of the aphids. This affects photosynthetic ability and results in eventual plant death. The biology and ecology of cowpea aphids varies depending on climate and soil, under favourable conditions a generation may take only 13 days and adults live from 6-15 days and may produce more than 100 progenies (Singh et al., 1996). The life-cycle of aphids is by asexual reproduction. They give birth to nymphs which then develop into adult since aphids are viviparous (Githiri et al., 1996).

2.4.1.2 Effect of aphids on cowpea (Vigna unguiculata Koch)

Aphids are partly responsible for pre-harvest pod damage in the field and they primarily infest seedlings, although large populations also infest flower buds, flowers and pods (Githiri et al., 1996). They cause direct damage to the plant by removal of its phloem tissues (sap). Apart from physical damage, aphids are believed to transmit virus to cowpea plants and transmission causes aphid borne mosaic viruses (Giga, 2001). Small and isolated populations have no major impact on cowpea yield, but large populations can cause leaf distortion, stunted growth, and poor nodulation of root systems and in some cases, result in plant mortality and low yield (Singh and Singh, 1990). Cowpea aphids also reduce photosynthetic capacity of the crop because of colonies that cover the stems and leaves during feeding and thereby shading the crop from receiving sunlight and thus reducing growth noticed by distortion of leaves (Jackai et al., 2001). The mode of aphid feeding on its substrate has been greatly studied (Giga, 2001).

2.4.1.3 Mode of aphid feeding

Cowpea is mostly attacked by several insect pests of which the most serious are the sucking piercing pests (Aphids) as revealed by Jackai et al. (2001). The cowpea aphids feed on young, succulent terminal growth by piercing plant tissue and penetrating the phloem with needle-like mouthparts (Githiri et al., 1996). They suck fluid or sap from the stem, terminal shoots, petioles, flowers, seeds and pods (Kitch et al., 1999). They also inject a powerful toxin into the plant when feeding and in situations where the populations are large, stunting and plant death results (Walling, 2008). These insects are responsible for the damage of the crop during vegetative stage in the field. Heavy feeding affects the growth of the crop causing stunted growth, pod set reduction, delay of flower initiation and plant maturity (Asiwe, 2009). In many cases, aphid feeding causes significant damage to the plant and it appears that aphids are able to escape plant defences while moving their stylets intercellularly and by manipulating their host through secretion of saliva into the phloem sieve elements (Walling, 2008). These sap-sucking insects cause damages and losses in susceptible cowpea lines by modifying metabolism of the plant (Siyunda et al., 2022a).

2.4.1.4 Damages and losses caused by cowpea aphids (Aphis craccivora Koch)

Aphids cause a serious damage to cowpea which in most cases result in low yield. The damage that is caused by aphids is mainly due to a number of causes, including loss of plant sap and clogging of leaf surfaces (Githiri et al., 1996). However, large populations can turn leaves yellow and stunted growth of shoots (Bugg et al., 2008). They also cause leaf curling which is a common symptom of aphid infestation that occurs when a colony attacks the underside of a leaf, causing the leaves to desiccate (Githiri et al., 1996). The curling behaviour provides protection for the aphid colony, but the leaf becomes useless to the plant (Asiwe, 2009). The damages of aphids on cowpea cause a disease called aphid borne mosaic virus (GRDC, 2010).

2.4.1.5 Aphid borne mosaic virus

Aphids can damage cowpea by spreading viruses or causing direct damage when feeding on plants (GRDC, 2010). Feeding damage is caused by large population of aphids, but virus transmission can occur before aphids are seen to be present (Asiwe, 2009). Crops affected by aphids are noticed by yellowing leaves and thus plant death (Soleymane et al., 2013). Viruses are widespread in pulses and can cause significant economic losses, especially when extensive infection occurs in early crop growth (GRDC, 2010). Transmission through seed is increased by planting affected seed year after year and the resistance of cowpea against sap-sucking insect pests (aphids) has been greatly studied (Kitch et al., 1999).

2.4.1.6 Resistance of cowpea lines against cowpea aphids

Breeding for aphid resistance is needed to reduce further losses from cowpea aphid outbreaks in the field (Singh et al., 1996). Singh and Singh (1990) revealed that the use of resistant lines is the cheapest, most effective and sustainable way to control insect pest in the production area. Resistance to aphid attack is characterized by a lower and isolated insect population density or fewer damage symptoms on the resistant plants (Giga, 2001). This can also be seen by the development of new leaves (trifoliate) even under heavy attack and thus the crop continues to flower and form pods (Singh et al., 1996). The other threatening insect pest of cowpea includes cowpea beetle (bruchid).

2.4.2 Cowpea weevils (*Callosobruchus maculatus* F.)

2.4.2.1 Biology and ecology of cowpea beetle

Cowpea beetle belongs to the order Coleoptera and family Bruchidae. They are hard bodied insect that are less than 1 cm in length (Siyunda et al., 2022b). They vary both in size and colour and it is a field-to-storage pest (Oluwafemi et al., 2013). The female beetles are dark coloured and larger while the male bruchids are light coloured and smaller in size (Siyunda et al., 2022b). It is important to understand the biology and ecology of bruchid in order to evaluate the level of resistance and susceptibility of different cowpea lines. Cowpea beetle have the capacity to lay eggs on the seeds and when they hatch, the larvae burrow into the seed and consume the endosperm of seeds (Singh and Singh, 1990). The entire life cycle of cowpea bruchid takes about 35 days (Siyunda et al., 2022b). During development, the larva feeds on the interior of the seed, consuming the tissue leaving exit holes through which it exits when it becomes an adult. The holes make it easy to recognize infested seeds and also to determine the seed resistance through seed damage index (Singh and Singh, 1990). The matured adult emerges after a larval period of 3 to 7 weeks, depending on climatic conditions (Toriola, 2010).

The life cycle of cowpea beetle consists of egg, larva, pupa and adult. The eggs are small, clear, shiny and smooth and oval shaped. They are normally glued firmly to the surface of pods and seeds (Toriola, 2010). The newly laid eggs are translucent grey and inconspicuous and when they hatch, the empty eggs shells become white and clearly visible to the naked eye. Eggs hatch within 5-6 days of oviposition. Upon hatching, the larvae bite through the base of the eggs and bore into the seeds where they spend the whole life cycle feeding on the seed (Siyunda et al., 2022b). The larvae pupate inside the seed and pupation takes place in the seed. Pupation takes about 7 days to complete. The emerged adult from the seed does not live longer than 12 days (Singh and Singh, 1990). During this time the females lay more than hundred eggs. The optimum temperature for egg-laying is about 30 to 35° C (Siyunda et al., 2022b).

2.4.2.2 Effect of bruchids on cowpea (Vigna unguiculata F.)

Cowpea suffers heavily from insects, both in the field and when grains are stored after harvest (Joana and Gungula, 2010). According to Redden and McGuire (1983) the simplest reliable variable to use for evaluation of resistance to bruchids is the percentage of undamaged seed with zero emergence holes. In addition to yield loss in the field, cowpea also suffers considerable loss in storage due to bruchids (Mwila et al., 2022). The cowpea bruchid not only causes a reduction in dry weight but it also reduces grain quality and seed viability making it unfit for human consumption as well as for planting and thus causing substantial reduction in market value (Singh and Singh, 1990).

Bruchids change the flavour and nutritive value of grains which reduce the marketability and acceptability of pulses (Singh and Singh, 1990). They are not easily seen in the field but they are visible after harvesting of the crop from the field (Huignard et al., 1985). Shade et al. (1990) are of the opinion that bruchids can destroy about 80% of cowpea grains in storage when the seeds are not treated with insecticides. Singh and Singh (1990) revealed that within a storage period of 100 days, the resistant varieties show about 25% damaged seeds in contrast to 95% damage for the susceptible varieties. Bruchids are responsible for seed weight loss and quality in storage (Singh and Singh, 1990). The mode of bruchid feeding on cowpea seeds has been done in several studies.

2.4.2.3 Mode of bruchid feeding

In stored seeds, cowpea bruchid causes irreparable damage to the endosperm and reduces nutritive value and quality of seeds (Obopile et al., 2011). The damage is caused by larvae feeding inside the seed by consuming the cotyledons and when adults emerge, they leave circular exit holes (Devereau et al., 2002). This is a practical situation where the seeds are often seen with holes especially on susceptible cowpea lines and the damage reduces the weight and may render the seeds to be unfit for human consumption (Shade et al., 1990). The resistance of cowpea against storage insect pest has been measured (Singh and Singh, 1990).

2.4.2.4 Resistance of cowpea lines against bruchids

The resistance of cowpea to bruchid in storage is measured by the delayed, staggered and lower emergence levels of insects (Singh and Singh, 1990). Literature has revealed that grains with hard seed coat, rough texture and wrinkled shape are more resistant to bruchid infestation than the soft, smooth and fine-textured grains (Singh et al., 1996). Bruchid infestations start in the field on pods but population growth of bruchids increase following threshing when eggs can be laid directly on the seeds in storage because they are oviparous (Siyunda et al., 2022b). Giga (2001) stressed that bruchid larvae feed and develop inside the seed endosperm and emerge as adults after 3-4 weeks. Singh and Singh (1990) screened several cowpea varieties for bruchid resistance and reveal that it is not morphological characters such as seed weight and seed colour but the nutritional value of the seeds which will determine its acceptability or consumption. It has been reported that variables such as percentage adult emergence, developmental period and percentage seed weight loss are the most reliable indicators for resistance of cowpea to damage by *C. maculatus* (Mogbo et al., 2004). Cowpea bruchid causes significant damages and losses that are greatly discussed.

2.4.2.5 Damage and losses caused by bruchids

The cowpea weevil (bruchid) is a serious pest of stored grain legumes. According to Oluwafemi et al. (2013) cowpea bruchid is an insect pest capable of causing high yield loss both in quantity and quality of the seeds. Shade et al. (1990) estimated that the dry weight loss due to damage by *C. maculatus* exceed 29%. Additionally, bruchid infestation can impact seed quality, reduces germination ability and quality for both planting and consumption (Toriola, 2010). There are several control measures of insect pests of cowpea.

2.5 Control of insect pests of cowpea

Various control measures are adopted to suppress insect pest population which include use of synthetic insecticides (chemical control), biological control or integrated pest management (IPM), cultural practices, double bagging and host-plant resistance. The use of chemical control is the quickest method to control aphids yet it is not environmentally friendly due to adulteration of both soil and water (Asiwe, 2009). Biological control involves the use of natural enemies or predators, parasites and pathogens to control cowpea insect pests. Cultural practices/methods used to manage cowpea insect pest includes methods such as crop rotation, intercropping and sanitation. The other control measure of insect pest of cowpea is by host plant resistance (Ego, 2011). The use of host plant resistant is the most important method adopted because it is environmentally friendly. This is because it does not involve the use of synthetic chemicals that pose danger to crops and soil.

2.5.1 Host-plant resistance to insect pests of cowpea

The most economical and environmentally friendly strategy of controlling insect pests both in-field and in-storage is through Host Plant Resistance (Babura and Mustapha, 2012). In both, in-field plants and stored-grains, several factors lead to the production of resistance against infestation in insect pests. These include hardness of seeds which is thought to make insect penetration more difficult thus providing protection (Siyunda et al., 2022b). The texture of the seed coat of cowpea seed may have negative influence on the oviposition of the cowpea weevil; the quantity and quality of nutritional constituents (Ahmed et al., 2007). Through host plant resistance, more cowpea breeding lines can be developed by crossing resistant varieties with susceptible lines.

2.6 Plant defence against insect herbivory

Host plant resistance is an important form of plant defense against insect herbivory and is widely supported in crop protection against insect pests and diseases (Sharma and Vaishampayan et al., 2009). The constitutive plant defence is present in plants irrespective of the external stimuli, while the induced defence is stimulated by insect feeding and/or the elicitor application (War et al. 2012). Moreover, plants manage the resources between defence and growth by eliciting anti-herbivore defence only when necessary (Karban, 2011). A meta-analysis of genetic correlation between plant resistance to multiple enemies has shown positive correlations if both the compared species are generalist herbivores or both are specialist herbivores (Maffei et al., 2012). Plant resistance to herbivores showed positive genetic correlation from herbivores with different feeding habits, such as gall inducers and leaf miners (War et al., 2011), miners and leaf folders (Scott et al., 2010), and leaf folders and gall inducers (Pieterse et al., 2012). Mechanism of resistance in the pairwise comparison between insects of different feeding guilds, such as phloem-feeding and leaf-chewing herbivores, showed the lowest genetic correlation (Leimu and Koricheva, 2006). Both morphological and biochemical defences in plants are important to withstand insect attack (Siyunda et al., 2022b). Although morphological defence is primarily used by plants against insect pests, the biochemical-based defence is considered more effective as it directly affects insect growth and development (Kariyat et al., 2013). Some plant metabolites indirectly defend plants by recruiting the natural enemies of the insect pests, such as parasitoids and predators (Arimura et al., 2009). Induced resistance in plants against biotic stresses is attributed to the phenylpropanoid and octadecanoid pathways mediated by salicylic acid (SA) and jasmonic acid (JA), respectively (Zhao et al., 2009). These pathways produce a number of plant defensive secondary metabolites in intermediate steps (He et al., 2011), which affect insect growth and development and also release volatiles that attracts the insect's natural enemies (Howe and Jander, 2008).

2.6.1 Specialized metabolites in plant defence

According to Mahanil. (2008), specialised metabolites for which insecticidal or anti-insect activity has most often been proven are isoprene derived terpenoids followed by alkaloids and phenolic compounds as cited by Boulogne et al. (2012). Less reported on, but also significant, are acylsugars and cyanogenic glycosides (Constabel and Barbehenn, 2008). Although not regarded as a specialised metabolite, direct defence against insects can also be based on the synthesis of defence proteins or polymers including cuticular waxes (Gonzales-Vigil et al., 2011). Estimates on the divergence of specialised metabolites reach over 200, 000 different structures (Pichersky and Lewinsohn, 2011). They branch off from primary metabolism (e.g., amino acids, fatty acids, hormones) (Degenhardt et al., 2009) and have precursor pathways that are rather conserved among plant species (Chen et al., 2011). The diversification in the end-products is, however, largely the result of (stereo-)isomeric configuration, skeleton rearrangements and modifications of the final product which can be versatile (Bleeker et al., 2011). With >20, 000 papers on metabolites exhibiting insecticidal properties published since the 1980s, the mass of information is overwhelming (Radha, 2013). However, after critical review, Isman and Grieneisen (2014) concluded that only one third of these publications were based on appropriate chemical characterizations of the tested (putative) insecticides and even less (27%) included positive controls. Moreover, the majority of these are based on assays with artificial diets, often supplied with a relatively high dose of a (purified) metabolite. Nawrot and Harmatha (2012), reviewed the literature describing plant-derived metabolites acting on insects in the scope of protecting post-harvest products, which might be less relevant for application in the field.

2.6.2 Biochemical focus of the current study

In this study, the researcher focused on endogenously produced anti-insect metabolites with potential for application in breeding, due to evidence for a chemotype-to-phenotype correlation in plants (Berberet et al., 2009), where metabolites can either be toxic (Radha, 2013), repellent (Huynth et al., 2015), or inhibit development or feeding rates of the insect (Lourenco et al., 2018). The metabolites under consideration included alkaloids, phenolics, tannins, and sugar compounds, using multiple insect resistance approaches unlike the previous studies that have been extensively based on the single insect pests' approach which is proving to be unsustainable (Lourenco et al., 2018).

2.7 Heritability

Heritability (h^2) can be defined as the ratio of genetic variance to total phenotypic variance (Barelli et al., 1999). This number can range from 0 (no genetic contribution) to 1 (all differences on a trait reflect genetic variation). It provides effective mean for predicting future evolutionary response to selection (Bhateria et al., 2006). 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. The additive genetic value of genotype, called the breeding value, is the sum of the average effects of all the alleles the genotype carries (Falconer and Mackay, 1996). Broad sense heritability, defined as $H^2 = V_G/V_P$, captures the proportion of phenotypic variation due to genetic values that may include effects due to dominance and epistasis. On the other hand, narrow sense heritability, $h^2 = V_A/V_P$, captures only that proportion of genetic variation that is due to additive genetic values (V_A). Note that often, no distinction is made between broad sense heritability and narrow sense heritability; however, narrow sense is most important in plant selection programs, because of response to selection depends on additive genetic variance.

Moreover, resemblance between progenitor and offspring is mostly driven by additive genetic variance (Bhateria et al., 2006). On the other hand, selecting under optimal conditions especially high inputs increases genetic variance relative to environmental variance and thus increases heritability of yield and its components. Heritability estimates in crops are classified as high (>0.50), medium (0.30-0.50), and low (<0.30) (Bhateria et al., 2006).

Previous genetic studies using TVu 2027 as donor suggested that maternal genes are involved in the inheritance of resistance to bruchid (Miesho et al., 2018). The same study highlighted involvement of a major recessive gene and modifiers, and also noted that either dominant or interactive effects were more important than additive types of gene effects (Miesho et al., 2018). Dobie. (1981) and Redden. (1983) reported paternal and embryonic genotypic effect in certain backcross combinations of F_3 generation and digenic control of resistance in one of their cross and monogenic control in another cross, in conjunction with one or more modifier or minor gene loci. Furthermore, Adjadi et al. (1985) reported that resistance to bruchid resulted from two recessive genes.

2.8 Focus of the current study

The study of genetic parameters such as heritability of different production traits and genetic correlations in cowpea is needed, for the formulation of effective breeding plans and for the estimation of breeding values. The study of heritability, using metabolites/chemical models linked to genes of interest for plant traits are rare (Miesho et al., 2018). It is important to understand the heritability of resistance to aphid and bruchid character, and the gene action controlling it to help breeders select suitable parents for the breeding program. Therefore, the aim of the present study was; firstly, to identify cowpea genotypes that exhibit multiple resistance to aphid and bruchid infestation. Secondly, to identify aphid and bruchid resistant metabolites linked to the genes associated with multiple insect resistance in cowpea. Lastly, to determine the heritability of genes associated with production of metabolites linked to multiple insect resistance in cowpea.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Project and study sites description

Two experimental sites were used to assess the resistance of 110 cowpea lines in terms of their reactions to cowpea aphids and bruchids and also to determine the metabolites responsible for their resistance. This project was part of the cowpea breeding project being executed by the University of Zambia (UNZA). The non-restricted aphid screening was conducted in the field at UNZA (Lat= -15.39058^o, Long = 28.32813^o), and the restricted aphid screening was conducted in the greenhouse at Natural Resources Development College (NRDC) ((Lat= -15.3833^o, Long= 28.3667^o),) in Lusaka Province, Zambia. Bruchid screening was conducted in the insectarium laboratory, in the Department of Plant Science, School of Agricultural Sciences, at the University of Zambia. The extraction and qualitative analyses of metabolites were conducted in the chemistry laboratory, Department of Chemistry, School of Natural Sciences, University of Zambia. The quantitative metabolite analyses were conducted by the Food and Drugs laboratory, in Lusaka, Zambia. All the experimental sites used, are based in Lusaka which is in agro-ecological region II. Lusaka is in the southern part of the central plateau at an elevation of about 1,279 meters. Primarily due to its high altitude, Lusaka features a humid subtropical climate according to Koppen climate classification (ZMD, 2021). Its coldest month, June, has a monthly mean temperature of 14.9 °C (58.8 °F) (ZMD, 2021). Lusaka features hot summers and cool winters, with cold conditions mainly restricted to nights in June and July. The hottest month is October, which sees daily average high temperatures at around 32 °C (90 °F) (ZMD, 2021). There are three main seasons: a warm monsoon season between November and March, a dry winter between April and August, and a hot summer in September and October. Region II is characterized by average annual rainfall of 800-1000 mm and covers 42% of Zambia’s land area (ZMD, 2021).

3.2 Germplasm Source

The germplasm used consisted of three parental lines namely; Musandile (MS-parent), Bubebe (BB-parent), and Lutembwe (LT-parent), one landrace variety namely; Namuseba, 101 advanced mutant lines (7th generation mutants), and 5 purelines from the International Institute of Tropical Agriculture (IITA) located in Lusaka, Zambia. The parental lines were obtained from Zambia Agricultural Research Institute (ZARI) located in Lusaka, Zambia, while the mutants were provided by the Department of Plant Sciences under the School of Agricultural Sciences, at the University of Zambia, Lusaka.

3.3 Experiment 1: Aphid Screening

3.3.1 Aphid Culturing

Aphid (*Aphis craccivora* Koch) culturing was done using insect-proof cages. The cage was 2 meters in length, 1-meter in width, and 1.4 m in height. It was covered with fine saran mesh, able to allow the exchange of air but insect-proof. The cage had 4 compartments each measuring 1 meter in length, 1 meter in width, and 1.4 meters in height. Three polyethylene pots (2Ltr v/pot) filled with loam soil (7Kg/pot) were placed in each compartment. A susceptible cowpea line against *A. craccivora* Koch (TVu 3236) was used as a substrate in the culturing process. Each pot had three plants and each plant was infested with aphids 7 days after planting. In order to maintain and build the aphid population, after 2 weeks the polyethylene pots in the cages were replaced with new polyethylene pots consisting of three 14 days old plants/pot. The protocol used for aphid culturing was adopted from Togola et al. (2020).

3.3.2 Measuring Scales for Aphid Screening

The parameters that were measured in the current study for both unrestricted and restricted aphid screening methods included; plant survival rate, aphid population build-up, number of aphids per plant, and plant vigour. The readings of these variables were recorded at 5, 9, 13, 17, and 21 days after the infestation of aphids onto cowpea seedlings aged 7 days old (Togola et al., 2020). Plants that survived aphid infestations were allowed to grow and produce seeds. Three different measuring scales were employed to measure the parameters under consideration as below;

3.3.2.1 Aphid Population

The measuring scale recommended by Soleymane et al. (2013), for aphid population build-up was used. The scale had a meter rating from 0 to 5, where 0 represented the complete absence of aphids, the score of 1 represented few individual aphids, the score of 2 represented just a few small individual colonies of aphids, the score of 3 represented aphids in several small colonies, 4 denoted aphids in large individual colonies and the meter rate of 5 represented aphid colonies that were large and continuous.

3.3.2.2 Plant Vigour

The measuring score for plant vigour was done using a measuring scale recommended by Obopile (2006), though slightly modified. The scale had a meter range of 1 to 3, where the score of 1 represented cowpea plants that looked weak and had developed senescence, the score of 2 represented cowpea plants that showed medium growth, and the score of 3 represented cowpea plants that were growing vigorously.

3.3.2.3 Number of Aphids/Plant

The measuring scale used for this parameter is as recommended by Soleymane et al. (2013). The measuring scale had a metering rate of 1 to 9; 1 represented the number of aphids ranging between 0 to 4, the measuring score of 3 represented the number of aphids ranging between 5 to 20 aphids per plant, and the score of 5 represented the number of aphids ranging from 21 to 100 aphids per plant, the score of 7 denoted the number of aphids ranging from 101 to 500 aphids per plant and lastly, the measuring score of 9 represented the number of aphids above 500 aphids per plant.

3.3.2.4 Plant Survival Rate

The percentage survival rate of plants was calculated based on the plants that survived on the particular scoring day. The standard was 3 plants per polyethylene pot (Soleymane et al., 2013).

3.3.3 Non-Restricted Method (Open Field screening method)

The field screening of 110 cowpea genotypes was conducted at the School of Agricultural Sciences field station located at the University of Zambia. Polyethylene plastic pots (2Ltr) were used for this field experiment. Loam soil was collected in the non-utilized area of the field station. Each polyethylene pot of each genotype represented a plot in each block. Each polyethylene pot had three plants. At 7 days after planting, plants were infested with 4 cultured aphids at the 5th instar stage. Scoring was done at 5, 9, 13, 17, and 21 days after infestation.

3.3.4 Restricted Method (No choice test)

The 21 cowpea lines that had shown aphid resistance traits during the open field screening method were then advanced to the 8th mutation generation and screened using the no-choice test at NRDC greenhouse. The materials consisted of 21 cowpea genotypes advanced from the open field screening method. These 21 that exhibited the aphid resistance trait during the open field screening method were selected from an initial set of 110 genotypes and thus were advanced to validate their resistance. The same insect-proof cages used in the culturing of aphids at UNZA were used in the no-choice test at NRDC. Each compartment of the cage (1 meter in length, 1-meter in width, and

1.4 meters in height) housed a single genotype consisting of three polyethylene pots (2Ltr/pot) filled with sterilized top soils (Togola et al., 2020). The intended number of plants per polyethylene pot was 3. In order to reduce plant failure, 5 seeds were planted per pot. The maximum number of plants allowed to grow in each pot was 3, thus in pots where germination was at 100%, thinning of 2 plants was done in order to accommodate the intended number of plants in the polyethylene pot.

3.3.5 Experimental design and analysis for experiment 1

Randomized complete block design (RCBD) was used for open field screening method and completely randomized design (CRD) was used for restricted screening method. In each method, each genotype was regarded as a treatment replicated three times. R version 3.5.1 was used to conduct the tests at alpha 0.05

3.4 Experiment 2: Bruchid Screening

3.4.1 Stock culture of bruchid

The study was carried out in the Department of Plant Science, University of Zambia, Lusaka, Zambia in 2021. Culturing of the bruchid population was done at the University of Zambia, in the Insectarium laboratory. The population was maintained at 28^0C (±2^0C) and Relative Humidity of 70±5% (Sarwar, 2012). A disinfected susceptible genotype, Namuseba was used as substrate in the culturing of the insect population. Adult insects were placed in twelve glass jars that were sterilized at 55°C for 4hrs in an oven (SDO/225/DIG model) before use. The jars were covered tightly with muslin affixed with rubber bands in order to prevent the adults from escaping (Choudhary et al., 2015). The cowpea seeds (150 g) were placed in each jar and infested with 20 bruchids. The jars were kept under laboratory conditions undisturbed and mass-cultured up to the third generation (Senthilraja and Patel, 2021). The freshly emerged adults of *C. maculatus* were used for genotype screening (Sarwar, 2012).

3.4.2 Experimental cowpea seed

The undamaged and clean cowpea seeds for each genotype were examined using a hand lens to make sure that all the seeds were clean and undamaged and that no eggs had been laid on them. The seeds of all 110 test genotypes were then kept at -5 ^{0}C for one week in the deep freezer and thereafter left for 24 h under ambient laboratory conditions (Sarwar, 2012).

3.4.3 No-choice test

Relative resistance and susceptibility of cowpea genotypes against *C. maculatus* were determined under a no-choice test (Giga and Smith, 1981). One-hundred seeds from each genotype were weighed and kept separately in 250ml plastic containers and two pairs of adult beetles (1or 2 days old, consisting of 50% male and 50% female) were put in each container, separately (Senthilraja and Patel, 2021). Each container was covered on the top with a two-fold muslin cloth fastened with a rubber band to avoid insects from escaping and still provide sufficient aeration (Sarwar, 2012). After infesting the plastic containers with the beetles, the insects were discarded after 7 days, to ensure maximum oviposition (Choudhary et al., 2015).

3.4.3.1 No. of eggs laid/100 seeds

The number of eggs laid on each genotype was counted after 7 days of the release of *C. maculatus*. A hand lens was used in the counting process for easy visibility.

3.4.3.2 Adult emergence (%)

Adult emergence was recorded every 24h daily. The formula used for calculating adult emergence was the standard formula suggested by Sharma and Thakur (2014).

$$\text{Adult emergence (\%)} = \frac{\text{No. of adults emerged}}{\text{Number of eggs laid}}\ x\ 100$$

3.4.3.3 Developmental period (days)

The developmental period was considered as the time taken from oviposition to adult emergence (Sharma and Thakur, 2014).

3.4.3.4 Growth index

The calculation of the growth index was done using the formula suggested by Singh and Pant (1955) as shown below;

$$\text{Growth Index} = \frac{\text{Adult emergence(\%)}}{\text{Developmental Period (days)}}$$

3.4.3.5 Percent infestation

The percent infestation was calculated using the standard formula and categorized according to the suggestion of Deshpande et al. (2011) (Table 1).

$$\text{Percent Infestation} = \frac{\text{No. of seeds with emergent holes}}{\text{Total number of seeds observed}}\ x\ 100$$

Table 1: Resistance/susceptibility rating based on percent seed infestation

S. No.	**Class**	**Per cent infestation**
1	Highly resistant	0-20
2	Moderately resistant	21-40
3	Least susceptible	41-60
4	Moderately susceptible	61-80
5	Highly susceptible	81-100

3.4.3.6 Weight loss (%)

The formula suggested by Sharma and Thakur (2014), was used for the calculation of the percent loss in weight as shown below. The final weight for each genotype was taken with a single pan electric balance (JLab SINGLE PAN) independently for each treatment.

$$\text{Weight loss (\%)} = \frac{\text{Initial Weight of Seeds (g)} - \text{Final Weight of Seeds (g)}\ \text{x}\ 100}{\text{Initial Weight of Seeds (g)}}$$

3.4.3.7 Frass produced

This was done by separating healthy and damaged grains from dust material by passing each sample (genotype) through a sieve. Thereafter, the frass (dust material) of each treatment was weighed separately using the single pan electric balance (JLab SINGLE PAN) (Sarwar, 2012).

3.4.4 Experimental design and analysis for experiment 2

The experimental design used in the study was a completely randomized design (CRD) with 3 replications (Steel and Torrie, 1980). R version 3.5.1 was used to conduct the tests at alpha 0.05

3.5 Experiment 3: Metabolite Analysis

3.5.1 Identification of Metabolites

The extraction and qualitative analysis of metabolites were conducted in the chemistry laboratory, Chemistry Department, School of Natural Sciences, University of Zambia, while the quantitative metabolite analysis was done by the Food and Drugs Laboratory, in Lusaka, Zambia. The materials included; 1 resistant genotype against *A. craccivora* and *C. maculatus*, 2 susceptible genotypes against *A. craccivora* and *C. maculatus*, 1 genotype susceptible to *A. craccivora* but resistant to *C. maculatus*, and 1 genotype susceptible to *C. maculatus* but resistant to *A. craccivora*.

3.5.1.1 Sample Preparation

The leaf sample to be analyzed initially involved the planting of five selected genotypes namely; BBBT1-11, LT10-7-1-12-1, LT11-5-2-2-18, LT11-5-2-2-2, and MS1-8-2-6-8-1 in insect-proof cages at NRDC. To stimulate the secretion of the metabolites in cowpea leaves and seeds, cowpea genotypes had aphids infested at 7 days old after planting. The infected plants had some leaves plucked 14 Days After Infestation (DAI) and washed carefully to remove any physical trace of aphids on the leaves. The plucked leaf samples were oven dried using an electric dryer (SDO/225/DIG model) at 45 ^{o}C for 72 hrs and ground to powder. The powdered leaf sample was then used in the metabolite extraction process. The protocol used in leaf sample preparation was adopted from Togola et al., (2020). The remaining part of the plant for the selected genotypes after plucking some leaves, was allowed to grow and bear seeds. Harvested seeds were dried to an acceptable moisture content level (12%) and ground to powder using a grinder (Geno/Grinder-SPEX). The powdered seed sample was then used in the metabolite extraction process.

3.5.1.2 Metabolite Determination

The metabolites considered included; alkaloids, phenolics, tannins and sugars. On the basis of tests for insect resistance conducted, seeds were classified as resistant or susceptible to *A. craccivora* and *C. maculatus* or both.

The protocol used in the determination of alkaloid content was according to the description of Harbone (1973) with slight modification. 5g of the powdered sample was weighed using the electronic balance (JLab SINGLE PAN model). The sample was placed in the 250mL beaker and 200mL of 10% acetic acid in ethanol, was also added to the 250mL beaker and allowed to settle for 4 minutes. The solution was then filtered to obtain the extract, which had to be concentrated to ¼ of the original volume using a water bath. Drops of concentrated ammonium hydroxide were added to the extract. The process of adding concentrated ammonium hydroxide was done until precipitation had been completed. The solution was allowed to settle, in order to collect the precipitate. Thereafter, diluted ammonium hydroxide was used to wash the collected precipitate which was then filtered. The residue was then dried and weighed. According to the protocol of Harbone (1973), the dried residue is the alkaloid.

Folin Ciocalteu assay was used in the determination of the total content of phenolics of ethanol/water extract. This was done according to the description by Waterman and Mole (1994). 0.25mL of the hydro-alcoholic extract was placed in the volumetric flask (25mL) and distilled water (5mL) was added to the volumetric flask (Salawu et al., 2014). 1.25mL of the reagent (Folin-Ciocalteu's phenol reagent) was added to the volumetric flask and thoroughly mixed with hydro-alcoholic extract and distilled water. 3.75mL of sodium carbonate solution of 20%(w/v) concentration was added to the volumetric flask after 2 mins. Thoroughly mixing of the content was done after which distilled water was again added to the capacity (volume) of the flask and mixing was done. A duration of 2hrs was allowed after adding sodium carbonate solution. Thereafter, the absorbance was measured using a lambda EZ150 spectrophotometer (Perkin Elmer, USA) at 760nm. Tannic acid was used as the standard and the expression of the results was done as milligrams (mg) of tannic acid equivalent/gram (g) of the sample.

In order to extract soluble carbohydrates (sugars), 0.5 g of the sample was mixed with 80% ethanol in the ratio 1/30 (sample/solvent, g/ml) at 70 ^{o}C for 15 min under agitation. The extract was cooled and centrifuged for 10 mins at 10,000 x g and 10 ^{o}C. The supernatant was recovered and the residues were extracted two more times using the same procedure. Supernatants were pooled and filtered through a 0.45um syringe filter prior to HPLC analysis. Total Sugar Content (TSC) was determined by HPLC using a Dionex Ultimate 3000 system equipped with corona detector (electrospray) and a diode array detector. A shodex Asahipak NH2P-50 of 250 mm x 4.6 mm x 5 um with a mobile phase composed of pure water (phase A) and pure acetonitrile (phase B) was used. Setting was done with an isocratic elution program (30% phase B, 70% phase A) at a flow rate of 1ml min^{-1} and a column temperature of 30^{0}C. Injection was set at 210 nm. Calibration curves were realized in triplicate.

Tannin analysis was carried out on 10 g of dry seed coat from different cultivated Vigna lines according to Van de Casteele et al. (2019). The extraction was performed with boiling methanol–ethanol-water (4:4:2, v/v/v) under nitrogen; the solvent was evaporated under a vacuum and the residue dissolved in water (crude extract). Tannins were separated from non-tannins with 1% gelatin in 10% NaCl solution and the pellet, containing tannins, was solubilized in 50% acetone (pH of 3) at 37 ◦C. Gelatin was then removed, increasing the acetone concentration up to 90%. The acetone was evaporated under a vacuum, total tannins were dissolved in water and the hydrolysable fraction was separated from the condensed one using formaldehyde at 1.6 mg mL^{-1} final concentration. Tannins were assayed by Folin–Ciocalteau spectrophotometric method and results were expressed as catechin equivalents. The presence of condensed tannins was also proved by the red colour formation after heating the crude extracts with n-butanol–HCl–Fe (III).

3.5.1.3 Experimental design and analysis for experiment 3

Completely randomized design with each treatment replicated three times was used as a design. The data collected was subjected to analysis of variance (ANOVA) for test of significance using R version 3.5.1. Variables that displayed significant differences were separated using Turkey's HSD test. Treatment means were considered significant when $P \leq 0.05$ at 5% level. Correlation analysis was also conducted to acertain which metabolite was associated with multiple resistance trait at alpha 0.05.

3.5.2 Metabolite Heritability Analysis

3.5.2.1 Plant genetic materials

In this study, two (2) populations were developed and the crossing procedure and population advancement from F_1 to F_3 was adopted from Ouedraogo et al. (2021).

Cowpea line LT10-7-1-12-1 (female parent), susceptible to both *A. craccivora* and *C. maculatus*, was crossed with BBBT1-11 (male parent) resistant to both *A. craccivora* and *C. maculatus* to generate F1 progenies for population 1 (Figure 1). The F_1 individuals from the cross (LT10-7-1-12-1 x BBBT1-11) were self-pollinated to produce 160 F_2 individuals, which were advanced to F_3. The F_1 (98 plants), F_2 (160 plants), and F_3 (198 plants) were analyzed for the identified metabolite content (phenolic compounds) to determine their heritability.

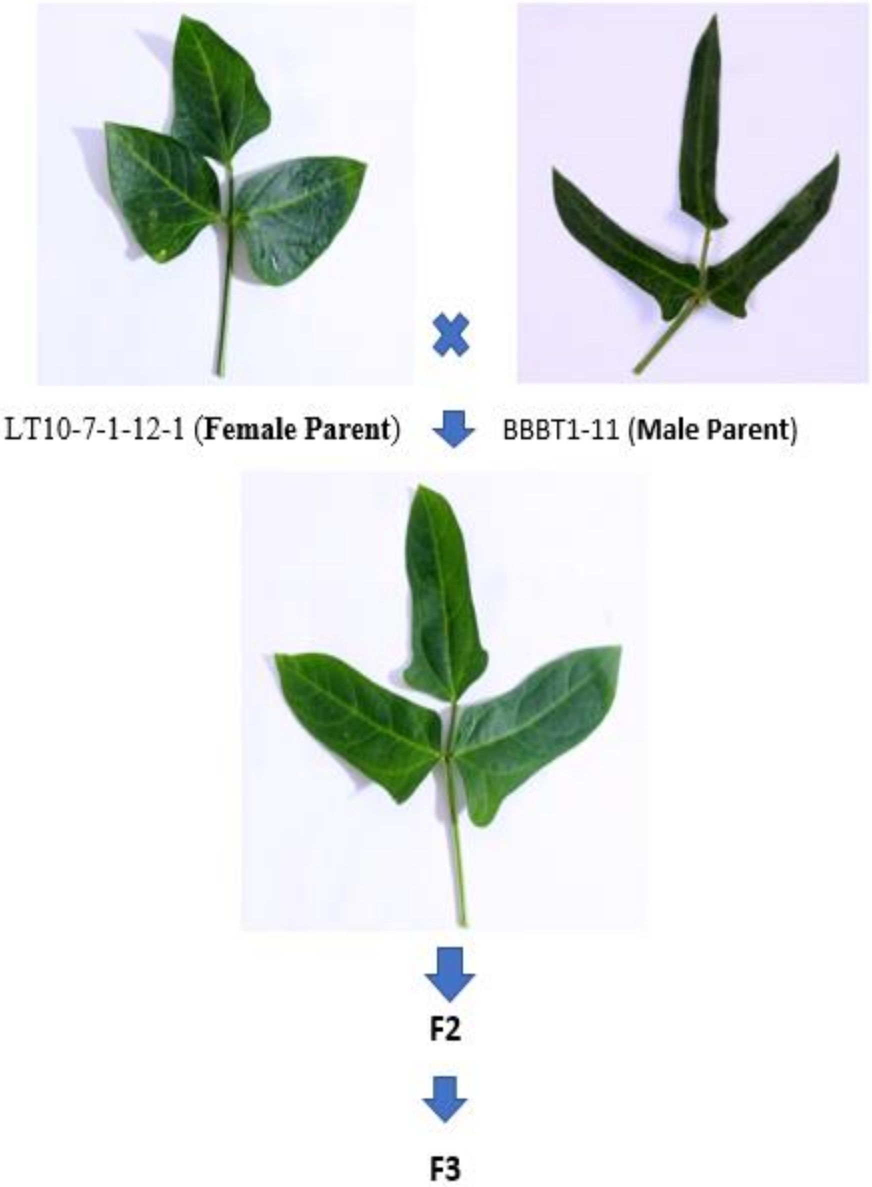

Figure 1: Cross between LT10-7-1-12-1 and BBBT1-11, and population advancement

Similarly, cowpea line LT11-5-2-2-18 (female parent), susceptible to both *A. craccivora* and *C. maculatus*, was crossed with BBBT1-11 (male parent) resistant to both *A. craccivora* and *C. maculatus* to generate F_1 progenies for population 2 (Figure 2). The F_1 individuals from the cross (LT11-5-2-2-18 x BBBT1-11) were self-pollinated to produce 97 F_2 individuals, which were advanced to F_3. The F_1 (68 plants), F_2 (97 plants), and F_3 (178 plants) were analyzed for the identified metabolite content (phenolic compound) to determine their heritability.

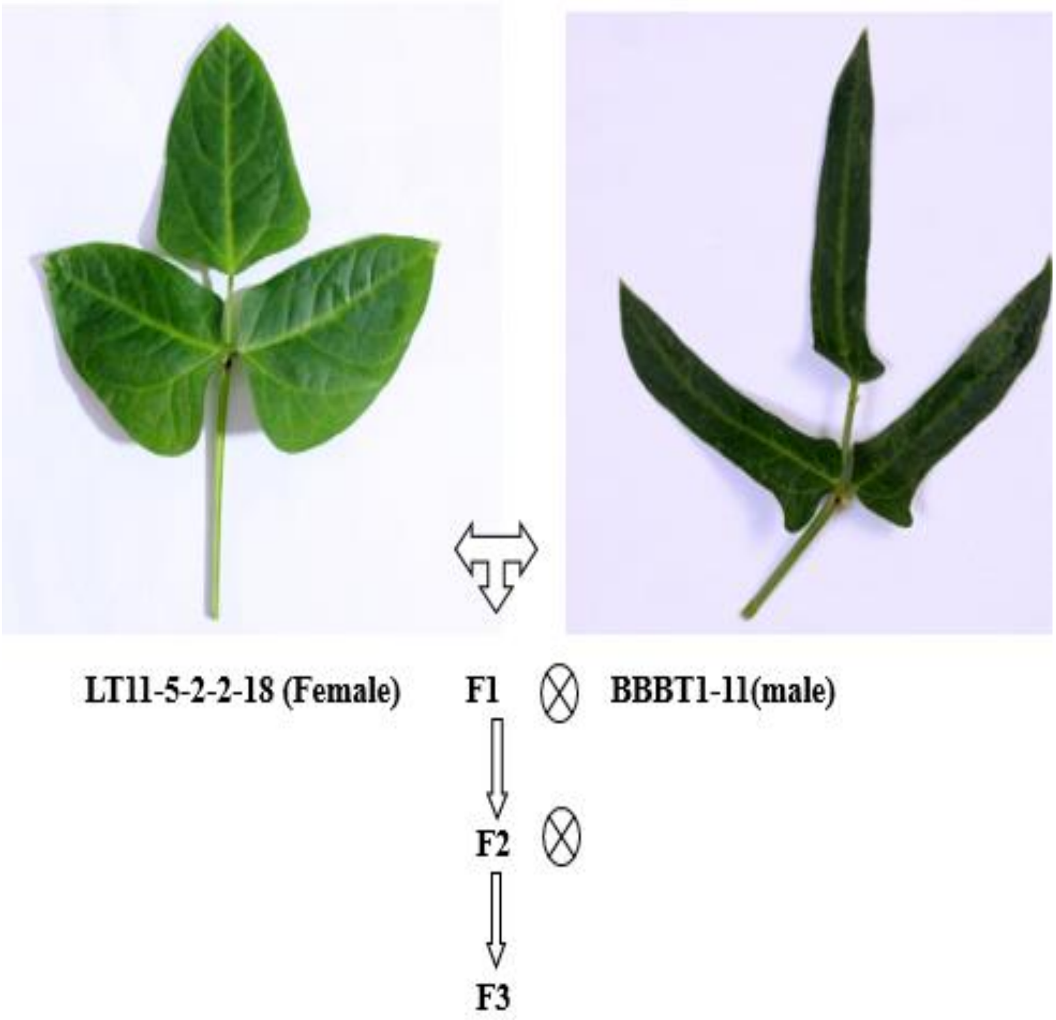

Figure 2: Cross between LT11-5-2-2-18 and BBBT1-11, and population advancement

3.5.2.2 Greenhouse screening process/sample preparation

The screening was conducted using insect-proof cages. There were three plants per pot. 7-day-old cowpea seedlings were infested, each with 5 aphids using a camel hair brush. Water was supplied to the plant directly at the foot to prevent aphid stalling. Plants remained under aphid infestation throughout the test. To avoid water stagnation, the bottoms of the pots were provided with apertures. The same procedure for sample preparation under metabolite determination was also followed to determine the metabolites (section 3.5.1.1.) in all plant populations i.e., F_1, F_2, and F_3.

3.5.2.3 Data collection and analyses for heritability of metabolites.

Chi-squared tests for goodness-of-fit were used to evaluate deviations in the observed data from the expected segregation ratios. These expected ratios were as follows: 3:1 (one dominant gene or two linked genes), 15:1 (two duplicated genes/two complementary genes), and 63:1 (three independent genes). Chi-squared tests for association were used to determine whether there was an association between the metabolite levels (Total Phenolic Content) and phenotypic data (multiple insect resistance). R version 3.5.1 was used at alpha 0.05.

CHAPTER FOUR

RESULTS

4.1 Aphid screening

4.1.1 Non-restrictive method

4.1.1.1 Average Aphid Score

Table 2 presents the average aphid score for the selected genotypes at different intervals as defined by DAI (Days After Infestation). The cowpea genotypes showed significant differences from each other at a 5% level of significance, in terms of the number of aphids per plant. The cowpea line BBBT1-4, recorded the highest aphid score of 3.1 at 5 DAI. This line was not significantly different from cowpea lines; BB-parent (2.8), LT10-7-1-12-1 (2.8), MS1-8-2-2-5-1 (2.5), and MS-parent (1.9). The cowpea lines BBBT1-11 and LT4-2-4-1-2 recorded the lowest aphid score of 1.0 at 5 DAI, which was similar with MS-parent (1.9), LT-parent (1.7), and MS1-8-2-6-8-1 (1.3). At 9, 13, 17, and 21 DAI, cowpea line BBBT1-11 recorded the lowest aphid scores of 1.0, 1.2, 1.4 and 1.0, respectively.

Table 2: Average aphid score for cowpea genotypes under non-restrictive screening method

	DAI				
Genotype	**5**	**9**	**13**	**17**	**21**
BB-Parent	2.8	4.4	7.3	7.5	6.8
BBBT1-11	1.0	1.0	1.2	1.4	1.0
BBBT1-4	3.1	7.5	8.3	8.7	8.3
LT-Parent	1.7	4.2	6.8	6.2	5.8
LT4-2-4-1-2	1.0	1.7	2.1	1.5	1.2
LT10-7-1-12-4	2.8	7.2	7.8	8.2	7.9
MS-Parent	1.9	4.5	5.8	6.3	5.9
MS1-8-2-6-8-1	1.3	1.7	2.3	1.8	1.5
MS1-8-2-2-5-1	2.5	6.8	7.8	7.9	7.2
P-value	0.01	0.00	0.00	0.02	0.01
CD at 5%	1.33	1.39	1.88	1.49	1.68

DAI; days after infestation, CD: critical difference

The lower aphid scores recorded on BBBT1-11 were, however, not significantly different from the aphid score on LT4-2-4-1-2 at 9 (1.7), 13 (2.1), 17 (1.5) and 21 (1.2) DAI and also on MS1-8-2-6-8-1 at 9 (1.7), 13 (2.3), 17 (1.8), and 21 (1.5) DAI. From 9 DAI to 21 DAI, cowpea line BBBT1-4 recorded the highest aphid scores of 7.5 (9 DAI), 8.3 (13 DAI), 8.7 (17 DAI), and 8.3 (DAI) respectively. All the parental lines and some of the mutant lines such as BBBT1-4, LT10-7-1-12-4 and MS1-8-2-2-5-1 as listed in Table 2 showed levels of susceptibility compared to mutant lines BBBT1-11 and MS1-8-2-6-8-1 which indicated good levels of resistance against aphids.

4.1.1.2 Aphid Population Build-up Score

Table 3 presents the rate of aphid population build-up on seedlings of selected cowpea genotypes. Generally, aphid population build-up was very rapid among susceptible genotypes such as LT10-7-1-12-4 which was fully colonized from 7 to 9 DAI. At 5 DAI, there were fewer differences in terms of aphid population build-up between susceptible and resistant cowpea lines, with LT10-7-1-12-4 (2.8) and MS1-8-2-2-5-1 (2.1) having higher aphid population build up than line BBBT1-11 (0.3) which had the lowest.

Table 3: Average aphid population build-up for cowpea genotypes (non-restrictive screening)

	DAI				
Genotype	**5**	**9**	**13**	**17**	**21**
BB-Parent	1.5	2.6	3.3	3.0	2.6
BBBT 1-11	0.3	0.5	1.0	0.9	0.4
BBBT1-4	1.8	3.5	4.3	3.7	3.3
LT-Parent	1.3	2.2	3.2	4.2	3.1
LT4-2-4-1-2	0.8	1.0	1.1	1.0	0.9
LT10-7-1-12-4	2.8	3.9	4.8	4.2	4.0
MS-Parent	1.0	2.5	3.8	3.3	2.9
MS1-8-2-6-8-1	1.0	1.1	1.5	1.0	0.8
MS1-8-2-2-5-1	2.1	3.2	3.8	3.8	3.2
P-value	0.01	0.00	0.00	0.02	0.01
CD at 5%	1.52	0.81	0.99	1.78	1.68

DAI; days after infestation, CD: critical difference

The mutant line LT10-7-1-12-4, recorded significantly higher aphid population build-up from 9 to 21 DAI at 9 DAI (3.9), 13 DAI (4.8), 17 DAI (4.2), and at 21 DAI (4.0). Genotype LT10-7-1-12-4, was not significantly different from BBBT1-4 (3.5) and MS1-8-2-2-5-1 (3.2) at 9 DAI, BBBT1-4 (4.3), MS-parent (3.8) and MS1-8-2-2-5-1 (3.8) at 13 DAI, and BB-parent (3.0), MS-parent (3.3), LT-parent (4.2), BBBT1-4 (3.7) and MS1-8-2-2-5-1 (3.9) at 17 DAI. Unlike LT10-7-1-12-4, mutant line BBBT1-11, recorded the lowest readings of the aphid population build up at 9 DAI (0.5), 13 DAI (1.0), 17 DAI (0.9), and at 21 DAI (0.4) respectively. All the selected cowpea lines showed a decreasing trend in aphid population build-up after the 13th day from infestation except for LT-Parent and MS1-8-2-2-5-1, however, the rate at which the population build up decreased varied significantly at 5% level of significance.

4.1.1.3 Plant Vigour Analysis at 21 DAI

Table 4 presents the results obtained, showing that the plant vigour of different cowpea varieties was significantly different ($P<0.05$). Cowpea lines BBBT1-11, LT4-2-4-1-2, and MS1-8-2-6-8-1 scored the highest plant vigour of 3.0. Cowpea line LT10-7-1-12-1 recorded the lowest score of 1.0. Genotypes BBBT1-4 (1.2) and MS1-8-2-2-5-1 (1.3) had similar plant vigour at 21 DAI.

Table 4: Plant vigour for cowpea genotypes under non-restrictive screening method at 21 DAI

Genotype	**Average Score**
BB-Parent	1.9
BBBT 1-11	3.0
BBBT1-4	1.2
LT-Parent	2.2
LT4-2-4-1-2	3.0
LT10-7-1-12-4	1.0
MS-Parent	1.7
MS1-8-2-6-8-1	3.0
MS1-8-2-2-5-1	1.3
P-value	0.00
CD at 5%	0.67

CD: critical difference

4.1.1.4 Percentage survival of seedlings from 5 to 21 DAI

Susceptible and resistant cowpea lines exhibited significant differences ($P<0.05$), from 13 DAI to 21 DAI (Table 5). At 21 DAI, BBBT1-4 recorded the lowest percentage survival rate of 33%. Cowpea line LT10-7-1-12-4 which recorded 44.4% was not significantly different from the mutant lines BBBT1-4. Cowpea lines BBBT1-11, LT4-2-4-1-2, and MS1-8-2-6-8-1 recorded a survival percentage rate of 100%, which is a good indication of resistance against aphid attack.

Table 5: Average survival rate for cowpea genotypes under non-restrictive screening method

	DAI				
Genotype	**5**	**9**	**13**	**17**	**21**
BB-Parent	100	100	100	77	55
BBBT 1-11	100	100	100	100	100
BBBT1-4	100	77	77	67	33
LT-Parent	100	100	88	88	77
LT4-2-4-1-2	100	100	100	100	100
LT10-7-1-12-4	100	100	67	67	44
MS-Parent	100	100	100	100	77
MS1-8-2-6-8-1	100	100	100	100	100
MS1-8-2-2-5-1	100	100	100	89	89
P-value	>0.05	>0.05	0.00	0.00	0.00
CD at 5%	ns	ns	23.12	17.81	11.31

DAI; days after infestation, CD: critical difference

4.1.2 Restrictive method

4.1.2.1 Average Aphid Score

Table 6 presents the average aphid score for the genotypes at different intervals as defined by DAI. The resistant and susceptible cowpea genotypes showed significant differences from each other at a 5% level of significance, in terms of the number of aphids per plant. The cowpea lines LT4-2-4-1-1, LT11-3-3-12 and Sanzi, recorded the highest aphid score of 4.3 at 5 DAI. These lines were not significantly different from cowpea lines; Lunkhwakwa (3.7), LT 11-5-1-1-4 (3.7), TVu 2027 (3.0), Namuseba (3.0), BB7-9-7-5-1 (3.0), BB7-9-7-5-4 (3.7), Lukusuzi (3.7), BB10-4-2-3-1 (3.0), BB10-4-2-3-3 (3.0), LT3-8-4-1-4 (3.0), and MS 1-8-2-6-8-1 (3.0). The cowpea line BB10-4-2-3-

2 recorded the lowest aphid score of 1.0 which was similar to BBBT1-11, BBBT1-5 and LTBT1-5 with aphid scores of 1.7, 2.3 and 2.3 respectively. At 9, 13, 17 and 21 DAI, cowpea line BB10-4-2-3-2 recorded the lowest aphid scores of 1.0, 1.0, 1.0 and 0.7 respectively.

Table 6: Average aphid score for cowpea genotypes under restrictive screening method

	DAI				
Genotype	**5**	**9**	**13**	**17**	**21**
BBBT 1-11	1.7	3.0	1.7	1.0	1.0
BB10-4-2-3-2	1.0	1.0	1.0	1.0	0.7
Namuseba	3.0	3.7	3.7	3.0	1.0
BB7-9-7-5-4	3.7	5.0	4.3	3.0	3.0
BB10-4-2-3-1	3.0	4.3	3.7	3.0	1.7
Lukusuzi	3.7	7.0	7.0	5.7	3.7
BB7-9-7-5-1	3.0	6.3	6.3	5.0	4.3
LT4-2-4-1-2	3.0	7.0	6.3	5.0	5.0
MS1-8-2-6-8-1	3.0	6.3	6.3	5.0	5.0
BBBT1-7	3.0	5.0	7.0	5.7	5.0
LTBT1-5	2.3	5.7	6.3	5.0	5.0
LT3-8-4-1-4	3.0	5.0	7.0	7.0	5.7
BB10-4-2-3-3	3.0	5.0	6.3	5.7	5.0
IT07K205-8	3.0	5.0	5.7	7.7	4.3
BBBT1-5	2.3	5.0	7.0	5.7	5.0
TVu 2027	3.0	5.0	5.0	5.0	3.7
LT11-5-1-1-4	3.7	7.0	5.7	7.7	7.7
Lunkhwakwa	3.7	5.7	6.3	5.0	5.0
Sanzi	4.3	5.7	5.7	7.0	7.0
LT11-3-3-12	4.3	5.7	7.0	9.0	9.0
LT4-2-4-1-1	4.3	7.0	6.3	7.7	7.0
P-value	0.01	0.00	0.00	0.02	0.01
CD at 5%	1.33	1.39	1.88	1.49	1.68

DAI; days after infestation, CD: critical difference

The lower aphid scores recorded by BB10-4-2-3-2 were not significantly different from the aphid score of BBBT1-11 on 13 DAI (1.7), 17 DAI (1.0) and 21 DAI (1.0) and also for Namuseba (1.0) and BB10-4-2-3-1 (1.7) on 21 DAI. At 9 DAI, cowpea lines LT4-2-4-1-1, LT4-2-4-1-2, Lukusuzi and LT11-5-1-1-4 had the highest aphid score of 7.0 which was significantly different at 5% level of significance, from BB10-4-2-3-2 (1.0), BBBT1-11 (1.7), BBBT1-5 (2.3) and LTBT1-5 (2.3). On 13, 17 and 21 DAI, LT11-3-3-12 recorded the highest aphid scores of 7.0, 9.0 and 9.0 respectively. Cowpea lines BB10-4-2-3-2 and BBBT1-11 exhibited aphid resistance consistently from 5 to 21 DAI while cowpea line such as Sanzi, TVu 2027, LT4-2-4-1-1, and LT4-2-4-1-2 showed some indications of susceptibility against aphid attack.

4.1.2.2 Aphid Population Build-up Score

Table 7 presents the rate of aphid population build-up on seedlings of some cowpea genotypes. Aphid population build-up was very rapid and the plants were fully colonized within 7 to 9 days after infestation (DAI). At 5 DAI, there were fewer differences in terms of aphid population build-up between susceptible and resistant cowpea lines. 12 cowpea lines recorded the lowest aphid population build-up score of 1.0 at 5 DAI, this included cowpea lines; BBBT 1-11, BBBT1-5, BB10-4-2-3-1, BB10-4-2-3-2, and BB10-4-2-3-3, BB7-9-7-5-1, LT4-2-4-1-2, MS1-8-2-6-8-1, LTBT1-5, LT3-8-4-1-4, and IT07K205-8. These cowpea lines differed significantly from cowpea lines LT11-3-3-12 and LT11-5-1-1-4 that had the highest score of 2.0. Nevertheless, LT11-3-3-12 and LT11-5-1-1-4 recorded the aphid population build-up score, not significantly different at a 5% level of significance from the score of cowpea lines BBBT1-7, Lunkhwakwa and Sanzi that recorded 1.5, 1.7 and 1.7 respectively. At 9, 13, 17, and 21 DAI, cowpea lines BBBT1-11, BB10-4-2-3-1, BB10-4-2-3-2, and Namuseba recorded the lowest scores that were not significantly different. At 9 DAI, the highest score was recorded by cowpea line LT4-2-4-1-2 with a score of 4.0, at 13 DAI the highest score was recorded by Lukusuzi with a score of 4.0, at 17 the highest score was recorded by LT3-8-4-1-4 with the score of 4.0 and lastly at 21 DAI, LT11-5-1-1-4 recorded the highest score of 4.7. It is worth acknowledging, that differences were significantly increasing as DAI was increasing. A high score was observed between 9 and 13 DAI while between 13 and 21 DAI, the rate of aphid population build-up decreased.

Table 7: Aphid population build-up for cowpea genotypes under restrictive screening method

	DAI				
Genotype	**5**	**9**	**13**	**17**	**21**
BBBT1-11	1.0	1.7	1.0	1.0	0.3
BB10-4-2-3-2	1.0	1.0	1.0	1.0	0.7
Namuseba	1.0	1.3	1.0	1.0	1.0
BB7-9-7-5-4	1.3	2.7	2.0	2.3	1.3
BB10-4-2-3-1	1.0	1.7	1.7	1.0	1.0
Lukusuzi	1.3	4.0	4.0	3.3	1.3
BB7-9-7-5-1	1.0	3.7	3.7	3.0	2.0
LT4-2-4-1-2	1.0	4.0	3.7	3.0	2.7
MS1-8-2-6-8-1	1.0	3.7	3.7	3.0	2.7
BBBT1-7	1.5	3.0	4.0	2.5	2.5
LTBT1-5	1.0	3.3	3.7	3.0	2.0
LT3-8-4-1-4	1.0	3.0	4.0	4.0	2.7
BB10-4-2-3-3	1.0	3.0	3.7	3.0	2.3
IT07K205-8	1.0	2.7	3.7	3.7	2.3
BBBT1-5	1.0	2.3	4.0	3.0	2.0
TVu 2027	1.7	3.0	3.0	2.3	1.7
LT11-5-1-1-4	2.0	3.0	4.0	4.0	4.7
Lunkhwakwa	1.7	2.7	3.7	2.7	2.7
Sanzi	1.7	2.7	3.7	3.3	2.3
LT11-3-3-12	2.0	3.0	4.0	4.0	3.3
LT4-2-4-1-1	1.3	2.7	3.7	3.7	3.3
P-value	0.00	0.04	0.03	0.01	0.00
CD at 5%	0.55	0.81	0.92	0.93	1.11

DAI; days after infestation, CD: critical difference

4.1.2.3 Plant Vigour Analysis at 21 DAI

Table 8 presents the results obtained, showing that the plant vigour of different cowpea varieties was significantly different (P<0.05). Cowpea lines BBBT1-11, BB10-4-2-3-2, and LT11-5-1-1-4 scored the highest plant vigour of 3.0. Though not significantly different from cowpea lines BB10-4-2-3-1(2.3), Namuseba (2.3) and LTBT1-5 (2.7). Cowpea line BBBT1-7 recorded the lowest score of 1.0. However, BBBT1-7 was not significantly different at 5% level of significance, from cowpea lines LT4-2-4-1-1 (1.3), LT4-2-4-1-2 (1.7), LT11-3-3-12 (1.7), and Sanzi (1.2)

Table 8: Plant vigour for cowpea genotypes under restrictive screening method at 21 DAI

Genotypes	Average Score
BBBT1-11	3.0
BB10-4-2-3-2	3.0
Namuseba	2.3
BB7-9-7-5-4	2.0
BB10-4-2-3-1	2.3
Lukusuzi	2.0
BB7-9-7-5-1	2.0
LT4-2-4-1-2	1.7
MS1-8-2-6-8-1	2.0
BBBT1-7	1.0
LTBT1-5	2.7
LT3-8-4-1-4	1.7
BB10-4-2-3-3	2.0
IT07K205-8	1.3
BBBT1-5	1.3
TVu 2027	1.3
LT11-5-1-1-4	3.0
Lunkhwakwa	2.0
Sanzi	1.2
LT11-3-3-12	1.7
LT4-2-4-1-1	1.3
P-value	0.03
CD at 5%	0.99

CD: critical difference

4.1.2.4 Percentage survival of seedlings from 5 to 21 days after infestation

Table 9, indicates that the plant survival rate of susceptible and resistant cowpea varieties was not significantly different ($P>0.05$) at 5 and 9 DAI. The cowpea lines were significantly different ($P<0.05$), beginning at 13 DAI to 21 DAI.

Table 9: Plant survival rate (%) for cowpea genotypes under restrictive screening method

	Days After Infestation (DAI)				
Genotype	**5**	**9**	**13**	**17**	**21**
BBBT1-11	100	100	100	100	100
BB10-4-2-3-2	100	100	100	100	100
Namuseba	100	100	100	100	87
BB7-9-7-5-4	100	88	88	66	55
BB10-4-2-3-1	100	100	100	100	100
Lukusuzi	100	100	100	87	87
BB7-9-7-5-1	100	100	100	100	100
LT4-2-4-1-2	100	100	100	100	100
MS1-8-2-6-8-1	100	100	87	87	62
BBBT1-7	100	100	100	100	50
LTBT1-5	100	100	100	100	66
LT3-8-4-1-4	100	100	100	100	87
BB10-4-2-3-3	100	100	100	88	77
IT07K205-8	100	100	77	77	44
BBBT1-5	100	100	100	100	88
TVU 2027	100	100	80	80	20
LT11-5-1-1-4	100	100	100	100	100
Lunkwakwa	100	100	100	100	88
Sanzi	100	100	100	100	88
LT11-3-3-12	100	100	88	77	77
LT4-2-4-1-1	100	100	100	100	77
P-value	0.28	0.12	0.00	0.00	0.00
CD at 5%	ns	ns	21.99	18.97	19.46

At 21 DAI, TVu 2027 and IT07K205-8 recorded the lowest percentage survival rate of 20 and 44.4 respectively. Cowpea line IT07K205-8 which recorded 44.4 was not significantly different from the mutant lines BBBT1-7 (50%), BB7-9-7-5-4 (55.5%) and MS1-8-2-6-8-1 (62.5%). Cowpea lines BBBT1-11, BB10-4-2-3-1, BB10-4-2-3-2, BB7-9-7-5-1, LT4-2-4-1-2, and LT11-5-1-1-4 recorded a survival percentage rate of 100%, which is a good indication of resistance against aphids.

4.2 Bruchid screening

4.2.1 No choice test

The results obtained in the study revealed that 110 cowpea genotypes screened using the no-choice laboratory condition showed significant differences ($P<0.05$) in the expression of resistance to *C. maculatus*. Significant differences were observed among the genotypes for the number of eggs laid per 100 seeds of each genotype, adult emergence, developmental period, growth index, percentage infestation, weight loss, and frass produced.

4.2.1.1 No. of eggs laid/100 seeds

Significant variations in the number of eggs per 100 seeds of different cowpea genotypes, occurred at a 5% level of significance, from 13.01 in BBBT1-11 to 135.12 in MS-Parent (Table 10). Significantly less oviposition was seen on BBBT1-11(13.01 eggs/100 seeds) which was similar to the mutant genotypes LT11-5-2-2-2(18.27), LT11-5-2-2-4(19.61), LTBT1-4(27.61), LT11-5-2-2-10(24.81), LT11-5-2-2-20(18.67), BB10-4-2-3-1(14.67), BB10-4-2-3-3(24.33), BB7-9-7-5-3(20.07), MS1-8-2-6-6-2(14.67), and BBBT1-7(28.67). The genotype MS1-8-2-6-9-1 along with the other 43 genotypes not shown were more preferred by *C. maculatus* with significantly greater number of eggs laid per 100 seeds (Table 10).

4.2.1.2 Adult emergence

The percent adult emergence of *C. maculatus* on different cowpea genotypes varied significantly from 12.72% to 88.11% (Table 10). The lowest adult emergence was recorded on BBBT1-11 (12.72%) which was statistically at par with LT11-5-2-2-7 (16.71%) and LT11-5-2-2-10 (15.34%) indicating resistance. The highest adult emergence was recorded on LTBT1-5 (88.11%) which was similar to MS1-8-2-6-9-1 (84.01%) and LT parent (86.31%) indicating susceptibility to *C. maculatus*.

4.2.1.3 Development Period

The developmental period varied significantly ($P<0.05$) between susceptible and resistant varieties (Table 10). Longer developmental period (>30 days) was associated with resistant varieties such as BBBT1-11 while shorter development period (<30 days) was associated with susceptible varieties such as BB10-4-2-3-3. The shortest developmental period was recorded for BB10-4-2-3-3 and MS-parent at 19 days, which was statistically at par with 83 other genotypes out of 110 genotypes screened against *C. maculatus* and these indicated susceptibility. The longest developmental period was recorded for the mutant genotype LT11-5-2-2-4 (31 days) which was statistically at par with 9 genotypes out of 110 genotypes screened against *C. maculatus.*

4.2.1.4 Weight loss

The results on weight loss caused by *C. maculatus* to the seeds of different cowpea genotypes varied significantly (Table 10). The lowest weight loss of 0.94%, was recorded for the mutant genotype LT11-5-2-2-2, which was statistically at par with LT11-5-2-2-4 (2.68%), LT11-5-2-2-10 (2.69%), LT11-5-2-2-20 (3.66%), BB10-4-2-3-1 (1.76%), BB10-4-2-3-3 (0.96%), BB7-9-7-5-3 (2.12%), MS1-8-2-6-6-2 (3.17%), BBBT1-11 (1.05%), IT99K241-2 (2.03%) and IT99K573-2-1 (2.24%) indicating resistance to *C. maculatus*. Significantly ($P<0.05$) higher weight loss was recorded for LT16-7-2-5-1 (58.58%) which was statistically at par with MS1-8-2-6-9-1 (55.86%) and other two unlisted genotypes, indicating susceptibility to *C. maculatus.*

4.2.1.5 Frass produced

Genotypes BBBT1-11, BB10-4-2-3-1 and BB10-4-2-3-3 had the lowest frass (0.23g) which was not different from mutant genotypes LT11-5-2-2-2 (0.27g), LT11-5-2-2-4 (0.41g), LT11-5-2-2-10 (0.37g), LT11-5-2-2-20 (0.43g), BB7-9-7-5-3 (0.37g), MS1-8-2-6-6-2 (0.47g), and BBBT1-7 (0.43g) and also the pure lines IT99K241-2-1 (0.42g) and IT99K573-2-1 (0.37g) indicating resistance to *C. maculatus* (Table 10). The genotype LT16-7-2-5-1 recorded the highest amount of frass produced (6.53g) which can be attributed to more grain damage caused by *C. maculatus*.

4.2.1.6 Growth index

The data concerning the growth index of *C. maculatus* on different cowpea genotypes varied from 0.42 recorded for BBBT1-11 to 4.40 recorded for LTBT1-5 (Table 10). The highest growth index was recorded for LTBT1-5 which was statistically at par with 35 genotypes out of the 110 genotypes screened against *C. maculatus.*

Table 10: Averages of parameters measured during the evaluation of cowpea genotypes against bruchids

Genotypes	**No. eggs/ 100 seeds**	**AEP**	**DP**	**GI**	**PI**	**WLP**	**FP**
LT-Parent	**102.11**	**86.31**	**20**	**4.25**	**43.03**	**27.77**	**1.73**
LTBT1-4	27.61	63.01	27	2.31	13.33	5.51	0.69
LTBT1-5	118.21	88.11	20	4.40	93.22	44.01	5.07
LT11-5-2-2-2	18.27	27.01	30	0.89	13.11	0.94	0.27
LT11-5-2-2-4	19.61	54.72	31	1.76	16.02	2.68	0.41
LT11-5-2-2-10	24.81	15.34	27	0.56	13.33	2.69	0.37
LT11-5-2-2-20	18.67	32.33	24	1.37	8.67	3.66	0.43
LT16-7-2-5-1	123.17	80.31	20	4.08	95.45	58.58	6.53
BB-Parent	**110.34**	**78.21**	**20**	**3.91**	**40.67**	**15.89**	**1.63**
BBBT1-1	124.31	49.72	24	2.04	95.01	37.94	4.33
BBBT1-3	36.17	19.71	31	0.64	12.33	5.63	0.53
BBBT1-7	28.67	42.32	22	1.95	4.02	6.94	0.43
BBBT1-11	13.01	12.72	30	0.42	10.03	1.05	0.23
BB7-9-7-5-3	20.07	61.02	26	2.35	11.01	2.12	0.37
BB10-4-2-3-1	14.67	42.31	30	1.39	13.01	1.76	0.23
BB10-4-2-3-3	24.33	79.72	19	4.19	14.01	0.96	0.23
MS-Parent	**135.12**	**79.41**	**19**	**4.20**	**78.45**	**36.11**	**4.11**
MS1-8-2-6-6-2	14.67	32.32	20	1.61	8.03	3.71	0.47
MS1-8-2-6-9-1	128.67	84.01	22	3.82	90.33	55.86	5.41
IT99K241-2	67.67	51.71	21	2.42	13.04	2.03	0.42
IT99K573-2-1	47.67	79.31	22	3.55	17.02	2.24	0.37
S. Em±	1.87	1.57	0.40	0.18	0.68	0.49	0.09
CD at 5%	17.57	4.48	5.21	0.37	9.39	2.90	0.42
CV %	18.16	9.48	3.12	0.47	11.21	7.90	1.34

ADP, Adult Emergence Percent; DP, Development Period (days); GI, Growth Index; PI, Percent Infestation; WLP, Weight Loss Percent; FP, Frass Produced (grams).

4.2.1.7 Percent Infestation

Percent infestation by *C. maculatus* among different cowpea genotypes, varied from 4.02% recorded for BBBT1-7 to 95.42% recorded for LT16-7-2-5-1 (Table 10). Significantly less damage was recorded for the mutant line BBBT1-7 at 4.02%, which was statistically at par with LT11-5-2-2-2 (13.11%), LTBT1-4 (13.33%), LT11-5-2-2-10 (13.33%), LT11-5-2-2-20 (8.67%), BBBT1-11 (10.03%) and IT99K241-2 (13.04%). The highest damage was recorded for the mutant line LT16-7-2-5-1 at an infestation of 95.42%, which was statistically at par with MS1-8-2-6-9-1 (90.33%), LTBT1-5 (93.22%), BBBT1-1 (95.01%) and other 22 genotypes not listed in Table 10. Based on percent infestation, 15 genotypes were classified as highly resistant, 19 genotypes were classified as moderately resistant, 16 genotypes were classified as least susceptible while moderately susceptible genotypes were 21 and highly susceptible genotypes were 39 (Table 11). Genotype BBBT1-7 was classified as highly resistant though it recorded some degree of damage of 4.02%. The classification of the genotypes is based on the earlier classification reported by Miesho et al. (2018) and Senthilraja and Patel (2021).

Table 11: Resistance/susceptibility rating based on percent infestations

Class	**Percent Infestation**	**Number of genotypes**
Highly resistant	0-20	15
Moderately resistance	21-40	19
Least susceptible	41-60	16
Moderately susceptible	61-80	21
Highly susceptible	81-100	39

4.2.2 Relationship between Growth Index of C. maculatus and various parameters

The relationship between growth index and various growth parameters of *C. maculatus* in different cowpea genotypes (Table 12) showed that growth index had a negative association which was highly significant with the developmental period (r= -0.83**), highly significant positive association with adult emergence (r=0.85**), non-significant weak positive association with the number of eggs laid per 100 seeds (r=0.18), non-significant positive relationship with percent infestation (r=0.07), non-significant positive relationship with weight loss (r=0.21) and frass produced (r=0.41). Similar results were reported by Senthilraja and Patel (2021), who found a significant negative association (r= -0.50**) between growth index with the developmental period and a significant positive association (r= 0.76**) of growth index with adult emergence. Similar observations were reported in the studies by Tripathi et al. (2015) and Miesho et al. (2018).

The developmental period had a significant negative association with adult emergence (r= -0.54**) and a negative significant association with the number of eggs laid per 100 seeds (r= -0.65**). Other parameters also recorded a significant moderate negative association with developmental period viz., percent infestation (r= -0.49**), weight loss (r= -0.49**) and frass produced (r= -0.58**). Appleby and Credland (2004), attributed negative association of developmental period with parameters such as adult emergence, percent infestation, weight loss, and frass to delayed development of *C. maculatus*, which resulted in increased post-embryonic mortality, reduced percent infestation, and consequently, reducing weight loss and frass produced per resistant cowpea genotype. In the current study, the results are in conformity with the report by Miesho et al. (2018) who noted negative significant association (r= -0.67) between developmental period and the number of eggs laid and suggested that this was due to the intraspecific competition arising from the greater number of eggs laid which reduced the adult emergence, percent infestation, weight loss, and finally, frass produced.

Table 12: Correlation matrix of growth parameters of bruchids on cowpea genotypes

Parameters	**GI**	**DP**	**AEP**	**No. of eggs**	**PI**	**WLP**	**FP**
GI	-	-0.83**	0.85**	0.18	0.07	0.21	0.41
DP		-	-0.54**	-0.65**	-0.49**	-0.49**	-0.58**
AEP			-	0.23	0.34	0.48**	0.43**
No. of eggs				-	-0.08	0.31	0.06
PI					-	0.69**	0.55**
WLP						-	0.89**
FP							-

***Highly significant at 1 percent level; AEP, Adult Emergence Percent; DP, Development Period (days); GI, Growth Index; PI, Percent Infestation; WLP, Weight Loss Percent; FP, Frass Produced.*

4.3 Metabolite Analysis

4.3.1 Identification of metabolites

4.3.1.1 Qualitative Metabolite Analyses

The results on the qualitative estimation of secondary metabolites associated with resistance to *A. craccivora* and *C. maculatus* in cowpea are presented in Table 13.

Qualitative differences were observed in all the metabolites under consideration across different genotypes. Mutant lines BBBT1-11 and MS1-8-2-6-8-1 recorded a moderate presence of the total alkaloid content both in the leaf and seed samples. Mutant lines LT11-5-2-2-18 and LT11-5-2-2-2 recorded low presence of total alkaloids in the leaf while a moderate presence of alkaloid content was recorded by both genotypes in the seed samples. Mutant line LT10-7-1-12-1 susceptible to both aphids and bruchids recorded a lower presence of total alkaloids in both the leaf and seed samples. Mutant lines LT10-7-1-12-1 and LT11-5-2-2-18 susceptible to both aphids and bruchids indicated similar qualitative traits interms of total sugars, total phenolics, and total tannins. However, they indicated differences in the alkaloid content in the seed samples (Table 13).

Table 13: Qualitative analysis of cowpea genotypes showing different response to aphids and bruchids

	Status		**TAC**		**TPC**		**TTC**		**TS**	
Genotypes	**A**	**B**	**leaf**	**seed**	**leaf**	**seed**	**leaf**	**seed**	**leaf**	**seed**
BBBT1-11	R	R	++	++	+++	+++	+	+	+	+
LT10-7-1-12-1	S	S	+	+	++	++	+++	+++	++	++
LT11-5-2-2-18	S	S	+	++	++	++	+++	+++	++	++
LT11-5-2-2-2	S	R	+	++	+++	+++	+++	+++	++	++
MS1-8-2-6-8-1	R	S	++	++	++	+++	+	+	+	+

+++ = present (high); ++ = present (moderate); + = present (Low); A=Aphids; B=Bruchids; R=Resistant; S=Susceptible; TAC; Total Alkaloid Content, TPC; Total Phenolic Content, TTC; Total Tannin Content, TS; Total Sugars.

4.3.1.2 Quantitative Metabolite Analyses

The analysis of variance showed significant differences in all the variables under consideration across all the genotypes in the study (Table 14).

Table 14: ANOVA for differences in leaf biochemical traits among the studied cowpea

Source of variation	**DF**	**TAC**	**TPC**	**TTC**	**TS**
Genotypes	4	3.71***	38.49***	116.87***	0.12***
Error to residue	10	3.18	9.89	13.63	0.01

**** Significant at p<0.001 respectively, TAC; Total Alkaloid Content, TPC; Total Phenolic Content, TTC; Total Tannin Content, TS; Total Sugars.*

Table 15: Leaf biochemical constituents in resistant and susceptible cowpea genotypes

Genotypes	**Aphids**	**Bruchids**	**TAC (%)**	**TPC (mg/100g)**	**TTC (mg/100g)**	**TSC (g/Kg)**
BBBT1-11	R	R	53.12	175.23	111.41	17.02
LT10-7-1-12-1	S	S	11.84	71.23	313.01	54.67
LT11-5-2-2-18	S	S	17.31	62.45	282.11	42.81
LT11-5-2-2-2	S	R	28.89	113.24	130.93	42.12
MS1-8-2-6-8-1	R	S	48.11	98.61	112.23	24.63
S. Em±			3.64	7.32	5.71	3.45
CD at 5%			5.97	21.23	26.71	7.81
CV (%)			9.31	13.12	17.05	11.34

R; Resistant, S; Susceptible, TAC; Total Alkaloid Content, TPC; Total Phenolic Content, TTC; Total Tannin Content, TSC; Total Sugar Content.

The TAC ranged between 11.84% to 53.12% (Table 15). The lowest reading was recorded in the mutant line LT10-7-1-12-1 (11.84%), susceptible to both aphids and bruchids, and the highest reading was recorded in BBBT1-11 (53.12%). Although, BBBT1-11 recorded the highest reading in terms of total alkaloid content, it was not significantly different from mutant line MS1-8-2-6-8-1 (48.11%), susceptible to bruchids but resistant to aphids. It is also worth noting that, mutant lines that were susceptible to both aphids and bruchids (LT10-7-1-12-1 and LT11-5-2-2-18) recorded total alkaloid content readings that were not significantly different from each other (Table 15).

Significant differences were recorded in TPC among different genotypes. TPC ranged between 62.45mg/100g to 175.23mg/100g. All the genotypes significantly differed apart from the genotypes that were susceptible to both aphids and bruchids (LT10-7-1-12-1 and LT11-5-2-2-18). Mutant line BBBT1-11, recorded the highest TPC of 175.23mg/100g and the lowest reading was recorded by LT11-5-2-2-18 (62.45mg/100g).

The TTC were significantly high among different lines. Mutant line LT10-7-1-12-1 (313.01mg/100g), recorded the highest level of tannins followed by LT11-5-2-2-18 (282.11mg/100g). Mutant line BBBT1-11 (111.41mg/100g) was insignificantly different from MS1-8-2-6-8-1 (112.23 mg/100g).

The TSC ranged between 17.02g/Kg to 54.67g/Kg. The lowest reading was recorded in the mutant line BBBT1-11 (17.02g/Kg), resistant to both aphids and bruchids, and the highest reading was recorded in LT10-7-1-12-1 (54.67g/Kg), susceptible to both aphids and bruchids attack. Although, LT10-7-1-12-1 (54.67g/Kg) and LT11-5-2-2-18 (42.81g/Kg) were susceptible to both aphids and bruchids, they were significantly different in terms of TSC. It is also worth noting that, mutant lines that were susceptible to aphids (LT10-7-1-12-1, LT11-5-2-2-18 and LT11-5-2-2-2) recorded TSC readings that were significantly different from the lines that were resistant to aphids (BBBT1-11 and MS1-8-2-6-8-1).

The analysis of variance for seed biochemical traits showed significant differences in all the variables under consideration across all the genotypes in the study (Table 16).

Table 16: ANOVA for differences in seed biochemical traits among the studied cowpea

Source of variation	**DF**	**TAC**	**TPC**	**TTC**	**TS**
Genotypes	4	22.71***	58.49***	217.87***	7.45×10^{-4}***
Error to residue	10	2.23	5.62	13.63	6.94×10^{-4}

**** Significant at p<0.001, TAC; Total Alkaloid Content, TPC; Total Phenolic Content, TTC; Total Tannin Content, TS; Total Sugars.*

Table 17: Seed biochemical constituents in resistant and susceptible cowpea genotypes

Genotypes	**Aphids**	**Bruchids**	**TAC (%)**	**TPC (mg/100g)**	**TTC (mg/100g)**	**TSC (g/Kg)**
BBBT1-11	R	R	64.43	196.12	132.82	24.12
LT10-7-1-12-1	S	S	13.84	84.21	356.01	67.31
LT11-5-2-2-18	S	S	44.23	72.12	342.11	52.12
LT11-5-2-2-2	S	R	42.11	129.09	167.43	48.23
MS1-8-2-6-8-1	R	S	53.42	101.11	138.93	27.63
S. Em±			3.45	11.41	9.67	5.67
CD at 5%			17.81	17.98	35.21	12.44
CV (%)			8.84	13.01	17.18	11.56

R; Resistant, S; Susceptible, TAC; Total Alkaloid Content, TPC; Total Phenolic Content, TTC; Total Tannin Content, TS; Total Sugar Content.

The TAC ranged between 13.84% to 64.43% (Table 17). The lowest reading was recorded in the mutant line LT10-7-1-12-1 (13.84%), susceptible to both aphids and bruchids, and the highest reading was recorded in BBBT1-11 (64.43%). Although, BBBT1-11 recorded the highest reading in terms of total alkaloid content, it was not significantly different from mutant line MS1-8-2-6-8-1 (53.42%), susceptible to bruchids but resistant to aphids. It is also worth noting that, mutant lines that were susceptible to both aphids and bruchids recorded total alkaloid content readings that were significantly different from each other. Significant differences were recorded in TPC among different genotypes (Table 17). TPC ranged between 72.12mg/100g to 196.12mg/100g. All the genotypes significantly differed apart from the genotypes that were susceptible to both aphids and bruchids (LT10-7-1-12-1 and LT11-5-2-2-18). Mutant line BBBT1-11, recorded the highest TPC of 196.12mg/100g and the lowest reading was recorded by LT11-5-2-2-18 (72.12 mg/100g). The TTC significantly varied among different lines as shown in Table 17. Mutant line LT10-7-1-12-1 (356.01 mg/100g), recorded the highest level of tannins followed by LT11-5-2-2-18 (342.11 mg/100g). Mutant line BBBT1-11 (132.82 mg/100g) was insignificantly different from MS1-8-2-6-8-1 (138.92 mg/100g) and LT11-5-2-2-2 (168.43 mg/100g). The TSC ranged between 24.12 g/Kg to 67.31 g/Kg. The lowest reading was recorded in the mutant line BBBT1-11 (24.12 g/Kg), resistant to both aphids and bruchids, and the highest reading was recorded in LT10-7-1-12-1

(67.31 g/Kg). Although, LT10-7-1-12-1 (67.31 g/Kg) and LT11-5-2-2-18 (52.12 g/Kg) were susceptible to both aphids and bruchids, they were significantly different in terms of TSC. A similar pattern of behaviour observed in the leaf samples in terms of TSC, was also observed in the seed samples of the genotypes in the study. mutant lines that were susceptible to aphids (LT10-7-1-12-1, LT11-5-2-2-18 and LT11-5-2-2-2) recorded TSC readings that were significantly higher compared to the lines that were resistant to aphids (BBBT1-11 and MS1-8-2-6-8-1).

4.3.1.3 Correlation Analyses

The correlation analyses of the dependent variable (resistance/susceptibility trait) and the independent variables (TAC, TPC, TSC and TTC) at the leaf and seed levels are shown in Table(s) 18 and 19. TAC and TPC indicated a positive association with resistance against aphids and bruchids in cowpea, while TSC and TTC indicated a negative association.

Table 18: Correlation analysis for cowpea metabolites and resistance/susceptibility (leaf)

Independent Variables	**Dependent variables (analyzed separately)**					
	Resistance			**Susceptibility**		
Metabolite content (leaves)	correlation coefficient (r)	Asymp Sig (2 sided) p-value (unadjusted estimates)	Asymp Sig (2 sided) p-value (adjusted estimates)	correlation coefficient (r)	Asymp Sig (2 sided) p-value (unadjusted estimates)	Asymp Sig (2 sided) p-value (adjusted estimates)
TAC	0.31^{ns}	0.391	0.687	-0.18^{ns}	0.446	0.783
TPC	0.88***	<0.0001	1.1×10^{-5}	-0.76***	<0.0001	2.3×10^{-5}
TSC	-0.78***	<0.0001	3.7×10^{-7}	0.78***	<0.0001	1.5×10^{-7}
TTC	-0.48**	0.002	0.028	0.81***	<0.0001	2.4×10^{-8}

TAC; Total Alkaloid Content, TPC; Total Phenolic Content, TTC; Total Tannin Content, TS; Total Sugar Content.

The positive association between TAC and resistance was non-significant while the positive association between TPC and resistance was highly significant. In regard to susceptibility, TAC and TPC indicated a negative association while TSC and TTC positively and significantly correlated with susceptibility. The results in the current study indicate phenolic content (TPC) among other metabolites, to be highly associated with the multiple resistance trait against aphids and bruchids in cowpea mutant genotypes understudy.

Table 19: Correlation analysis for cowpea metabolites and resistance/susceptibility (seed)

Independent Variables	**Dependent variables (analyzed separately)**					
	Resistance			**Susceptibility**		
Metabolite content (seeds)	Correlation coefficient (r)	Asymp Sig (2 sided) p-value (unadjusted estimates)	Asymp Sig (2 sided) p-value (adjusted estimates)	Correlation coefficient (r)	Asymp Sig (2 sided) p-value (unadjusted estimates)	Asymp Sig (2 sided) p-value (adjusted estimates)
TAC	0.28^{ns}	0.211	0.725	-0.11^{ns}	0.526	0.863
TPC	0.87***	<0.0001	3.1×10^{-7}	-0.86***	<0.0001	1.2×10^{-8}
TSC	-0.89***	<0.0001	2.7×10^{-7}	0.84***	<0.0001	3.2×10^{-7}
TTC	-0.41**	0.021	0.048	0.78***	<0.0001	1.7×10^{-5}

TAC; Total Alkaloid Content, TPC; Total Phenolic Content, TTC; Total Tannin Content, TS; Total Sugar Content.

4.3.2 Heritability Analyses

4.3.2.1 Goodness of fit test

To determine the genetic ratio that best fit the observed data, a threshold of 0.001 was used (Table 20). This threshold is often used for statistical tests in plant breeding (Betensky, 2019). The assumptions for the number of genes involved in the heritability of the trait (multiple insect resistance) exhibited by BBBT1-11 are adopted from the study by Ouedraogo et al. (2021).

Table 20: Test for genetic ratios

Parents	Assumptions	Test Ratio	N. P	O.V R	O.V S	E.V R	E.V S	df	dfi	X^2	p-value
A × B	1 dominant gene or 2 linked genes	3:1	197	190	7	148	49	1	196	47.92^{***}	$<7.6x10^{-6}$
A × B	2 Duplicat / 2 Compli. genes	9:7	197	190	7	111	86	1	196	128.79^{***}	$<2.1x10^{-7}$
A × B	≥3 genes	63:1	197	190	7	194	3	1	196	5.42^{*}	0.049
C × B	1 dominant gene or 2 linked genes	3:1	178	173	5	133	45	1	177	47.58^{***}	$<7.6x10^{-6}$
C × B	2 Duplicate/ 2 Compli. genes	9:7	178	173	5	100	78	1	177	121.61^{***}	$<2.1x10^{-7}$
C x B	≥3 genes	63:1	178	173	5	175	3	1	177	1.36^{ns}	0.193

Abbreviations: A., LT10-7-1-12; B., BBBT1-11; C., LT11-5-2-2-18; Compl., complementary; df, degree of freedom of ration categories; dfi, degree of freedom of individuals; duplicat., duplicate; E.V., expected value; N.P., total number of plants; O.V., observed value; R, resistant; S, susceptible.

Population 1 (A × B) and population 2 (C × B), had p-values less than 0.05 except for the third assumption (≥3 genes) (Table 20). However, these results could be grouped into two categories. The first category concerns tests where the P value was lower than 0.001, and the second category concerns tests where the P value was higher than 0.001. Thus, for Population 1, in the case of one dominant gene or two linked genes without crossing over (3:1) being responsible for the multiple insect resistant trait, the difference between the observed and expected values was highly significant ($\chi2 = 47.92$; P value $< 7.6 \times 10^{-6}$). In the same population, the observed and expected values were significantly different for cases where there were two duplicate or two complementary genes ($\chi 2 = 128.79$ and P value $< 2.1 \times 10^{-7}$). However, In the same population, for equal or more than 3 genes (63:1), the difference between the observed and expected values was not significant at the 0.01 threshold ($\chi 2 = 5.42$; P value = 0.049). For Population 2 (C × B), in the case of one dominant or two linked genes, the observed and expected data were significantly different ($\chi 2 = 47.58$; P value $< 7.6 \times 10^{-6}$). In the case involving two duplicate or complementary genes, the difference between the observed and expected values was significant ($\chi 2 = 121.61$, P value $< 2.1 \times 10^{-7}$). However, In the same population, for equal to or more than 3 genes (63:1), the difference between the observed and expected values was not significant at the 0.01 threshold ($\chi 2 = 1.36$; P value = 0.193).

4.3.2.2 Determination of association between the phenotypic and phenolic data

The association between the phenotypic data (resistance and susceptibility of cowpea progenies) and the phenolic content was determined for both population 1 and population 2. In population 1, six (6) F_3 plants out of 197 were found unmatched (Table 21), and in population 2, five (5) F_3 plants were found unmatched as well (Table 22).

Table 21: Distribution of susceptible and resistant F_3 plants for population 1

		Phenolic content		
Trait	**Unmatched**	**High**	**Low**	**Total**
Phenotypic Resistant	4	181	5	190
Phenotypic Susceptible	2	2	3	7
Total	6	183	8	197

Table 22: Distribution of susceptible and resistant F_3 plants for population 2

		Phenolic content		
Trait	**Unmatched**	**High**	**Low**	**Total**
Phenotypic Resistant	2	169	2	173
Phenotypic Susceptible	3	1	1	5
Total	5	170	3	178

For population 1, the difference between the observed and expected data were significant (χ2 = 39.85; P value $<1.0 \times 10^{-5}$) and for population 2, the difference between the observed and expected data were also significant (χ2 = 27.66; P value $<1.0 \times 10^{-5}$) (Table 23).

Table 23: Association between phenolic levels and phenotypic data

Population	χ2	df	df1	p-value
Population 1	39.85	1	190	$<1.0\times10^{-5}$
Population 2	27.66	1	172	$<1.0\times10^{-5}$

4.3.2.3 Mode of inheritance based on phenotyping and phenolic levels

The results of the chi-squared test for goodness-of-fit of combined phenotyping and the assumed genotyping data are shown in Table 24. Based on the results in Table 20, the test was limited to three genes or more; the rest of the assumptions (one dominant gene or two linked genes, and two duplicate or complementary genes) were unlikely.

With a test ratio equal to 63:1 for both, Population 1 (A × B) and Population 2 (C × B), the difference between the observed and expected data were not significant (χ2 = 1.35; P value = 0.931 for Population 1 and χ2 = 0.339; P value = 0.456 for Population 2) (Table 24).

Table 24: Determination of the number of genes based on F_3 individuals

Parents	**Assumptions**	**Test Ratio**	**N. P**	**O.V**		**E.V**		**df**	**dfi**	**X^2 Value**	**p-value**
				R	**S**	**R**	**S**				
A x B	≥3 genes	63:1	191	186	5	188	3	1	190	1.35^{ns}	0.931
C x B	≥3 genes	63:1	173	171	2	170	3	1	172	0.339^{ns}	0.456

Abbreviations: A., LT10-7-1-12; B., BBBT1-11; C., LT11-5-2-2-18; df, degree of freedom of ration categories; dfi, degree of freedom of individuals; E.V., expected value; N.P., total number of plants; O.V., observed value; R, resistant; S, susceptible.

The screening of the F_3 individuals of population 1 and population 2 with aphids and bruchids and later analyzing their phenolic levels, confirmed the result of phenotyping. For a genotypic ratio of

63:1, the p-value was not significant, confirming that in both populations, three or more genes are involved in the expression of this trait (multiple insect resistance trait). Thus, the trait can be classified as a complex trait.

CHAPTER FIVE

DISCUSSION

5.1 Aphid screening

5.1.1 Non-restrictive method

5.1.1.1 Average Aphid Score

Some of the plants such as BBBT1-4 (susceptible genotype), were fully colonized within four to nine days after infestation. Significant differences at a 5% level of significance, occurred at 5 DAI and onwards between resistant and susceptible cowpea lines. The findings are consistent with Arturo et al. (1988), in which the researcher reported significant differences in the cumulative number of progenies per aphid female from the fourth day and onwards in a cross between the susceptible line and the two resistant cowpea lines. Ofuya (1993) and Soleymane et al. (2013), also reported significant differences in the number of aphids in susceptible and resistant cowpea varieties. The leaves of cowpea line BBBT1-4 that showed susceptibility in the present study turned yellow, became stunted and started dying at 9 DAI onwards, this agrees with Bata et al. (1987) and Soleymane et al. (2013). The present study had some genotypes such as BBBT1-11, LT4-2-4-1-2, MS1-8-2-6-8-1, that indicated resistance against aphids, this is in conformity with Soleymane et al. (2013), who also reported the genotype IT97K-556-6 having some level of resistance as it withstood the aphid pressure. The constant resistance observed in the cowpea lines BBBT1-11, LT4-2-4-1-2, and MS1-8-2-6-8-1, maybe due to resistance which is expressed through antibiosis and antixenosis compounds that can disturb the functioning of aphids, and also the growth and development of aphids. Therefore, cowpea lines BBBT1-11, LT4-2-4-1-2, and MS1-8-2-6-8-1 suggest high levels of antixenosis and antibiosis. These lines were the least favourable to aphid infestation and BBBT1-4 was the most favourable. According to Teetes (2007) as cited in Ouedraogo et al. (2018), stated that antixenosis and antibiosis activities often result in the rise in insect mortality or reduced longevity and reproduction in most insects.

5.1.1.2 Aphid Population Build-up Score

In the present study, cowpea line BBBT1-11, recorded the lowest scores of aphid population build-up consistently. This line may be classified as a resistant variety against *A. craccivora* as indicated by Giga (2001), who stated that resistance to aphid attack can be characterized by lower insect population density. As observed in the current study, differences in the spread of aphid colonies and variations in the aphid population density on different cowpea lines may be attributed to varying levels of sucrose between susceptible and resistant cowpea lines.

5.1.1.3 Plant Vigour Analysis at 21 DAI

Cowpea lines such as BBBT1-11, LT4-2-4-1-2 and MS1-8-2-6-8-1 out of the 9 selected lines showed higher plant vigour/growth while BB-Parent and LT-Parent lines indicated medium promising vigour (Table 4). The remaining 4 cowpea lines (BBBT1-4, LT10-7-1-12-4, MS1-8-2-2-5-1 and MS-parental line) out of 9 lines showed poor performance. According to Arturo et al. (1988), cowpea lines with medium to good performance managed to resist the severity of an aphid attack. Giga (2001), also reported that resistant varieties can grow vigorously with no or fewer damage symptoms compared to susceptible varieties, regardless of the insect pressure. Singh et al. (1996), further stated that resistance in plants, can be seen by the development of new leaves (trifoliate) even under attack and thus the crop continues to flower and form pods. The observation by Giga (2001) and Singh et al. (1996) are in accordance with the observations made in the current study concerning genotypes such as BBBT1-11 and LT4-2-4-1-2 that exhibited resistance against aphid attack by growing vigorously compared to genotypes such as BBBT1-4 that recorded poor growth.

5.1.1.4 Percentage survival of seedlings from 5 to 21 DAI

The present findings are supported by the findings of Ouedraogo et al. (2018), who recorded the lowest average survival percentage of 33% and the degree of infestation equal to 3.6 in the genotype N°2300 and also the highest survival rate of 93.3% and the degree of infestation equal to 1.4 in the genotype IT97K-556-6. A lower average survival percentage may be an indication of susceptibility to aphid attack in cowpea plants, which might be a result of lower levels of antibiosis activities that failed to reduce the development and multiplication of aphids. Thus, BBBT1-4 and LT10-7-1-12-4 exhibited signs of lower levels of antibiosis activities while cowpea lines BBBT1-11, LT4-2-4-1-2, and MS1-8-2-6-8-1 exhibited signs of higher levels of antibiosis activities, which enabled the plants to develop new leaves (trifoliate) even under aphid attack.

5.1.2 Restrictive method

5.1.2.1 Average Aphid Score

The results of the current study in terms of aphid score recorded under the restrictive screening method, were consistent with those recorded under non-restrictive screening method. The multiplication of aphids was rapid on cowpea lines that showed susceptibility such as Sanzi, TVU2027, LT4-2-4-1-1, LT11-3-3-12 and LT4-2-4-1-2. The findings are in tandem with the observations of Soleymane et al. (2013) who reported significant differences in the number of aphids in susceptible and resistant varieties. Soleymane et al. (2013) reported the genotype IT97K-556-6 having some level of resistance against aphid attack as it withstood the aphid pressure. The constant resistance observed in the cowpea lines BBBT1-11, maybe due to resistance which is expressed through antibiosis and antixenosis compounds that can disturb the functioning of aphids, and also the growth and development of aphids. Therefore, cowpea lines BBBT1-11 and BB10-4-2-3-2 suggest high levels of antixenosis and antibiosis. These lines were the least favourable to aphid infestation and LT11-3-3-12 was the most favourable. According to Teetes (2007) as cited in Ouedraogo et al. (2018), stated that antixenosis and antibiosis activities often result in the rise in insect mortality or reduced longevity and reproduction in most insects.

5.1.2.2 Aphid Population Build-up Score

In the present study, cowpea lines BBBT1-11, BB10-4-2-3-1, BB10-4-2-3-2, and Namuseba recorded the lowest scores of aphid population build-up consistently, though was not significantly different at the 5% level of significance. These lines may be classified as resistant varieties against *A. craccivora* as indicated by Giga (2001), who stated that resistance to aphid attack can be characterized by lower insect population density. As observed in the current study, differences in the spread of aphid colonies and variations in the insect population density on susceptible and resistant cowpea lines may be attributed to varying levels of sucrose (Togola et al., 2020).

5.1.2.3 Plant Vigour Analysis at 21 DAI

The present findings on plant vigour analysis are supported by the findings of Giga (2001) who reported that resistant varieties can grow vigorously with no or fewer damage symptoms compared to susceptible varieties, regardless of the insect pressure. Singh et al. (1996) states that resistance in plants, can be seen by the development of new leaves (trifoliate) even under attack and thus the crop continues to flower and form pods. Arturo et al. (1988), further state that the cowpea lines that showed good performance managed to resist the severity of aphid attack and thus can be classified as resistant varieties.

5.1.2.4 Percentage survival of seedlings from 5 to 21 days after infestation

TVu 2027 and IT07K205-8 exhibited lower levels of antibiosis activities while cowpea lines BBBT1-11, BB10-4-2-3-1, BB10-4-2-3-2, BB7-9-7-5-1, LT4-2-4-1-2, LT11-5-1-1-4 exhibited high levels of antibiosis activities, that enabled the plants to develop new leaves (trifoliate) even under aphid attack. A lower average survival percentage expressed by TVu 2027 and IT07K205-8 may be an indication of susceptibility to aphid attack in cowpea plants, which might be a result of lower levels of antibiosis activities that failed to reduce the development and multiplication of aphids.

5.2 Bruchid screening

5.2.1 No choice test

The results obtained in the study revealed that 110 cowpea genotypes screened using the no-choice laboratory condition showed significant differences ($P<0.05$) in the expression of resistance to *C. maculatus.*

5.2.1.1 No. of eggs laid/100 seeds

Significant variations in the number of eggs per 100 seeds of different cowpea genotypes, occurred at a 5% level of significance, from 13.01 in BBBT1-11 to 135.12 in MS-Parent (Table 10). The genotype MS1-8-2-6-9-1 along with the other 43 genotypes not shown were more preferred by *C. maculatus* with significantly greater number of eggs laid per 100 seeds (Table 10). Sharma and Thakur (2014), reported that some cowpea seeds were more preferred for egg-laying by *C. maculatus* than other cowpea lines. Similar results about oviposition preference of *C. maculatus* on chickpeas and green gram have been reported by Shaheen et al. (2006), Patil et al. (2009), Raghuwashi et al. (2016), Tripathi et al. (2015), and Waghmare and Bantewad (2020). Therefore, the current findings are in accordance with the earlier researchers. The differences in oviposition preference exhibited by *C. maculatus*, may be attributed to different morphological traits of the cowpea seed.

5.2.1.2 Adult emergence

In terms of adult emergence, similar results were reported by Senthilraja and Patel (2021) with regards to susceptible and resistant cowpea genotypes against *C. maculatus*. The resistance of genotypes such as BBBT1-11, and LT11-5-2-2-10 may be attributed to varying sorts of reserve protein and vicilin which cannot be metabolized by the midgut proteinases of insects, thereby reducing the food supply of the larvae and interfering with *C. maculatus* development.

5.2.1.3 Development Period

The findings in the current study were in accordance with the study by Senthilraja and Patel (2021) who recorded the shortest developmental period of 20 days for the genotype GC1710. Similarly results from Tripathi et al. (2015) showed that the developmental period was significantly shorter for susceptible genotypes and significantly longer for resistant genotypes of cowpea. The longer developmental period of *C. maculatus* in cowpea genotypes may be attributed to the antibiosis resistance, leading to the reduction of adult emergence. This was the case also in the study by Smith and Clement (2012), in which the resistant cowpea genotype was found to possess antibiosis resistance against *C. maculatus*.

5.2.1.4 Weight loss

The results indicated that weight loss was much influenced by the levels of susceptibility in different cowpea genotypes (Senthilraja and Patel, 2021). The significant susceptibility levels of LT16-7-2-5-1 and MS1-8-2-6-9-1 may be attributed to variable traits such as low levels of reserve proteins and vicilin, thus were more preferred to *C. maculatus* compared to other genotypes. The study by Deshpande et al (2011), indicates that variation in seed weight loss due to *C. maculatus* infestation occurred mainly due to the variations in adult emergence and percent infestation levels. The observation by Deshpande et al. (2011) is in accordance with the observations made in the current study. Cowpea line LT16-7-2-5-1 that recorded the highest weight loss (58.58%), also recorded the highest percent infestation (95.45%) and adult emergence (80.37%).

5.2.1.5 Percent Infestation

Based on percent infestation, 15 genotypes were classified as highly resistant, 19 genotypes were classified as moderately resistant, 16 genotypes were classified as least susceptible while moderately susceptible genotypes were 21 and highly susceptible genotypes were 39 (Table 11). Genotype BBBT1-7 was classified as highly resistant though it recorded some degree of damage of 4.02%. The classification of the genotypes is based on the earlier classification reported by Miesho et al. (2018) and Senthilraja and Patel (2021). Variation in percent infestation between susceptible and resistant cowpea genotypes has been reported by several authors such as Sarwar (2012), Deshpande et al. (2011), and Augustine et al. (2018). The variation in the susceptibility and resistant levels of the cowpea genotypes in the current study, could be linked to the association between some physiochemical characteristics of the cowpea seeds.

5.2.1.6 Growth index

The highest growth index was recorded for LTBT1-5 (4.40) which was statistically at par with 35 genotypes out of the 110 genotypes screened against *C. maculatus* while the lowest growth index was recorded for BBBT1-11 (0.42). The low growth index of BBBT1-11 indicated resistance while the high growth index of LTBT1-5 indicated susceptibility against attack from bruchids. The current findings are in tandem with the study by Umrao and Verma (2003). The resistance against bruchids indicated by BBBT1-11 may be attributed to the low levels of digestible protein content compared to susceptible genotypes such as LTBT1-5 that might have high levels of digestible protein content.

5.2.1.7 Frass produced

The results concerning frass produced, are in line with Sarwar (2012), who reported a direct relationship (correlation) between frass produced and parameters such as percent infestation (grain damage) and adult emergence. A genotype CH-86/02 which recorded the significantly highest percent infestation of 87% also recorded the significantly highest frass weight of 0.24g, this coincides with the present findings.

5.2.2 Relationship between Growth Index of C. maculatus and various parameters

The significant positive associations between adult emergence percent and weight loss percent (r=0.48**) and frass produced (r=0.43**) and also the significant positive association between percent infestation and weight loss percent (r=0.69**) and frass produced (r=0.55**) were observed in the current study. Sarwar (2012) and Senthilraja and Patel (2021), in their respective studies reported similar observations with resistant genotypes being able to delay the development of *C. maculatus*. The observed delay in the development of *C. maculatus* in resistant genotypes, might have been due to varying types of reserve protein and vicilin which cannot be metabolized by the midgut proteinases of insects, thereby reducing the food supply of the larvae and interfering with *C. maculatus* development.

5.3 Metabolite Analysis

5.3.1 Identification of metabolites

The insignificant difference in the total alkaloid content expressed between BBBT1-11 (resistant to both aphids and bruchids) and MS1-8-2-6-8-1 (resistant to aphids and susceptible to bruchids) at the leaf and seed level, nullifies alkaloids from being responsible for the multiple resistant traits expressed by mutant line BBBT1-11. A combined presence of tannins (free and condensed) was high in mutant lines LT10-7-1-12-1 and LT11-5-2-2-18 (both of which are susceptible to aphids and bruchids). Therefore, even tannin content might not be responsible for multiple resistant traits. The results of the current study in terms of tannin content, correlate with the results reported by Lattanzio et al. (2005). Lattanzio et al. (2005), reported that cowpea lines in his study had seed coats that contained condensed tannins that were positively correlated to their colour but not to their resistance against weevils. Therefore, in his study non-condensed tannins were attributed to the resistance of bruchids than condensed tannins. In the current study, since the recorded tannins were high in susceptible genotypes compared to the resistant genotypes in both leaf and seed samples, it is highly likely that the higher tannin content recorded in susceptible genotypes had a high level of condensed tannins than the non-condensed tannins. Therefore, tannins are equally nullified to be responsible for the multiple insect resistance exhibited by BBBT1-11 genotype.

TSC were equally high in susceptible genotypes than in resistant lines (either against aphids or bruchids). The findings concerning TSC of the current study are in accordance with the findings of Togola et al. (2020), who reported low levels of TSC in resistant varieties against aphids compared to the susceptible varieties that had significantly higher levels of sucrose. In the study by Togola et al. (2020), the HPLC analyses proved that low sucrose levels in the cowpea genotypes, was related with high resistance to aphids. In the current study, aphid resistance exhibited by genotypes such as BBBT1-11 and MS1-8-2-6-8-1 may be attributed to the low level of sucrose. However, TSC may not be responsible for the multiple insect resistance exhibited by BBBT1-11. This is because, though BBBT1-11 and MS1-8-2-6-8-1 have similar levels of TSC and are both resistant towards aphids, MS1-8-2-6-8-1 was susceptible to *C. maculatus* while BBBT1-11 was resistant against *C. maculatus.*

Among the metabolites under consideration in the current study TPC (phenolic compounds), highly indicated the responsibility of being associated with the multiple insect resistant trait expressed by mutant line BBBT1-11. This is because, BBBT1-11 recorded highly significant levels of phenolic compounds compared to all the genotypes, unlike other metabolites considered in the present study. The findings of the current study are related to the findings of Adams-Philip et al. (2010), who stated that plant phenolic compounds confer various physiological functions for the plant to survive and to adapt to environmental disturbances at the pre-post (vegetative) and postharvest (seed) stages. Lattanzio et al. (2005) reported the role that phenolics play in insect defence mechanism. In the investigation by Lattanzio et al. (2005), a positive relationship was found between resistance characteristic to aphids and total phenolic compound content of cowpea lines. In the current study, feeding by the aphids on BBBT1-11 (resistant to both aphids and bruchids) might have induced the production of the phenolic compounds. Phenolic compounds might have exerted toxicity upon being ingested by the aphids and when stored in the seed, affected larval development and also the response of the adult *C. maculatus* negatively. Thus, the multiple resistance against aphids and bruchids exhibited by BBBT1-11 might be attributed to the presence of phenolic compounds.

5.3.2 Heritability Analyses

According to Ouedraogo et al. (2021), the non-significant difference observed between the observed and the expected values for the third assumption in both population 1 and population 2, is an indication of the likelihood of the third assumption (≥3 genes) being the case, in which 3 or more genes were involved in the heritability of the multiple insect resistance trait exhibited by BBBT1-11 from F_1 to F_3 progenies. The remaining ratios tested were less likely to fit the observed data as it can be seen in their P values being very small. The screening of the F_3 individuals of population 1 and population 2 with aphids and bruchids and later analyzing their phenolic levels, confirmed the result of phenotyping. For a genotypic ratio of 63:1, the P value was not significant, confirming that in both populations, three or more gene are involved in the expression of this trait (multiple insect resistance trait). Thus, the trait can be classified as a complex trait.

Insect resistance has three components: resistance/ tolerance, antibiosis, and antixenosis. One or more genes may be involved in the expression of each component (Ouedraogo et al., 2021). Depending on the screening method, the expression of one or several resistance components may be captured. Both antibiosis and antixenosis involve a plant response and a pest response, but in the case of resistance, only a plant response is involved (Smith, 2005). In host plant resistance to insects, antixenosis and antibiosis may be involved simultaneously, but in different proportions (Obopile and Ositile, 2010). The survival rate under insect infestation is an important indicator for assessing insect resistance. Antibiosis and antixenosis are also important for host plant resistance. For instance, plants produce a number of metabolites in response to insects, pathogens, and other stress factors (Sharma and Vaishampayan, 2009). They act as antifeedants and affect insects by reducing their growth and development. Each of the components of host plant resistance to insects has a genetic basis. Plant survival under aphid and bruchid infestation is part of insect resistance. Given the number of genes involved in the expression of this trait, it is controlled by quantitative trait loci (QTLs).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

Significant differences at a 5% level of significance were observed among 110 genotypes under consideration with regard to aphid and bruchid resistance. In general, parental lines were outperformed by mutant lines with regard to aphid and bruchid resistance. Mutant line MS1-8-2-6-8-1 was identified as a genetic resource that is highly resistant to aphids only while LT11-5-2-2-2 was identified as a genetic resource that is highly resistant to bruchids only. The genetic resource identified to be resistant to both aphids and bruchids was BBBT1-11, the mutant line derived from Bubebe (parent material). Among the metabolites under consideration (i.e., alkaloids, phenolics, tannins, and total sugars), phenolics were identified to be highly associated with multiple resistant trait against aphids and bruchids expressed in the mutant line BBBT1-11. The trait possessed by the mutant line BBBT1-11 is under the control of three or more genes (a few major genes). Therefore, according to the findings of the current study, multiple resistance against aphids and bruchids exists in some cowpea lines such as BBBT1-11, and the trait is associated with the phenolic content. Suffice it to say, the trait is highly heritable as it is controlled by a few major genes (three or more genes).

6.2 Recommendations

The following recommendations have been made; the identified genetic materials that are resistant to aphids only, bruchids only, and both aphids and bruchids should be utilized in breeding programs with a target of breeding insect-resistant cowpea genotypes. There is also a need to identify the actual phenolic group of metabolites responsible or associated with the trait of multiple resistance against aphids and bruchids. Once identified, there might be a need to isolate the phenolic group and feed it on both aphids and bruchids to determine whether the phenolic group directly affects the aphids and bruchids. Further research should also be conducted to genotype the resistant material and identify the QTLs responsible for the trait observed in BBBT1-11 (resistant to both aphids and bruchids).

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LIST OF APPENDICES

Appendix 1: Aphid Score

Line	**Genotype**	**Days After Infestation (DAI)**				
		5	**9**	**13**	**17**	**21**
L1	LT-PRT	1.7	4.2	6.8	6.2	5.8
L2	LTBT1-1	2.3	3.3	4.5	3.4	2.1
L3	LTBT1-2	1.8	4.3	5.4	5.4	3.4
L4	LTBT1-3	2.1	2.4	3.1	4.2	3.1
L5	LTBT1-4	3.4	4.5	5.6	7.1	4.3
L6	LTBT1-5	1.0	1.2	1.2	1.2	1.1
L7	LTBT1-6	2.3	2.5	3.4	4.7	3.2
L8	LTBT1-7	2.1	3.4	4.3	3.4	3.1
L9	LT11-5-2-2-1	2.3	2.5	2.7	2.1	1.4
L10	LT11-5-2-2-2	3.4	3.6	4.3	3.2	2.3
L11	LT11-5-2-2-3	2.3	2.5	3.1	3.3	2.1
L12	LT11-5-2-2-4	1.7	2.3	7.1	5.3	4.2
L13	LT11-5-2-2-5	2.4	3.4	3.6	3.7	3.2
L14	LT11-5-2-2-6	1.8	2.4	3.2	4.2	3.2
L15	LT11-5-2-2-7	2.7	2.8	3.4	3.1	2.2
L16	LT11-5-2-2-8	2.3	3.4	5.6	5.2	4.8
L17	LT11-5-2-2-9	2.3	2.6	3.2	3.3	2.1
L18	LT11-5-2-2-10	1.2	2.3	3.4	4.2	2.3
L19	LT11-5-2-2-11	3.2	3.5	4.5	3.2	2.2
L20	LT11-5-2-2-12	2.3	2.7	3.1	3.1	2.1
L21	LT11-5-2-2-13	1.7	2.3	3.1	2.8	2.2
L22	LT11-5-2-2-14	1.8	2.6	3.4	3.1	2.3
L23	LT11-5-2-2-15	2.3	2.9	4.7	2.9	2.5
L24	LT11-5-2-2-16	1.2	2.7	3.1	3.1	2.3

Aphid Score Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L25	LT11-5-2-2-17	2.3	5.6	6.3	3.5	3.2
L26	LT11-5-2-2-18	1.7	2.3	2.4	3.5	2.1
L27	LT11-5-2-2-19	1.1	2.1	3.4	2.1	1.3
L28	LT11-5-2-2-20	1.4	4.5	5.3	5.6	2.3
L29	LT11-5-2-2-21	2.3	3.7	3.6	3.4	2.2
L30	LT3-8-4-1-1	1.8	2.3	4.5	4.2	2.3
L31	LT3-8-4-1-2	2.3	2.5	4.5	3.4	2.4
L32	LT3-8-4-1-3	1.2	2.3	4.5	3.4	2.1
L33	LT3-8-4-1-4	1.1	1.2	1.3	1.1	1.0
L34	LT3-8-4-1-5	1.6	2.3	4.1	4.2	3.5
L35	LT3-8-4-1-6	1.8	2.3	3.2	3.4	2.8
L36	LT3-8-4-1-7	1.5	2.3	3.3	4.5	2.1
L37	LT3-8-4-1-8	1.2	2.3	3.4	3.4	2.1
L38	LT3-8-4-1-9	2.1	2.3	4.5	3.4	2.4
L39	LT10-7-1-12-1	2.3	7.2	7.3	7.4	7.1
L40	LT10-7-1-12-2	2.1	6.8	7.1	7.9	7.5
L41	LT10-7-1-12-3	2.0	5.2	6.1	6.7	5.3
L42	LT10-7-1-12-4	2.8	7.2	7.8	8.2	7.9
L43	LT10-7-1-12-5	2.7	4.5	5.2	6.7	4.8
L44	LT10-7-1-12-6	2.1	6.3	6.7	7.8	7.1
L45	LT10-7-1-12-7	2.5	3.4	4.5	6.7	6.1
L46	LT10-7-1-12-8	1.9	2.3	3.4	6.7	5.2
L47	LT11-5-1-1-1	1.7	5.6	6.5	7.8	3.4
L48	LT11-5-1-1-2	2.0	4.2	5.1	7.2	4.6
L49	LT11-5-1-1-3	2.2	3.2	4.4	4.8	3.8
L50	LT11-5-1-1-4	1.1	1.2	1.2	1.0	1.0
L51	LT11-3-3-12	1.0	1.1	1.1	1.0	1.0

Aphid Score Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L52	LT16-7-2-5-1	2.1	3.2	3.5	3.7	3.2
L53	LT16-7-2-5-2	2.2	3.1	3.4	3.6	3.1
L54	LT16-7-2-5-3	1.9	2.1	3.2	3.3	2.3
L55	LT16-7-2-5-4	1.3	2.1	3.2	3.4	2.4
L56	LT16-7-2-5-5	1.4	2.3	3.4	4.7	3.2
L57	LT3-8-4-6-1	2.3	4.4	5.6	6.7	5.4
L58	LT3-8-4-6-2	2.1	3.4	4.3	5.7	4.3
L59	LT3-8-4-6-3	3.4	4.5	5.6	7.3	3.2
L60	LT4-2-4-1-1	1.0	1.0	1.1	1.0	1.0
L61	LT4-2-4-1-2	1.0	1.7	2.1	1.5	1.2
L62	**BB-PRT**	2.8	4.4	7.3	7.5	6.8
L63	BBBT1-1	3.4	4.6	7.8	6.8	3.3
L64	BBBT1-2	2.9	4.7	6.4	6.8	3.4
L65	BBBT1-3	2.8	4.4	7.3	7.5	6.8
L66	BBBT1-4	3.1	7.5	8.3	8.7	8.3
L67	BBBT1-5	1.2	1.3	1.0	1.0	1.0
L68	BBBT1-6	1.8	3.5	4.5	8.3	4.5
L69	BBBT1-7	1.7	2.3	1.2	1.0	1.0
L70	BBBT1-8	3.3	7.5	8.3	7.4	5.4
L71	BBBT1-9	3.1	5.6	5.8	7.3	4.5
L72	BBBT1-10	3.1	7.5	8.3	8.7	8.3
L73	BBBT1-11	1.0	1.0	1.2	1.4	1.0
L74	BB7-9-7-5-1	1.0	1.1	1.2	1.0	1.0
L75	BB7-9-7-5-2	2.7	3.8	4.6	3.9	3.2
L76	BB7-9-7-5-3	2.4	3.9	4.5	4.1	3.2
L77	BB7-9-7-5-4	1.0	1.0	1.1	1.2	1.0
L78	BB7-9-7-5-5	3.2	3.4	5.6	4.5	4.3

Aphid Score Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	**9**	**13**	**17**	**21**
L79	BB7-9-7-5-6	2.8	3.4	4.7	4.5	3.8
L80	BB10-4-2-3-1	1.2	1.3	1.5	1.0	1.0
L81	BB10-4-2-3-2	1.0	1.2	1.2	1.1	1.0
L82	BB10-4-2-3-3	1.0	1.2	1.3	1.1	1.0
L83	BB14-16-2-2	2.1	3.7	4.1	5.6	3.8
L84	BBVN-1	1.9	4.5	4.1	5.1	4.1
L85	BBVN-2	2.7	4.1	5.6	4.8	4.2
L86	**MS-PRT**	1.9	4.5	5.8	6.3	5.9
L87	**MS1-8-2-2-5-1**	2.5	6.8	7.8	7.9	7.2
L88	MS1-8-2-6-6-1	3.2	3.3	4.5	3.4	2.1
L89	MS1-8-2-6-6-2	3.2	3.4	4.6	3.3	1.9
L90	MS1-8-2-6-8-1	1.3	1.7	2.3	1.8	1.5
L91	MS1-8-2-6-8-2	2.5	2.3	3.4	3.2	2.1
L92	MS1-8-2-6-8-3	1.5	2.4	3.1	2.3	3.1
L93	MS1-8-1-4-1	1.4	2.5	3.5	2.3	2.3
L94	MS1-8-1-4-2	1.6	2.5	4.5	2.7	2.1
L95	MS10-11-1-1-1	1.1	2.3	5.4	3.4	2.1
L96	MS1-8-2-2-5-1	1.7	1.9	3.1	4.5	3.1
L97	MS1-8-2-6-9-1	2.3	2.4	3.4	5.6	3.3
L98	IT93K452-1	3.4	3.2	3.4	5.6	2.0
L99	IT99K241-2	2.3	3.2	3.4	6.7	2.2
L100	IT99K573-2-1	2.3	2.8	4.5	5.0	3.3
L101	IT99K-7-21-2-2	3.4	2.4	4.1	5.6	3.4
L102	IT07K205-8	1.0	1.0	1.3	1.3	1.0
L103	Aloka Local	3.7	2.6	4.2	5.6	3.3
L104	Danilla	3.4	3.8	4.1	5.6	3.2
L105	TVu3236	2.3	3.7	3.4	5.6	3.1

Aphid Score Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L106	TVu2027	1.1	1.2	1.3	1.0	1.0
L107	Sanzi	1.3	1.3	1.5	1.1	1.1
L108	Lunkwankwa	1.0	1.3	1.4	1.2	1.2
L109	Lukusuzi	1.0	1.5	1.4	1.3	1.0
L110	Namuseba	1.0	1.5	1.3	1.2	1.1

Appendix 2: Aphid Population Build up

Line	**Genotype**	**Days After Infestation (DAI)**				
		5	**9**	**13**	**17**	**21**
L1	LT-PRT	1.3	2.2	3.2	4.2	3.1
L2	LTBT1-1	1.4	1.8	2.1	3.3	2.3
L3	LTBT1-2	1.2	1.6	1.8	3.8	2.5
L4	LTBT1-3	0.7	1.1	2.1	3.5	2.3
L5	LTBT1-4	1.3	2.3	2.4	3.3	3.6
L6	LTBT1-5	0.5	0.8	1.1	0.9	0.8
L7	LTBT1-6	1.4	1.8	2.3	3.2	2.3
L8	LTBT1-7	1.3	1.5	1.8	2.3	3.4
L9	LT11-5-2-2-1	1.2	2.3	3.4	4.4	4.2
L10	LT11-5-2-2-2	0.9	2.5	3.4	4.3	4.1
L11	LT11-5-2-2-3	1.1	2.4	3.2	4.3	3.8
L12	LT11-5-2-2-4	1.2	2.9	3.2	4.6	4.2
L13	LT11-5-2-2-5	2.3	3.2	3.3	3.8	3.2
L14	LT11-5-2-2-6	1.9	2.2	2.7	3.3	2.1
L15	LT11-5-2-2-7	2.1	2.7	2.9	3.4	2.1
L16	LT11-5-2-2-8	2.2	2.9	3.2	4.1	3.7
L17	LT11-5-2-2-9	2.0	2.7	3.2	4.3	3.2
L18	LT11-5-2-2-10	1.3	1.3	1.9	4.5	2.3
L19	LT11-5-2-2-11	1.5	2.3	2.4	3.4	3.2
L20	LT11-5-2-2-12	0.9	3.2	3.3	3.2	2.3
L21	LT11-5-2-2-13	1.2	3.2	3.6	3.2	2.5
L22	LT11-5-2-2-14	1.4	2.4	3.9	3.3	2.3
L23	LT11-5-2-2-15	0.3	2.5	3.8	2.3	2.1
L24	LT11-5-2-2-16	1.3	2.7	3.6	3.1	2.8
L25	LT11-5-2-2-17	1.5	3.0	3.4	2.7	2.2
L26	LT11-5-2-2-18	2.1	2.6	3.3	2.6	2.1
L27	LT11-5-2-2-19	1.8	2.3	2.8	2.4	1.8

Aphid Population Build up Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	**9**	**13**	**17**	**21**
L28	LT11-5-2-2-20	1.4	2.5	2.9	2.1	1.4
L29	LT11-5-2-2-21	1.2	1.8	3.2	2.3	1.3
L30	LT3-8-4-1-1	1.7	2.1	3.6	2.4	1.3
L31	LT3-8-4-1-2	1.8	2.5	2.9	2.1	1.7
L32	LT3-8-4-1-3	1.1	1.5	2.5	2.2	1.8
L33	LT3-8-4-1-4	0.3	0.4	1.0	0.9	0.6
L34	LT3-8-4-1-5	2.3	2.6	3.2	3.1	2.8
L35	LT3-8-4-1-6	2.5	3.6	3.7	3.2	2.5
L36	LT3-8-4-1-7	2.3	3.2	3.8	2.8	2.1
L37	LT3-8-4-1-8	1.2	1.7	2.3	3.4	3.1
L38	LT3-8-4-1-9	1.6	2.1	3.2	3.3	3.2
L39	LT10-7-1-12-1	2.3	3.2	3.3	3.2	2.8
L40	LT10-7-1-12-2	2.2	2.8	3.4	2.3	2.1
L41	LT10-7-1-12-3	2.1	2.8	3.3	2.1	1.9
L42	LT10-7-1-12-4	2.8	3.9	4.8	4.2	4.0
L43	LT10-7-1-12-5	1.2	2.3	3.4	3.7	4.1
L44	LT10-7-1-12-6	1.8	2.1	2.8	3.1	3.7
L45	LT10-7-1-12-7	2.1	2.3	2.5	2.8	3.2
L46	LT10-7-1-12-8	2.3	2.6	2.7	2.9	2.1
L47	LT11-5-1-1-1	2.1	2.8	2.9	3.1	2.3
L48	LT11-5-1-1-2	2.1	2.6	3.1	3.3	2.6
L49	LT11-5-1-1-3	1.8	2.1	3.2	3.3	3.7
L50	LT11-5-1-1-4	0.4	0.7	0.9	1.1	1.0
L51	LT11-3-3-12	0.5	0.8	1.1	1.4	0.7
L52	LT16-7-2-5-1	1.5	2.5	3.0	3.2	2.3
L53	LT16-7-2-5-2	1.6	2.1	2.7	2.8	2.1
L54	LT16-7-2-5-3	1.7	2.5	3.1	3.3	2.1

Aphid Population Build up Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	**9**	**13**	**17**	**21**
L55	LT16-7-2-5-4	0.7	2.7	3.3	3.4	4.1
L56	LT16-7-2-5-5	0.5	2.6	2.9	3.2	2.8
L57	LT3-8-4-6-1	0.3	1.6	1.9	3.3	3.9
L58	LT3-8-4-6-2	1.1	1.8	2.1	2.5	2.8
L59	LT3-8-4-6-3	1.2	1.8	2.1	3.4	3.4
L60	LT4-2-4-1-1	0.4	1.0	1.5	1.4	1.1
L61	LT4-2-4-1-2	0.8	1.0	1.1	1.0	0.9
L62	**BB-PRT**	1.5	2.6	3.3	3.0	2.6
L63	BBBT1-1	1.3	3.3	3.3	2.8	2.3
L64	BBBT1-2	1.2	3.2	3.4	2.5	2.1
L65	BBBT1-3	1.4	2.8	3.2	2.7	2.2
L66	BBBT1-4	1.8	3.5	4.3	3.7	3.3
L67	BBBT1-5	0.2	0.4	1.1	1.7	1.5
L68	BBBT1-6	1.2	2.2	2.8	3.2	2.1
L69	BBBT1-7	0.4	1.1	1.3	1.1	0.9
L70	BBBT1-8	0.4	1.1	1.7	2.1	2.1
L71	BBBT1-9	0.8	1.2	2.1	2.2	3.4
L72	BBBT1-10	1.1	1.6	2.3	1.4	2.3
L73	BBBT1-11	0.3	0.5	1.0	0.9	0.4
L74	BB7-9-7-5-1	0.5	0.9	1.1	1.5	1.7
L75	BB7-9-7-5-2	1.6	2.3	2.7	4.5	3.1
L76	BB7-9-7-5-3	1.8	2.1	3.2	4.3	2.8
L77	BB7-9-7-5-4	0.5	1.2	1.6	1.3	1.1
L78	BB7-9-7-5-5	2.1	2.2	3.7	3.8	3.3
L79	BB7-9-7-5-6	2.8	3.1	3.5	4.2	3.7
L80	BB10-4-2-3-1	0.2	0.5	0.7	1.1	1.0
L81	BB10-4-2-3-2	0.3	0.8	1.2	1.5	1.1

Aphid Population Build up Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	**9**	**13**	**17**	**21**
L82	BB10-4-2-3-3	0.3	1.2	1.6	1.1	1.2
L83	BB14-16-2-2	1.0	2.3	2.5	3.9	3.7
L84	BBVN-1	1.6	2.4	3.4	4.5	4.3
L85	BBVN-2	0.3	2.2	3.2	4.7	4.4
L86	**MS-PRT**	1.0	2.5	3.8	3.3	2.9
L87	**MS1-8-2-2-5-1**	2.1	3.2	3.8	3.9	3.2
L88	MS1-8-2-6-6-1	1.8	2.4	3.3	2.9	2.1
L89	MS1-8-2-6-6-2	1.9	2.3	3.4	2.9	2.2
L90	MS1-8-2-6-8-1	1.0	1.1	1.5	1.0	0.8
L91	MS1-8-2-6-8-2	1.9	2.3	2.4	2.7	2.4
L92	MS1-8-2-6-8-3	1.6	1.7	2.1	2.6	2.2
L93	MS1-8-1-4-1	1.8	2.1	2.4	2.9	3.2
L94	MS1-8-1-4-2	1.9	2.7	3.7	3.8	3.1
L95	MS10-11-1-1-1	1.1	2.1	3.3	3.5	3.1
L96	MS1-8-2-2-5-1	1.0	1.2	2.5	3.2	2.3
L97	MS1-8-2-6-9-1	0.2	1.1	2.4	3.3	2.7
L98	IT93K452-1	2.1	2.3	1.5	2.8	3.2
L99	IT99K241-2	1.7	2.4	2.3	3.2	3.1
L100	IT99K573-2-1	1.4	2.1	2.7	2.9	2.1
L101	IT99K-7-21-2-2	1.5	2.3	2.4	3.2	3.1
L102	IT07K205-8	1.1	1.3	1.5	1.2	1.1
L103	Aloka Local	1.8	2.3	2.6	2.9	2.1
L104	Danilla	1.9	2.5	2.8	3.0	2.1
L105	TVu3236	1.3	2.4	2.6	2.9	2.3
L106	TVu2027	1.3	1.4	1.7	1.1	1.0
L107	Sanzi	1.3	1.5	1.3	1.1	1.0
L108	Lunkwankwa	1.0	1.2	1.1	1.0	0.4

Aphid Population Build up Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L109	Lukusuzi	1.1	1.5	1.1	0.9	0.5
L110	Namuseba	1.2	1.4	1.5	1.1	0.9

Appendix 3: Plant Vigour

Line	**Genotype**	**Days After Infestation (DAI)**				
		5	**9**	**13**	**17**	**21**
L1	LT-PRT	3.0	3.0	3.0	2.8	2.2
L2	LTBT1-1	3.0	3.0	3.0	3.0	1.2
L3	LTBT1-2	3.0	3.0	3.0	3.0	2.5
L4	LTBT1-3	3.0	3.0	3.0	3.0	1.5
L5	LTBT1-4	3.0	3.0	3.0	3.0	1.2
L6	LTBT1-5	3.0	3.0	3.0	3.0	3.0
L7	LTBT1-6	3.0	3.0	3.0	3.0	1.2
L8	LTBT1-7	3.0	3.0	3.0	3.0	1.3
L9	LT11-5-2-2-1	3.0	3.0	3.0	3.0	1.5
L10	LT11-5-2-2-2	3.0	3.0	3.0	3.0	1.4
L11	LT11-5-2-2-3	3.0	3.0	3.0	3.0	1.8
L12	LT11-5-2-2-4	3.0	3.0	3.0	3.0	1.8
L13	LT11-5-2-2-5	3.0	3.0	3.0	3.0	1.8
L14	LT11-5-2-2-6	3.0	3.0	3.0	3.0	1.6
L15	LT11-5-2-2-7	3.0	3.0	3.0	3.0	1.6
L16	LT11-5-2-2-8	3.0	3.0	3.0	3.0	1.2
L17	LT11-5-2-2-9	3.0	3.0	3.0	3.0	1.0
L18	LT11-5-2-2-10	3.0	3.0	3.0	3.0	1.0
L19	LT11-5-2-2-11	3.0	3.0	3.0	3.0	1.0
L20	LT11-5-2-2-12	3.0	3.0	3.0	3.0	1.4
L21	LT11-5-2-2-13	3.0	3.0	3.0	3.0	1.0
L22	LT11-5-2-2-14	3.0	3.0	3.0	3.0	1.0
L23	LT11-5-2-2-15	3.0	3.0	3.0	3.0	1.0
L24	LT11-5-2-2-16	3.0	3.0	3.0	3.0	1.8
L25	LT11-5-2-2-17	3.0	3.0	3.0	3.0	1.3
L26	LT11-5-2-2-18	3.0	3.0	3.0	3.0	1.3
L27	LT11-5-2-2-19	3.0	3.0	3.0	3.0	1.4

Plant Vigour Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L28	LT11-5-2-2-20	3.0	3.0	3.0	3.0	1.5
L29	LT11-5-2-2-21	3.0	3.0	3.0	3.0	1.0
L30	LT3-8-4-1-1	3.0	3.0	3.0	2.3	1.0
L31	LT3-8-4-1-2	3.0	3.0	3.0	2.2	1.0
L32	LT3-8-4-1-3	3.0	3.0	3.0	2.3	1.8
L33	LT3-8-4-1-4	3.0	3.0	3.0	3.0	3.0
L34	LT3-8-4-1-5	3.0	3.0	3.0	3.0	1.0
L35	LT3-8-4-1-6	3.0	3.0	3.0	3.0	1.2
L36	LT3-8-4-1-7	3.0	3.0	3.0	3.0	1.5
L37	LT3-8-4-1-8	3.0	3.0	3.0	3.0	1.3
L38	LT3-8-4-1-9	3.0	3.0	3.0	3.0	1.0
L39	LT10-7-1-12-1	3.0	3.0	3.0	3.0	1.0
L40	LT10-7-1-12-2	3.0	3.0	3.0	3.0	1.3
L41	LT10-7-1-12-3	3.0	3.0	3.0	3.0	1.0
L42	LT10-7-1-12-4	3.0	3.0	3.0	1.8	1.0
L43	LT10-7-1-12-5	3.0	3.0	3.0	3.0	1.2
L44	LT10-7-1-12-6	3.0	3.0	3.0	3.0	1.3
L45	LT10-7-1-12-7	3.0	3.0	3.0	3.0	1.2
L46	LT10-7-1-12-8	3.0	3.0	3.0	3.0	1.2
L47	LT11-5-1-1-1	3.0	3.0	3.0	3.0	1.2
L48	LT11-5-1-1-2	3.0	3.0	3.0	3.0	1.5
L49	LT11-5-1-1-3	3.0	3.0	3.0	3.0	1.6
L50	LT11-5-1-1-4	3.0	3.0	3.0	3.0	3.0
L51	LT11-3-3-12	3.0	3.0	3.0	3.0	3.0
L52	LT16-7-2-5-1	3.0	3.0	3.0	3.0	1.0
L53	LT16-7-2-5-2	3.0	3.0	3.0	3.0	1.2
L54	LT16-7-2-5-3	3.0	3.0	3.0	3.0	1.3

Plant Vigour Cont’

Line	**Genotype**	**Days After Infestation (DAI)**				
		5	**9**	**13**	**17**	**21**
L55	LT16-7-2-5-4	3.0	3.0	3.0	3.0	1.2
L56	LT16-7-2-5-5	3.0	3.0	3.0	3.0	1.5
L57	LT3-8-4-6-1	3.0	3.0	3.0	3.0	1.6
L58	LT3-8-4-6-2	3.0	3.0	3.0	3.0	1.7
L59	LT3-8-4-6-3	3.0	3.0	3.0	3.0	1.8
L60	LT4-2-4-1-1	3.0	3.0	3.0	3.0	3.0
L61	LT4-2-4-1-2	3.0	3.0	3.0	3.0	3.0
L62	**BB-PRT**	3.0	3.0	3.0	2.1	1.9
L63	BBBT1-1	3.0	3.0	3.0	3.0	1.4
L64	BBBT1-2	3.0	3.0	3.0	3.0	1.5
L65	BBBT1-3	3.0	3.0	3.0	3.0	1.3
L66	BBBT1-4	3.0	3.0	3.0	1.6	1.2
L67	BBBT1-5	3.0	3.0	3.0	3.0	3.0
L68	BBBT1-6	3.0	3.0	3.0	3.0	1.2
L69	BBBT1-7	3.0	3.0	3.0	3.0	3.0
L70	BBBT1-8	3.0	3.0	3.0	3.0	1.3
L71	BBBT1-9	3.0	3.0	3.0	3.0	1.5
L72	BBBT1-10	3.0	3.0	3.0	3.0	1.3
L73	BBBT1-11	3.0	3.0	3.0	3.0	3.0
L74	BB7-9-7-5-1	3.0	3.0	3.0	3.0	3.0
L75	BB7-9-7-5-2	3.0	3.0	3.0	3.0	1.2
L76	BB7-9-7-5-3	3.0	3.0	3.0	3.0	1.4
L77	BB7-9-7-5-4	3.0	3.0	3.0	3.0	3.0
L78	BB7-9-7-5-5	3.0	3.0	3.0	3.0	1.2
L79	BB7-9-7-5-6	3.0	3.0	3.0	3.0	1.3
L80	BB10-4-2-3-1	3.0	3.0	3.0	3.0	3.0
L81	BB10-4-2-3-2	3.0	3.0	3.0	3.0	3.0

Plant Vigour Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L82	BB10-4-2-3-3	3.0	3.0	3.0	3.0	3.0
L83	BB14-16-2-2	3.0	3.0	3.0	3.0	1.3
L84	BBVN-1	3.0	3.0	3.0	3.0	1.5
L85	BBVN-2	3.0	3.0	3.0	3.0	1.6
L86	**MS-PRT**	3.0	3.0	3.0	2.1	1.7
L87	**MS1-8-2-2-5-1**	3.0	3.0	3.0	1.6	1.3
L88	MS1-8-2-6-6-1	3.0	3.0	3.0	3.0	1.3
L89	MS1-8-2-6-6-2	3.0	3.0	3.0	3.0	1.2
L90	MS1-8-2-6-8-1	3.0	3.0	3.0	3.0	3.0
L91	MS1-8-2-6-8-2	3.0	3.0	3.0	3.0	1.3
L92	MS1-8-2-6-8-3	3.0	3.0	3.0	3.0	1.4
L93	MS1-8-1-4-1	3.0	3.0	3.0	3.0	1.2
L94	MS1-8-1-4-2	3.0	3.0	3.0	3.0	1.1
L95	MS10-11-1-1-1	3.0	3.0	3.0	3.0	1.0
L96	MS1-8-2-2-5-1	3.0	3.0	3.0	3.0	1.0
L97	MS1-8-2-6-9-1	3.0	3.0	3.0	3.0	1.2
L98	IT93K452-1	3.0	3.0	3.0	3.0	1.3
L99	IT99K241-2	3.0	3.0	3.0	3.0	1.5
L100	IT99K573-2-1	3.0	3.0	3.0	3.0	1.3
L101	IT99K-7-21-2-2	3.0	3.0	3.0	3.0	1.4
L102	IT07K205-8	3.0	3.0	3.0	3.0	3.0
L103	Aloka Local	3.0	3.0	3.0	3.0	1.2
L104	Danilla	3.0	3.0	3.0	3.0	1.3
L105	TVu3236	3.0	3.0	3.0	3.0	1.1
L106	TVu2027	3.0	3.0	3.0	3.0	3.0
L107	Sanzi	3.0	3.0	3.0	3.0	3.0
L108	Lunkwankwa	3.0	3.0	3.0	3.0	3.0

Plant Vigour Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L109	Lukusuzi	3.0	3.0	3.0	3.0	3.0
L110	Namuseba	3.0	3.0	3.0	3.0	3.0

Appendix 4: Plant Survival Rate

Line	**Genotype**	**Days After Infestation (DAI)**				
		5	**9**	**13**	**17**	**21**
L1	LT-PRT	100	100	88	88	77
L2	LTBT1-1	100	100	100	100	89
L3	LTBT1-2	100	100	100	100	77
L4	LTBT1-3	100	100	100	100	100
L5	LTBT1-4	100	100	100	100	100
L6	LTBT1-5	100	100	100	100	100
L7	LTBT1-6	100	100	100	100	100
L8	LTBT1-7	100	100	100	100	100
L9	LT11-5-2-2-1	100	100	100	100	100
L10	LT11-5-2-2-2	100	100	100	100	77
L11	LT11-5-2-2-3	100	100	100	89	55
L12	LT11-5-2-2-4	100	100	100	100	77
L13	LT11-5-2-2-5	100	100	100	100	89
L14	LT11-5-2-2-6	100	100	100	100	66
L15	LT11-5-2-2-7	100	100	100	100	89
L16	LT11-5-2-2-8	100	100	100	100	66
L17	LT11-5-2-2-9	100	100	100	100	89
L18	LT11-5-2-2-10	100	100	100	89	89
L19	LT11-5-2-2-11	100	100	100	89	89
L20	LT11-5-2-2-12	100	100	100	100	89
L21	LT11-5-2-2-13	100	100	100	100	77
L22	LT11-5-2-2-14	100	100	100	100	77
L23	LT11-5-2-2-15	100	100	100	100	100
L24	LT11-5-2-2-16	100	100	100	100	100
L25	LT11-5-2-2-17	100	100	100	100	100
L26	LT11-5-2-2-18	100	100	100	100	100
L27	LT11-5-2-2-19	100	100	100	100	77

Plant Survival Rate Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L28	LT11-5-2-2-20	100	100	100	100	77
L29	LT11-5-2-2-21	100	100	100	89	66
L30	LT3-8-4-1-1	100	100	100	89	77
L31	LT3-8-4-1-2	100	100	100	89	77
L32	LT3-8-4-1-3	100	100	100	89	77
L33	LT3-8-4-1-4	100	100	100	100	100
L34	LT3-8-4-1-5	100	100	100	100	77
L35	LT3-8-4-1-6	100	100	100	100	89
L36	LT3-8-4-1-7	100	100	100	100	89
L37	LT3-8-4-1-8	100	100	100	100	89
L38	LT3-8-4-1-9	100	100	100	100	89
L39	LT10-7-1-12-1	100	100	100	89	44
L40	LT10-7-1-12-2	100	100	100	89	77
L41	LT10-7-1-12-3	100	100	100	89	55
L42	LT10-7-1-12-4	100	100	67	67	44
L43	LT10-7-1-12-5	100	100	100	89	77
L44	LT10-7-1-12-6	100	100	100	89	89
L45	LT10-7-1-12-7	100	100	100	89	89
L46	LT10-7-1-12-8	100	100	100	89	89
L47	LT11-5-1-1-1	100	100	100	89	77
L48	LT11-5-1-1-2	100	100	100	89	77
L49	LT11-5-1-1-3	100	100	100	89	77
L50	LT11-5-1-1-4	100	100	100	100	100
L51	LT11-3-3-12	100	100	100	100	100
L52	LT16-7-2-5-1	100	100	100	100	89
L53	LT16-7-2-5-2	100	100	100	100	89
L54	LT16-7-2-5-3	100	100	100	100	77

Plant Survival Rate Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L55	LT16-7-2-5-4	100	100	100	100	89
L56	LT16-7-2-5-5	100	100	100	100	77
L57	LT3-8-4-6-1	100	100	100	100	66
L58	LT3-8-4-6-2	100	100	100	100	66
L59	LT3-8-4-6-3	100	100	100	100	77
L60	LT4-2-4-1-1	100	100	100	100	77
L61	LT4-2-4-1-2	100	100	100	100	100
L62	**BB-PRT**	100	100	100	89	77
L63	BBBT1-1	100	100	100	77	55
L64	BBBT1-2	100	100	100	89	77
L65	BBBT1-3	100	100	100	89	77
L66	BBBT1-4	100	77	77	67	33
L67	BBBT1-5	100	100	100	100	100
L68	BBBT1-6	100	100	100	89	77
L69	BBBT1-7	100	100	100	100	100
L70	BBBT1-8	100	100	100	89	100
L71	BBBT1-9	100	100	100	89	100
L72	BBBT1-10	100	100	100	89	100
L73	BBBT1-11	100	100	100	100	100
L74	BB7-9-7-5-1	100	100	100	100	100
L75	BB7-9-7-5-2	100	100	100	89	77
L76	BB7-9-7-5-3	100	100	100	89	44
L77	BB7-9-7-5-4	100	100	100	100	100
L78	BB7-9-7-5-5	100	100	100	89	77
L79	BB7-9-7-5-6	100	100	100	89	55
L80	BB10-4-2-3-1	100	100	100	100	100
L81	BB10-4-2-3-2	100	100	100	100	100

Plant Survival Rate Cont'

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L82	BB10-4-2-3-3	100	100	100	100	100
L83	BB14-16-2-2	100	100	100	89	77
L84	BBVN-1	100	100	100	89	44
L85	BBVN-2	100	100	100	89	55
L86	**MS-PRT**	100	100	100	100	77
L87	**MS1-8-2-2-5-1**	100	100	100	89	89
L88	MS1-8-2-6-6-1	100	100	100	89	77
L89	MS1-8-2-6-6-2	100	100	100	89	77
L90	MS1-8-2-6-8-1	100	100	100	100	100
L91	MS1-8-2-6-8-2	100	100	100	89	77
L92	MS1-8-2-6-8-3	100	100	100	89	77
L93	MS1-8-1-4-1	100	100	100	100	89
L94	MS1-8-1-4-2	100	100	100	100	100
L95	MS10-11-1-1-1	100	100	100	100	100
L96	MS1-8-2-2-5-1	100	100	100	100	100
L97	MS1-8-2-6-9-1	100	100	100	100	100
L98	IT93K452-1	100	100	100	100	100
L99	IT99K241-2	100	100	100	100	100
L100	IT99K573-2-1	100	100	100	100	100
L101	IT99K-7-21-2-2	100	100	100	89	77
L102	IT07K205-8	100	100	100	100	100
L103	Aloka Local	100	100	100	89	77
L104	Danilla	100	100	100	89	77
L105	TVu3236	100	100	100	89	77
L106	TVu2027	100	100	100	100	100
L107	Sanzi	100	100	100	100	100
L108	Lunkwankwa	100	100	100	100	100

Plant Survival Rate Cont’

Line	Genotype	Days After Infestation (DAI)				
		5	9	13	17	21
L109	Lukusuzi	100	100	100	100	100
L110	Namuseba	100	100	100	100	100

Appendix 5: Bruchid Analysis

Line	Genotype	eggs/100 seed	AEP	DP	GI	PI	WLP	FP
L1	LT-PRT	102.1	86.3	20.3	4.3	43.0	27.8	1.7
L2	LTBT1-1	116.7	78.2	21.2	3.7	39.1	25.2	1.5
L3	LTBT1-2	112.3	88.3	23.2	3.8	44.2	28.5	1.7
L4	LTBT1-3	23.4	87.1	21.2	4.1	43.6	28.1	1.7
L5	LTBT1-4	27.6	63.0	27.3	2.3	13.3	5.5	0.7
L6	LTBT1-5	118.2	88.1	20.0	4.4	93.2	44.0	5.1
L7	LTBT1-6	54.3	78.2	21.8	3.6	39.1	25.2	1.5
L8	LTBT1-7	79.3	78.9	22.1	3.6	39.5	25.2	1.6
L9	LT11-5-2-2-1	34.2	67.3	20.1	3.3	33.7	21.7	1.3
L10	LT11-5-2-2-2	18.3	27.0	30.3	0.9	13.1	0.9	0.3
L11	LT11-5-2-2-3	37.2	45.2	26.2	1.7	22.6	14.6	0.9
L12	LT11-5-2-2-4	19.6	54.7	31.1	1.8	16.1	2.7	0.4
L13	LT11-5-2-2-5	49.0	23.4	24.3	1.0	11.7	7.5	0.5
L14	LT11-5-2-2-6	112.3	70.1	24.3	2.9	35.1	22.6	1.4
L15	LT11-5-2-2-7	109.3	68.7	25.3	2.7	34.4	22.1	1.4
L16	LT11-5-2-2-8	98.2	67.0	24.6	2.6	33.5	21.2	1.3
L17	LT11-5-2-2-9	102.1	70.2	25.2	2.8	35.1	22.4	1.4
L18	LT11-5-2-2-10	24.8	15.3	27.3	0.6	13.3	2.7	0.4
L19	LT11-5-2-2-11	103.3	75.3	24.3	3.2	37.7	24.3	1.5
L20	LT11-5-2-2-12	112.1	72.1	23.6	3.1	36.1	23.3	1.4
L21	LT11-5-2-2-13	98.4	67.3	24.2	2.8	33.7	21.8	1.3
L22	LT11-5-2-2-14	88.7	56.3	22.3	2.5	28.2	18.1	1.1
L23	LT11-5-2-2-15	76.4	67.4	22.7	3.0	33.7	21.6	1.3
L24	LT11-5-2-2-16	79.3	56.4	23.1	2.4	28.2	18.3	1.1
L25	LT11-5-2-2-17	101.2	70.3	21.3	3.3	35.2	22.6	1.4
L26	LT11-5-2-2-18	102.4	79.3	20.2	3.9	39.7	25.7	1.6
L27	LT11-5-2-2-19	106.3	67.4	23.4	2.9	33.7	21.5	1.3

Bruchid Analysis Cont'

Line	Genotype	eggs/100 seed	AEP	DP	GI	PI	WLP	FP
L28	LT11-5-2-2-20	18.7	32.3	23.6	1.4	8.9	3.7	0.4
L29	LT11-5-2-2-21	67.7	56.3	22.3	2.5	28.2	18.3	1.1
L30	LT3-8-4-1-1	102.3	70.4	23.3	3.0	35.2	22.5	1.4
L31	LT3-8-4-1-2	98.2	57.5	22.1	2.6	28.8	18.4	1.1
L32	LT3-8-4-1-3	77.3	75.4	21.9	3.4	37.7	24.3	1.5
L33	LT3-8-4-1-4	78.9	34.5	22.3	1.5	17.3	11.1	0.7
L34	LT3-8-4-1-5	90.8	67.3	22.3	3.0	33.7	21.8	1.3
L35	LT3-8-4-1-6	102.3	71.2	23.4	3.1	35.6	23.1	1.4
L36	LT3-8-4-1-7	112.5	73.2	22.4	3.3	36.6	23.5	1.4
L37	LT3-8-4-1-8	98.3	56.7	20.1	2.8	28.4	18.3	1.1
L38	LT3-8-4-1-9	90.4	67.4	22.3	3.0	33.7	21.8	1.3
L39	LT10-7-1-12-1	118.3	83.0	20.1	4.1	41.5	26.6	1.6
L40	LT10-7-1-12-2	119.4	83.4	19.2	4.3	41.7	26.8	1.6
L41	LT10-7-1-12-3	123.7	73.0	22.3	3.3	36.5	23.5	1.4
L42	LT10-7-1-12-4	134.6	71.2	24.1	3.1	35.6	23.2	1.4
L43	LT10-7-1-12-5	120.2	70.3	20.1	3.5	35.2	22.6	1.4
L44	LT10-7-1-12-6	109.2	73.4	22.1	3.3	36.7	22.4	1.4
L45	LT10-7-1-12-7	98.5	67.4	22.3	3.2	33.7	21.9	1.3
L46	LT10-7-1-12-8	78.4	70.1	20.1	3.5	35.1	22.5	1.4
L47	LT11-5-1-1-1	112.4	67.4	23.1	2.8	33.7	21.6	1.3
L48	LT11-5-1-1-2	104.0	93.2	23.1	4.2	46.6	30.1	1.8
L49	LT11-5-1-1-3	78.3	91.2	21.4	4.3	45.6	29.4	1.9
L50	LT11-5-1-1-4	98.5	90.4	22.3	4.1	45.2	29.2	1.7
L51	LT11-5-1-1-5	101.4	87.3	22.3	3.9	43.7	28.2	1.7
L52	LT16-7-2-5-1	123.2	80.3	19.7	4.2	95.5	58.6	6.5
L53	LT16-7-2-5-2	89.5	85.3	21.3	4.1	42.7	27.5	1.7
L54	LT16-7-2-5-3	45.6	56.6	19.7	2.9	28.3	18.3	1.1

Bruchid Analysis Cont'

Line	**Genotype**	**eggs/100 seed**	**AEP**	**DP**	**GI**	**PI**	**WLP**	**FP**
L55	LT16-7-2-5-4	78.4	78.5	23.5	3.3	39.3	25.3	1.5
L56	LT16-7-2-5-5	102.2	89.4	21.8	4.1	44.7	28.8	1.8
L57	LT3-8-4-6-1	112.1	78.5	22.3	3.5	39.3	25.3	1.5
L58	LT3-8-4-6-2	98.7	67.3	21.3	3.2	33.7	21.8	1.3
L59	LT3-8-4-6-3	78.4	40.5	20.1	2.1	20.3	13.1	0.8
L60	LT4-2-4-1-1	80.5	56.7	20.8	2.7	28.4	18.3	1.1
L61	LT4-2-4-1-2	82.1	65.8	21.3	3.1	32.9	21.2	1.3
L62	**BB-PRT**	110.3	78.2	20.0	3.9	40.7	15.9	1.6
L63	BBBT1-1	124.3	49.7	24.3	2.0	95.0	37.9	4.3
L64	BBBT1-2	78.5	67.4	23.1	2.9	33.7	21.7	1.3
L65	BBBT1-3	36.2	19.7	30.7	0.6	12.3	5.6	0.5
L66	BBBT1-4	34.2	78.5	25.5	3.1	39.3	25.3	1.5
L67	BBBT1-5	34.5	67.5	26.4	2.6	33.8	21.8	1.3
L68	BBBT1-6	33.2	78.6	25.1	3.2	39.3	25.4	1.5
L69	BBBT1-7	28.7	42.3	21.7	2.0	4.0	6.9	0.4
L70	BBBT1-8	34.3	67.7	26.3	2.6	33.9	21.8	1.3
L71	BBBT1-9	89.5	67.5	21.3	3.2	33.8	21.9	1.4
L72	BBBT1-10	67.3	45.6	23.5	1.9	22.8	14.7	0.9
L73	BBBT1-11	13.0	12.7	30.3	0.4	10.0	1.1	0.2
L74	BB7-9-7-5-1	45.3	56.4	23.2	2.4	28.2	18.2	1.1
L75	BB7-9-7-5-2	34.6	56.3	20.1	2.8	28.3	18.3	1.1
L76	BB7-9-7-5-3	20.1	61.0	26.0	2.4	11.0	2.1	0.4
L77	BB7-9-7-5-4	45.6	45.7	20.1	2.3	22.9	14.7	0.9
L78	BB7-9-7-5-5	101.3	56.4	21.3	2.6	28.2	18.3	1.2
L79	BB7-9-7-5-6	110.2	34.4	22.3	1.5	17.2	11.1	0.7
L80	BB10-4-2-3-1	14.7	42.3	30.3	1.4	13.0	1.4	0.2
L81	BB10-4-2-3-2	67.5	35.3	23.1	1.5	17.7	11.4	0.7

Bruchid Analysis Cont'

Line	Genotype	eggs/100 seed	AEP	DP	GI	PI	WLP	FP
L82	BB10-4-2-3-3	24.3	79.7	19.0	4.2	14.0	1.0	0.3
L83	BB14-16-2-2	57.5	45.5	23.5	1.9	22.8	14.7	0.9
L84	BBVN-1	63.2	56.7	20.1	2.8	28.4	18.3	1.1
L85	BBVN-2	56.4	67.8	20.9	3.2	33.9	21.9	1.3
L86	**MS-PRT**	135.1	79.4	18.9	4.2	78.5	36.1	4.1
L87	**MS1-8-2-2-5-1**	112.3	87.7	21.9	4.0	43.9	28.3	1.7
L88	MS1-8-2-6-6-1	115.5	88.2	20.1	4.4	44.1	28.5	1.6
L89	MS1-8-2-6-6-2	14.7	32.3	20.0	1.6	8.0	3.7	0.5
L90	MS1-8-2-6-8-1	102.4	67.8	23.3	2.9	33.9	21.9	1.3
L91	MS1-8-2-6-8-2	98.7	78.6	24.2	3.2	39.3	25.4	1.5
L92	MS1-8-2-6-8-3	123.3	88.2	21.3	4.1	44.1	28.5	1.7
L93	MS1-8-1-4-1	110.3	82.1	23.2	3.5	41.1	26.5	1.6
L94	MS1-8-1-4-2	105.6	87.2	21.1	4.1	43.6	28.1	1.7
L95	MS10-11-1-1-1	104.3	78.3	20.1	3.9	39.1	25.3	1.4
L96	MS1-8-2-2-5-1	109.4	78.3	21.1	3.7	39.2	25.2	1.3
L97	MS1-8-2-6-9-1	128.7	84.0	22.0	3.8	90.3	55.9	5.4
L98	IT93K452-1	102.1	78.9	23.2	3.4	39.5	25.5	1.6
L99	IT99K241-2	67.7	51.7	21.3	2.4	13.2	2.0	0.5
L100	IT99K573-2-1	47.7	79.3	22.3	3.6	17.0	2.2	0.4
L101	IT99K-7-21-2-2	56.7	78.3	21.3	3.7	39.2	25.3	1.5
L102	IT07K205-8	45.6	74.5	23.4	3.2	37.3	24.0	1.6
L103	Aloka Local	57.5	60.5	22.1	2.7	30.3	19.5	1.3
L104	Danilla	67.8	56.4	22.4	2.5	28.2	18.2	1.2
L105	TVu3236	68.7	45.6	21.3	2.1	22.8	14.7	0.9
L106	TVu2027	98.3	45.4	23.4	1.9	22.7	14.6	1.1
L107	Sanzi	93.2	68.7	25.3	2.8	34.4	22.2	1.4
L108	Lunkwankwa	89.5	63.2	23.5	2.7	31.6	20.4	1.2

Bruchid Analysis Cont'

Line	**Genotype**	**eggs/100 seed**	**AEP**	**DP**	**GI**	**PI**	**WLP**	**FP**
L109	Lukusuzi	78.5	45.2	21.9	2.1	22.7	14.6	0.9
L110	Namuseba	122.3	79.5	20.1	4.0	39.8	25.6	1.6

Appendix 6: ANOVA tables

ANOVA for average aphid score at alpha 0.05 (5 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	4.02	2.01	3.00	0.054	3.03
Treatment	109	1177.95	10.81	16.15	0.01	1.12
Error	218	145.94	0.67			
Total	329	1327.91				

Decision: Since F cal (16.15) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average aphid score at alpha 0.05 (9 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	0.07	0.04	0.57	0.35	3.03
Treatment	109	707.52	6.49	92.71	0.00	1.12
Error	218	15.39	0.07			
Total	329	722.98				

Decision: Since F cal (92.71) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average aphid score at alpha 0.05 (13 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	0.19	0.10	1.43	0.051	3.03
Treatment	109	977.15	8.96	128.00	0.00	1.12
Error	218	15.84	0.07			
Total	329	993.18				

Decision: Since F cal (128.00) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average aphid score at alpha 0.05 (17 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	0.07	0.04	0.57	0.48	3.03
Treatment	109	707.52	6.49	92.71	0.02	1.12
Error	218	15.39	0.07			
Total	329	722.98				

Decision: Since F cal (92.71) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average aphid score at alpha 0.05 (21 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	1.10	0.55	11.00	0.02	3.03
Treatment	109	137.23	1.26	25.21	0.01	1.12
Error	218	11.34	0.05			
Total	329	149.67				

Decision: Since F cal (25.23) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average aphid population build-up at alpha 0.05 (5 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	0.86	0.43	0.67	0.25	3.03
Treatment	109	1509.11	13.85	21.64	0.01	1.12
Error	218	141.31	0.64			
Total	329	1651.28				

Decision: Since F cal (21.64) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average aphid population build-up at alpha 0.05 (9 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	1.55	0.78	9.75	0.01	3.03
Treatment	109	711.52	6.53	81.63	0.00	1.12
Error	218	17.91	0.08			
Total	329	730.98				

Decision: Since F cal (81.63) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average aphid population build-up at alpha 0.05 (13 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	0.02	0.01	0.14	0.78	3.03
Treatment	109	787.15	7.22	103.14	0.00	1.12
Error	218	16.24	0.07			
Total	329	803.41				

Decision: Since F cal (103.14) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average aphid population build up at alpha 0.05 (17 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	0.07	0.04	0.57	0.87	3.03
Treatment	109	707.52	6.49	92.71	0.02	1.12
Error	218	15.39	0.07			
Total	329	722.98				

Decision: Since F cal (92.71) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average aphid population build up at alpha 0.05 (21 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	0.09	0.05	8.33	0.00	3.03
Treatment	109	37.23	0.34	55.31	0.00	1.12
Error	218	1.34	0.006			
Total	329	38.66				

Decision: Since F cal (55.31) > F tab (1.12), therefore we reject the $H_{0.}$

ANOVA for average plant vigour analysis at alpha 0.05 (21 DAI)

Source of Variation	*df*	*SS*	*MS*	*F*	*P-value*	*F crit*
Block	2	0.61	0.31	0.36	0.87	3.03
Treatment	109	880.03	8.07	9.28	0.00	1.12
Error	218	189.14	0.87			
Total	329	1069.77				

Decision: Since F cal (34.39) > F tab (1.12), therefore we reject the $H_{0.}$