INHERITANCE OF BRUCHID (*Callosobruchus maculatus*) RESISTANCE IN COMMON BEANS (*Phaseolus vulgaris*)

BY

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DECLARATION

I, Natasha Mwila, declare that this dissertation represents my own work and that it has not been previously submitted for a degree, diploma or any other qualification at this or any other University.

Signature……

Date……..

APPROVAL

This dissertation of Natasha Mwila has been approved by the University of Zambia as partial fulfillment of the requirements for the award of the degree of Master of Science in Plant Breeding and Seed Systems.

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ABSTRACT

Beans (*Phaseolus vulgaris*) are an important food as a source of protein and fit well in the farming system of smallholders in Zambia. Unfortunately the grain is prone to storage losses mainly due to storage insects. Bruchid (*Callosobruchus maculatus)* attack on dry beans is a serious storage problem causing severe losses, distorting the taste and reducing the market value and acceptance to the consumers. In Zambia this is a major problem contributing to food insecurity in smallholder settings. Development of bruchid resistant varieties, therefore, is a key breeding objective. Prelude to the development of bruchid resistance is the need for understanding the genetics of bruchid resistance. This study, therefore, was carried out with specific objectives to evaluate common bean for bruchid resistance, identify phytochemicals related to bruchid resistance in common bean and establish the gene action controlling bruchid resistance in common bean.

The study was carried out at the University of Zambia (UNZA) involving crosses from two resistant and six susceptible genotypes in a North Carolina Design II. Beetle emergence was evaluated, mean development time derived and phytochemical analysis carried out using a thin layer chromatography.

Based on the susceptibility index, bean genotypes were categorized into resistant, moderately resistant and moderately susceptible. Carioca 38, Rab 608, Carioca x Lukupa and Carioca x Kalungu were identified as resistant, Rab 608 x Lukupa and Rab 608 x Kalungu were moderately resistant and Kalungu and Lukupa were moderately susceptible. Host preference for egg laying was exhibited by the bruchids and this was linked to the seed coat colour and seed size. Darker coloured large seeded genotypes showed more number of eggs laid (5 to 13) than lighter coloured small seeded (4 to 5.5). The seed coat thickness seemed to also play an important role in enhancing resistance in the bean varieties in this study, suggesting that increased seed coat thickness significantly reduced insect emergence on some genotypes and hence improves their resistance.

Reduced bruchid emergence and extended larval development periods in resistant genotypes suggest that antibiosis or anti feedant activity may be the actual resistance mechanisms.There was also a distinct presence of methyl esters, of R_f values ranging from 0.14 to 0.47, of fatty acids in the resistant varieties such as the Carioca 38 x Lukupa. This further confirmed the role phytochemicals can play in enhancing resistance in common beans. The adult emergence and the number of eggs laid significantly influenced, 74% and 18 %, respectively, the susceptibility index and were useful in explaining the resistance of the bean genotypes.

Carioca 38 and Rab 608 were both categorized as resistant with Carioca 38 showing no methyl esters which manifest in its F_2 progeny, while Rab 608 simply did not show any methyl esters in itself nor its progeny. The progeny of Carioca 38 showed resistance while that of Rab 608 was moderately resistant to the bruchids. This suggested different modes of resistance to bruchids for the two resistant bean parents. These results further suggested that Carioca 38 had multi resistance factors such as the seed color, seed size, seed coat thickness and the chemical constituents evident in its progeny.

The gene action for most of the bruchid resistance traits considered in this study (susceptibility index, number of eggs laid, number of insects emerged, seed coat thickness and protein content) were controlled by additive gene action. The general combining ability effects for Carioca 38 were significant and negative for adult emergence (-19.65), therefore, Carioca 38 would be good parent for continued use in selection and breeding for bruchid resistance. The heritability of the adult emergence was 79% and ultimately the susceptibility index was 53% indicating selection for these traits should be fairly easy as there is close correspondence between the genotype and the phenotype due to a relatively smaller contribution of the environment to the phenotype.

It can be concluded that the study demonstrated the presence of adequate genotypic variation among common bean genotypes on their resistance to bruchids (*C. maculatus*). This suggests that deliberate selection using superior genotypes in bruchid resistance identified targeting low number of eggs laid, low number of insects emerged, high protein content as well as the reduced seed coat thickness which explained variations could lead to development of appropriate varieties which could give lower susceptibility index in common bean varieties. This study also suggested that the resistance of bean genotypes to bruchids (*C. maculatus*) is a complex one as it is governed by several factors.

DEDICATION

I dedicate this work to my beloved family especially my late father (Andrew Mwila) and mother (Mable Nawila Mwila).

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LIST OF ABBREVIATIONS

CIAT	International Center for Tropical Agriculture
CIMMYT	International Maize and Wheat Improvement Center
FAO	Food and Agriculture Organization
FSRP	Food Security Research Program
TLC	Thin Layer Chromatography
ZARI	Zambia Agriculture and Research Institute
MFNP	Ministry of Finance and National Planning

CHAPTER ONE

1.0 INTRODUCTION

1.1 Importance

Beans are used as a food and indeed as a source of income in Zambia. As a food, beans are a source of nutrients contributing to human health. The fact that the crop is a legume also makes it an important component of sustainable agriculture in terms of soil amendment.

Common dry beans (*Phaseolus vulgaris* L*).* are the most important food legume for direct consumption in the world (Jones, 2007). It is grown worldwide for its edible bean, popularly consumed as dry, fresh and green, and can be kept for 3–4 years if stored in a cool, dry place, although with time, their nutritive value and flavor degrades and cooking times lengthen as they desiccate and harden (Rusike, 2012). A high per capita consumption of 13 to 40 kg yr^{-1} of dry beans has been reported in developing countries, especially within low-income families in urban and rural areas (Singh, 1999).

Beans are also consumed as substitutes for meats for the source of proteins. Beans are regularly used by institutions such as hospitals, prisons and schools to provide the required proteins. They are served quite often with rice and maize meal (Rusike, 2012). The key nutritional benefits of common beans are quite similar to those of soybeans except that they are relatively lower in fat content usually only 1 to 2%. Beans provide an important source of protein (~22%), vitamins (folate), and minerals (Ca, Cu, Fe, Mg, Mn, Zn, Mo, K) for human diets, especially in developing countries (Broughton *et al*.,

2003). Common beans also offer an excellent source of complex carbohydrate and fiber (Messina, 1999).

The common beans' contribution to heart health lies not just in their fiber, but in the significant amounts of antioxidants, folic acid, vitamin B6, and magnesium that they supply. Folic acid and B6 help lower levels of homocysteine and hence reduce risk factor for heart attack, stroke, or peripheral vascular disease (Wu X, 2004).

Intake of common beans is also protective against cancer. In one analysis of dietary data collected in USA by validated food frequency questionnaires in 1991 and 1995 from 90,630 women in the Nurses Health Study II, researchers found a significant reduced frequency of breast cancer in those women who consumed a higher intake of common beans or lentils (Adebamowo *et al*., 2004).

Besides being a major source of protein in human diets in most communities, beans are increasingly playing a major role in improving farmers' livelihoods as a source of income, in some cases for up to 45% of the households (Muimui, 2010; Kusolwa, 2007). In some cases, beans are considered as an extra source of income (secondary to others such as cereals, tobacco) and demand is rising for beans in the market. In some parts of Zambia, beans are even considered a high income crop relative to maize, especially when good yields are obtained (Rusike, 2012).

Beans are considered as a crop that can mitigate hunger in three countries Malawi, Tanzania and Zambia, thus is a major food security crop (Kusolwa, 2007). Hunger recurs every year in certain regions given the cropping cycles (once a year in Malawi and Zam-

bia). Beans play this role better as they are considered by families as a dependable and complete meal. In Zambia, beans are widely consumed countrywide among most households (MFNP, 2002). It also ranks second after maize and third in some places after maize and groundnuts as a food security crop, especially in the North Western and Northern Provinces, where they are consumed at least weekly or twice a week (Kusolwa, 2007).

A hallmark trait of legumes is their ability to develop root nodules and to fix N_2 in symbiosis with compatible rhizobia. This is often a critical factor in their suitability for the use in biological nitrogen fixation and it is due to this that beans is recommended for rotation in good crop management practices (such as conservation farming) among cereal and other crops mostly grown by farmers in Zambia (CSO/MACO/FSRP, 2004). Formation of symbiotically effective root nodules involves signaling between host and microsymbiont. Flavonoids and/or isoflavonoids released from the root of the legume host induce transcription of nodulation genes in compatible rhizobia, leading to the formation of lipochitooligosaccharide molecules that, in turn, signal the host plant to begin nodule formation (Long, 1996). Numerous changes occur in host and bacterial gene expression during infection, nodule development, and function (Vance, 2002), with approximately 100 host legume and rhizobial genes involved. Some 40 to 60 million metric tons (Mt) of N_2 are fixed by agriculturally important legumes annually (Smil, 1999; Delwiche, 1970).

The N_2 from legume fixation is essentially “free” Nitrogen for use by the host plant or by associated or subsequent crops. Furthermore, fertilizer N is frequently unavailable or costly to subsistence farmers, leaving them dependent on N_2 fixation by legumes or oth-

er N_2-fixing organisms. Therefore, most small scale farmers use rotation and intercropping with beans and other crops (Giller, 2001). Giller (2001)) suggests that rates of N_2 fixation of 1 to 2 kg N ha^{-1} growing season day^{-1}should be possible in all legumes.

Beans ranks second to groundnuts in terms of land area allocated to food legumes and the number of households growing the crop. It is estimated that over 14 million hectares of the world's arable land is dedicated to common bean production, which amounts to more than 11 million tons worldwide (Singh, 1999; FAOSTAT, 2004/2005).

The world total food legume area harvested under the focused crops stands at 61.5 m ha in 2006-08, which represents an increase of 10% from mid-1990s, and the total production in 2006-08 stands at 46.5 m tons, up by 24% from 1994-1996 level (FAOSTAT, 2004/2005). Annual production, including both dry and snap bean, exceeds 21 million metric tons (Mt), which represents more than half of the world's total food legume production (FAOSTAT, 2004/2005).

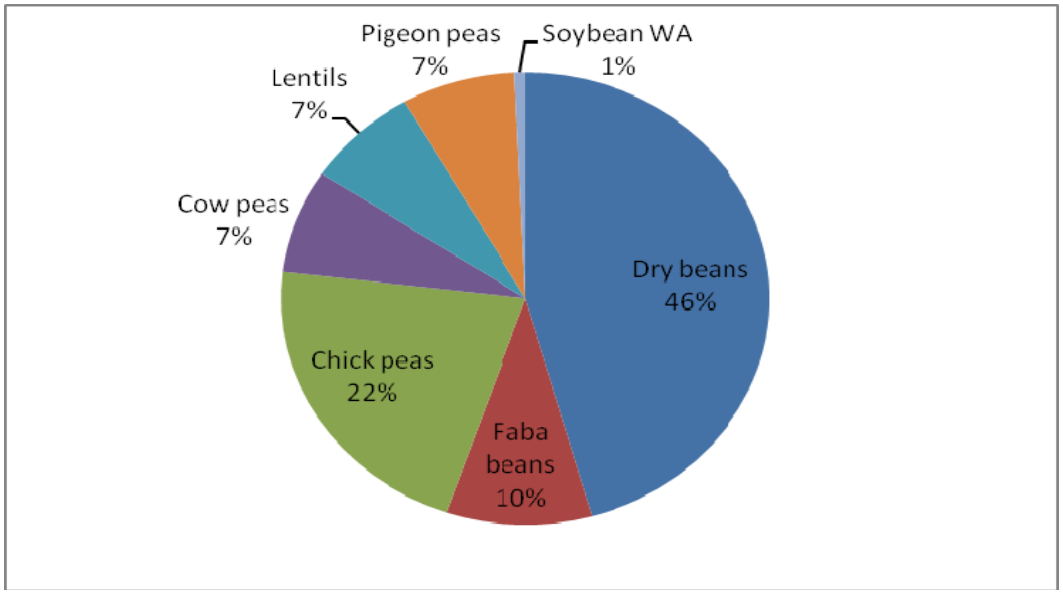


Figure 1: Shares of different legume crops in total global area and production, 2006-08, total world production = 47 m tons (CSO/MACO/FSRP, 2010).

Production of beans in Zambia is in the medium and high rainfall regions of the country mainly by small scale farmers (Misangu et al., 2001). However, although the CSO/MACO/FSRP (2004) survey indicates that beans are grown in all provinces in Zambia, the bulk of beans are produced in the Northern Province, accounting for 62% percent of the total production the other three provinces include Northwestern (8 percent); Central (11 percent); and Luapula (6 percent) (CSO/MACO/FSRP, 2010).

At national level, production statistics indicates an increasing pattern in the amounts of beans produced annually, in that national production in 2004 was estimated to be 600,000 tons per annum (CSO/MACO/FSRP, 2004) while that of 2006/07 agricultural season stood at 24,000 metric tons (Mt) (FEWSNET 2007). In 2008/9 Zambian farmers produced 95,333 Mt of beans representing a 12,000 Mt increase over the previous year (FAOSTAT, 2010).

Considering all the above factors of beans being an important crop, the production and productivity levels range from 200 kg to 800 kg per hectare against a potential of 3-4 tons/ha (Munyinda, 2013). Contributing to the low productivity are factors such as erratic rains, pests and diseases. Most of the common bean production is by resource-poor farmers where the crop is more vulnerable to attack by insect pests (Kusolwa, 2007).

Thus leading to severe losses of common dry bean in storage is the bruchid pest which causes severe losses. The extent of crop loss may vary depending on location, the species of weevil, storage conditions, and period of storage. The longer that beans are kept in warehouses (over three months) may lead to complete crop loss. However, the aver-

age worldwide loss caused by weevil (beetle) damage ranges from 7-50% of marketable beans (Slumpa and Ampofo, 1991).

High cost of pesticides, increasing adverse effects of pesticides on the environment, incorrect application of these pesticides on stored beans, and the difficulties in identifying bean bruchid resistance mechanisms early in breeding programmes have accelerated the post-harvest losses even amidst low yields already experienced especially in Zambia (Kusolwa, 2007).

Cultivar improvement for bruchid resistance is among the important strategies of mitigating biotic factors affecting productivity of beans in Sub-Saharan Africa (Kusolwa, 2007). Several studies have been done worldwide to evaluate bruchid resistance and most pointed to antibiosis but these however have been inconclusive (Murdock *et al*., 1990; Goossens *et al*., 2000; Zambre *et al*., 2005). In Zambia, screening of some varieties has been done and has revealed some varieties are resistant to the bruchid *Callosobruchus maculatus* (Sohati *et al*., 1994; Munyinda, 2013). It is important that the resistance to bruchids that is observed is studied further to understand the trait of interest and the gene action behind it. Dry bean cultivars that delay or prevent development of the predominant bruchid species will contribute to a stable supply of dry beans in the country. The objectives of the study were:

1. To evaluate common bean for *Callosobruchus maculatus* resistance.
2. To identify phytochemicals related to *Callosobruchus maculatus* resistance in common bean.
3. To establish the gene action controlling *Callosobruchus maculatus* resistance in common bean.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The bean beetle-bruchid

Bruchids are found in all major land masses except the Antarctica and New Zealand. There has been more speciation that has occurred in the tropical regions than in the temperate areas (Kingsolver, 2004) due to the optimum activity temperatures which are found in warmer climates.

Bruchids belong to the order Coleoptera in the family Bruchidae. Bruchids occur in several genus and species some being *Zabrotes subfasciatus, Acanthosclides obtectus, Callosobruchus maculatus, C. rhodesianus, C.analis* and *C.chinensis*. The genus *Callosobruchus* originated in Africa and Asia (Southgate, 1978). In Southern Africa *C. maculatus* and *C. rhodesianus* are the most prevalent (www. AfricanCrops.net, 2010; Southgate, 1978) although others such as *Z. subfasciatus* and *A. obtectus* are also prevalent and of economic importance in certain Southern African countries such as Malawi and Tanzania (Kusolwa, 2007; Kananji, 2007). In Zambia the most prevalent bruchid species is the *C. maculatus* (Sohati Pers. Commun, 2011, Chipabika Pers. Commun, 2013).

2.2 Damage

The adult beetles commonly referred to as weevils deposit eggs on the surface of bean pods or directly on the seeds, and the eggs hatch into larvae that burrow through the pods and into the seeds to feed on the nutritious cotyledons. The larvae remain inside the

seed during metamorphosis from larva to adult, when they emerge to continue the reproduction cycle even while beans are in storage.

Damage is directly related to the number of larvae that hatch and burrow into and feed within the seed. Each emerging adult leaves a serious perforation, with resultant weight loss of the seed. Adult bruchids cause no direct damage to the beans in storage, but females can lay 30 to 60 eggs that perpetuate the cycle depending on the species and host (Parsons and Credland, 2003).

Figure 2: Adult emergence and eggs laid of *Callosobruchus maculatus* in soybeans. Image Source: Clemson University – USDA Cooperative Extension Slide Series, USA 2013.

2.3 Losses incurred

Common bean production in the tropical and subtropical countries is constrained by many biotic and abiotic factors affecting crop productivity and crop quality during the growing season. Dry bean seeds being rich in proteins, carbohydrates, and lipids are sub-

ject to predation by post-harvest pests including bean weevils. Bruchids attack dry bean seeds both in the field before harvest and in storage warehouses (Kusolwa, 2007).

Most of the varieties which the farmers grow in Zambia are highly attacked by the bruchids causing severe losses. Other important losses include nutritional quality loss due to protein and carbohydrate degradation which highly affects the taste, low market value of edible bean seeds and eventually loss of seed viability. However it may even result in complete loss if bean is stored for a longer period (Parsons and Credland, 2003).

Though reports of economic damage vary, beetle damage in the warehouses can result in as much as a 48 % reduction in quality and quantity (Slumpa and Ampofo, 1991). A 5-20 % seed-weight loss has been reported, and bean seeds can sometimes be turned into hollow shells filled with powdered cotyledon and insect frass (Schoonhoven *et al.*, 1983; Schoonhoven and Cardona, 1986). Songa and Rono (1998) reported 40% weight loss and 80% quality loss, which made the beans not suitable for human consumption, after six months in storage on-farm. Losses ranging between 7% and 73% were reported in Colombia, Kenya and Tanzania (Silim, 1990). In Uganda, damage levels have been estimated to be 3% and 8% respectively for storage durations of 3 and 6 months (Silim *et al*., 1991). In Malawi, storage losses up to 38% have been reported (Chirwa, 2001).

2.4 RESEARCH STATUS

2.4.1 Worldwide

Breeding for bruchid resistance in common bean was begun at International Center for Tropical Agriculture (CIAT) in 1982. At CIAT more than 4000 cultivated beans have been screened for resistance to *Z. subfasciatus* and over 6000 for *A. obtectus*. No satisfactory levels of resistance were identified and a search was then made among the wild froms of *P.vulgaris* of Mexican origin which resulted in the discovery of very high levels of resistance to both bruchid species (Schoonhoven and Voysest, 1991).

There are several mechanisms of bruchid resistance identified in several studies that have been done in various crops and species. These include tolerance, antibiosis and non- preference.

2.5 RESISTANCE

Resistance may be justifiable if beans can be kept from weevil damage for at least 60-90 days or longer after harvest. In the absence of resistance and any control measures, weevil damage becomes apparent within 30-35 days after harvest or in storage (Kusolwa, 2007). Variation of resistance to a particular parasite may be expressed continuously or discontinuously in a segregating population depending on the number of resistance genes involved. Thus continuous variation between susceptibility and resistant entails many genes are involved. Discontinuous variation results in distinct well defined classes of resistance or susceptibility and resistant entails mono genic or oligo-genic control (Russell, 1978).

Resistance phenomenon is usually due to three situations; tolerance, antibiosis and non-preference.

Tolerance: the host plant appears to suffer little damage inspite of supporting a sizable pest population.

Antibiosis: The plant resists insect attack and has an adverse effect on the binomics of the insect pest. This adversely affects the development and reproduction of insects. This is related to the chemical and biological constituents of the seed such as the presence of certain amino acids which are linked to the trait of resistance to the bruchids. Most of several mechanisms of bruchid resistance identified in several studies point to antibiosis (Schoonhoven and Voysest, 1991) of biochemical nature and structural components of the seed. Antibiosis was expressed as reduced weevil emergence, longer larval developmental time and reduced progeny weight (Schoonhoven and Voysest, 1991).

Non-preference: Certain plants are less attractive to the pest for oviposition or feeding because of their texture, color, odor or taste, seed size, seed coat thickness (Nwanze and Horber, 1976; Brewer *et al*., 1983). This makes the plant unsuitable for colonization or oviposition of an insect.

The mechanisms of antibiosis and other forms of resistance are discussed in detail below;

2.5.1 TOLERANCE

There are conditions when these latter chemical defenses can be made inadequate, so bruchids are able to infest seeds. Firstly, many plants suffer reductions in defense com-

pounds during their developmental cycle. Secondly, just as plants evolve defenses, their predators evolve tools to evade those defense mechanisms (Franco *et al*., 2002). For example, it has been reported that a chemical mechanism is used by insects to overcome protein denaturing compounds, such as tannins (Konno *et al.*, 1997).

In this connection, Konno *et al.* (1997) observed that some Lepidoptera larvae secrete a large amount of free glycine in digestive juice to counter the protein denaturing activity of host plant tannins. As far as the second chemical defense considered, α-amylase inhibitor formed a complex with some α-amylases and, in this manner, was supposed to play a role in plant defense against insects (Ishimoto and Chrispeels, 1996; Jouanin *et al*., 1998). However, in nature, some bruchids can feed on plants producing α-amylase inhibitors because they possess a serine protease able to cleave some kinds of α-amylase inhibitors (Ishimoto and Chrispeels, 1996; Jouanin *et al*., 1998).

2.5.2 ANTI BIOSIS

Common bean contains significant amounts of seed storage proteins used for embryo and seedling development, as well as for defense against seed pests. Some of the well documented and important storage proteins in common bean seeds includes: phaseolin, lectins, phyto-haemagglutinins (PHA), trypsin inhibitors, and lectin-like proteins that include arcelins and α-amylase inhibitors. Phaseolin is among the most extensively studied major storage protein of common beans (Brown *et al*., 1982; Gepts, 1988) and has been used to explain the evolutionary relationship of different germplasm pools within *P. vulgaris* (Gepts 1988; Kami *et al*., 1995).

2.5.2.1 Phaseolin

Phaseolin is an important source of essential amino acids for animal nutrition, and unlike other bean seed storage proteins, is not associated with an antibiosis effect to insect pests. In addition to phaseolin, the second most common group of seed proteins in common beans are the loosely called lectins or phyto-haemaglutinins, as well as additional lectin-like proteins (Osborn *et al*., 1988b; Chrispeels and Raikhel, 1991).

The presence of phaseolin (vicilin-like 7S storage globulin) peptides in the seed coat of the legume *Phaseolus lunatus* L. (lima bean) was demonstrated by N-terminal amino acid sequencing. Utilizing an artificial seed system assay showed that phaseolin, isolated from both cotyledon and testa tissues of *P. lunatus*, was detrimental to the non-host bruchid *C. maculatus* with ED_{50} of 1.7 and 3.5%, respectively. The level of phaseolin in the seed coat (16.7%) was found to be sufficient to deter larval development of this bruchid. The expression of a *C. maculatus*-detrimental protein in the testa of non-host seeds suggests that the protein may have played a significant role in the evolutionary adaptation of bruchids to legume seeds (Macedo *et al*., 1993; Lattanzio *et al*., 2000).

2.5.2.2 Lectins and lectin like proteins (LLP'S)

Lectins a group of proteins possessing at least one non catalytic domain which binds reversibly to a specific mono or oligo saccharide have been considered as defensive compounds against cowpea weevil even if toxic effects of active lectins in some cases could be due to an α- amylase inhibitor presence. 18–20 plant α-amylase inhibitors are particularly abundant in cereals and leguminosae. Some wheat α- amylase inhibitors inhibit insect α-amylases strongly but do not inhibit mammalian α-amylases, suggesting that they

could be used as tools of engineered resistance of crop plants against pests. Bean α-amylase inhibitors, when added in low concentrations (1%) to artificial diet, proved to be toxic to the larvae of cowpea weevil and adzuki bean weevil (Pedra *et al*., 2003).

As seed storage proteins, they accumulate in cotyledons and provide a reserve for amino acids required in seed germination, and seedling development. Phyto-haemagglutinin (PHA) is the major lectin of beans and functions as a carbohydrate binding protein that defends plants against predation by most organisms, but is less effectively against cow-pea weevil *C. maculatus* (Murdock *et al*., 1990). Yet PHA may have a synergistic effect when combined with other anti-nutritional storage proteins in inhibition activity to pred-atory insects. Phyto-haemagglutinin is an anti-nutritional factor for mammals because it binds to the glycoproteins that line the intestinal tract thus inhibiting nutrient absorption (Broughton *et al*., 2003). Similarly, protease inhibitors in bruchids were suggested as potential anti-nutritional deterrents to larvae of *A. obtectus* and result in delayed growth and development (Campos *et al*., 2004).

2.5.2.3 Arcelins

Infestation studies were conducted at CIAT using different bean lines developed by the University of Wisconsin, for the presence and absence of the arcelin. Those lines positive for arcelin were resistant to *Z. subfasciatus* but susceptible to *A. obtectus*. Bean lines without arcelin were susceptible to both species (CIAT, 2005). A protein arcelin is postulated as the factor responsible for resistance in the wild *P.vulgaris* types. Four arcelin variants were identified arcelin1, 2, 3 and 4 (Schoonhoven and Voysest, 1991).

Arcelins were first found in a limited number of wild common bean accessions from Mexico (Osborn *et al*., 1988a, Osborn *et al*., 1988b). Arcelins are abundant seed storage proteins and they were discovered as a protein that was associated with inhibition of development of some species of bruchids. Approximately 10% of wild common bean accessions from Meso America possess arcelins. In addition to wild bean, tepary beans are also known to contain variants of arcelin proteins. This protein is absent in cultivated common bean (Chrispeels and Raikhel, 1991) presumably as a result of arcelins not making it through the domestication bottleneck.

Recent work based on genetic transformation indicated that arcelins may not be the only factors associated with high levels of resistance to bruchids (Goossens *et al*. 2000; Zambre *et al*. 2005). Alternatively, a multiple or synergistic interaction of arcelin with other factors may be involved, which would have not been transferred in a transformation process (Goosens *et al*., 2000). While these efforts have created lines with strong *Z. subfasciatus* resistance, they provide only weak to moderate resistance to *A. obtectus* (Cardona *et al*., 1990; Kornegay and Cardona 1991; Kornegay *et al*., 1993; Hartweck *et al*., 1997; Acosta-Gallego *et al*., 1998; Paes *et al*., 2000; Sales *et al*., 2000).

2.5.2.4 Trypsin inhibitors/tannins

Tannins, hydrolysable tannins and condensed proanthocyanidins are large polyphenolics whose molecular weights range from 500 to 4000 kDa and whose many hydroxyl groups interact with proteins, denaturing and precipitating them from solution (Haslam, 1998). Tannins may affect the growth of insects in three main ways: they have an astringent taste which affects palatability and decreases feed consumption, they form complexes

with proteins of reduced digestibility and they act as enzyme in-activators (Swain, 1977). Seed tissues contain tannins located mainly in a layer between the outer integument and the aleurone layer, while α-amylase inhibitors are located in cotyledons (Gatehouse *et al*., 1979; Lattanzio *et al*., 2000).

Gatehouse *et al.* (1979) concluded that resistance in some cowpea seed was derived from an elevated level of trypsin inhibitor within the seeds. However, some researchers suggest that the trypsin inhibitor alone does not account for bruchid resistance in cowpea, thus indicating a need for further investigations (Lattanzio *et al*., 2000).

2.5.2.5 Lipids

Lipids have also been found to be responsible for some resistance in beans. The influence of bean seed surface lipids on infestation of seeds by *A. obtectus* was investigated in Poland. The experiments were performed in dual-choice bioassays on three bean varieties: Blanka, Bor and Longina (Nietupski *et al*., 2005). Chemical analyses revealed the following groups of surface lipids: wax esters, long chain primary alcohols, n-alkanes, sterols, fatty acids, squalene, aldehydes, monoacylglycerols, ketones and fatty acid esters. Fatty acids and monoacylglycerols were found to deter bean weevil infestation, while alkan-1-ols acted as attractants.

2.5.3 NON- PREFERENCE

Studies on oviposition preferences of bruchids showed that bruchid species exhibited a marked preference for large seeded materials when mixtures of bean seeds of all sizes were infested. This resulted in many small seeded materials escaping infestation and a

bias toward selecting large seeded types (Schoonhoven and Voysest, 1991; CIAT, 1996).

It has been demonstrated that physical factors such as seed coat hardness and seed coat roughness confer resistance to bruchids (Giga and Smith, 2002). A hard seed coat may prevent larvae from successfully penetrating the seed, while a rough seed coat provides difficulties for *Z. subfasciatus* in particular, because it glues its eggs on the seed testa. Rough seeds are therefore less preferred for oviposition (Nwanze and Horber, 1976; Messina and Renwick, 1985). Lale and Kolo (1998) suggested that the presence of bio-chemical factors in the seed coat, irrespective of coat texture, may cause reduced ovipo-sition and the poor survival of bruchid eggs on some resistant cowpea varieties. Tannins in the seed coat (Deshpande, 1992) and trypsin inhibitors (Savelkoul *et al*., 1992) have been implicated in the resistance of seed to bean weevils.

Physical measurements were made of several pod and seed characteristics to ascertain whether the observed pod resistance was due to seed factors, pod-wall factors, or to in-teractions between the pod and seeds. Among the other pod and seed characteristics measured to identify major resistance factors, seed coat thickness was the one most highly correlated with pod resistance. The results suggested that interactions between pod-wall and seed coat characteristics play a large role in pod resistance of cowpeas to *C. maculatus* (Kitch. *et al*., 2011).

Reports on the effect of seed coat on oviposition and survival of *C maculatus* have been conflicting. For example, Nwanze and Horber (1976) suggested that causes of resistance in cowpea to *C. maculatus* might be categorized as non-preference during oviposition

and antibiosis during larval development. Antibiosis may not only be explained as a biochemical phenomenon, but it also involves physical components, namely the surface texture and structure of the seed coat, which affect larval penetration. Cowpea weevil prefers smooth coated seeds to wrinkled seeds for oviposition, and more first instar larvae successfully penetrate the seed coat in smooth than in rough seeds (Kitch *et al*., 2011). In contrast, Eddie and Amatobi, 2003, in their experiments on 22 cowpea varieties (five resistant, four moderately resistant, and 13 susceptible varieties), with and without seed coat, observed that seed coat has no value in protecting cowpea seed against attack by *C. maculatus*

The abundant literature references concerning the resistance mechanisms of plant tissues against insects strongly suggest that the ecological relationship between insects and plant tissues is a complex one with physical as well as chemical interactions. As far as the mechanism of seed resistance against bruchids is concerned, many strategies are used by seeds to protect themselves against insects:

(i) The seed may be too hard for newly hatched larva to penetrate, (ii) The seed may physically be too small or with an inconvenient shape for the larva to reach full size, (iii) The seed may contain too little food to support the larva and (iv) The seed may contain toxins or other substances in the cotyledons or its enveloping seed coat that inhibit the larval development (Kashiwaba. *et al*., 2003).

2.6 Gene action and inheritance of bruchid resistance

The inheritance of resistance to *A. obtectus* was studied by Kananji (2007) in a 6 x 6 complete diallel mating design. There were significant differences among genotypes for general combining ability (GCA) and specific combining ability (SCA). However, SCA

accounted for 81% of the sum of squares for the crosses, indicating predominance of the non-additive gene action contributing to bruchid resistance. A chi-square test for a single gene model showed that 5 of 13 F_2 populations tested fitted the 1:2:1 segregation ratio of resistant, intermediate and susceptible classes, respectively indicating partial dominance. Then eight F_2 populations did not conform to the two gene model of 1:4:6:4:1 segregation ratio of resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible classes, respectively. Average degree of dominance was in the partial dominance range in five F_3 populations, but in general resistance was controlled by over-dominance gene action in the F_2 populations. The additive-dominance model indicated that epistatic effects were not important in controlling the bruchid resistance. The frequency distribution of the 13 F_3 populations for resistance to *A. obtectus* provided evidence for transgressive segregation, suggesting that resistance is conditioned by more than one gene. Reciprocal differences were not significant in the F_2 generation seed; but were significant in four crosses in the F_3 generation seed for adult bruchid emergence, suggesting that maternal effects or cytoplasmic gene effects also played a role in the inheritance of resistance to the common bean weevil (Kananji, 2007).

Resistance to *A. obtectus* damage is accompanied by reduced starch content, and high content of an acidic polysaccharide (whose structure has not been elucidated). No protein cause for resistance was found. Inheritance of resistance to *A. obtectus* is recessive. Since the factor responsible for resistance is not a primary gene product and is expressed recessively, this factor is unsuitable for incorporation into breeding lines to be used for developing commercial cultivars (Hugo *et al*., 1990).

Arcelin alleles are reported to be inherited as single dominant genes thereby facilitating transfer (Osborn *et al*., 1988b). Studies conducted at CIAT using susceptible and resistant parental lines, F_1 hybrids and other reciprocals, and F_2 and F_3 progeny crosses revealed that the resistance to *A. obtectus* appeared recessively inherited and the genes controlling may reflect inheritance of the heteropolysaccharide (CIAT, 2005).

2.7 Advances in Zambia

In Zambia, the Department of Plant Science in the School of Agricultural Sciences at the University of Zambia initiated character improvement of common bean through induced mutation breeding in 2000. The research was carried out in collaboration with Zambia Agricultural Research Institute (ZARI) and the National Institute for Scientific and Industrial Research (NISIR). Gamma radiation was used and 150 Gray dose produced functional mutations (Munyinda, 2013).

Mutants were developed with desirable seed coat and pod colour. CA38-38-9-B (Carioca 38), CA18-22 and CA24-2-9-B1 had attractive white, cream and mauve seed coat colours respectively. Some mutants developed have shown resistance to insect storage pests and diseases. Carioca 38 has been found to be highly resistant to bean storage bruchid (*C. rhodesianus*) and cowpea bruchid (*C. maculatus*). The susceptibility index to bruchid attack was 0.00 for Carioca 38 while that of the parent was 0.027 (Chibowa, 2008). The identified resistant mutant derived lines could be used as parents to increase bruchid resistance in desirable bean varieties.

Another study evaluated F_2 crosses of common bean for bruchid *C. maculatus*. This study evaluated some important varieties such as Carioca 38 and critical crosses such as

Carioca 38 x Lyambai and Carioca 38 x Solwezi. The number of eggs laid on these varieties were similar and it was concluded that the Carioca 38 allowed eggs to be laid. Lyambai x Lukupa and Solwezi x Lukupa had the highest number of adult insects while the crosses between Carioca 38 x Lyambai and Carioca 38 x Solwezi followed with less. All the crosses were susceptible to *C. maculatus* but Carioca 38 was resistant (Zulu, 2010). Therefore in Zambia, very little has been established in the causes of resistance in varieties such as the Carioca 38 and Rab 608 where most of the farmers preferred varieties are so susceptible to the *C. maculatus* which results in severe losses.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The experiment had three phases namely, greenhouse, screen house and laboratory components. All phases were carried out at the University of Zambia (UNZA). The greenhouse experiment were carried out during the 2011/2012 season which involved crossing of the resistant and susceptible genotypes, while the screen house was used in advancing the F_1 to F_2 generation and was carried out in the 2012/2013 season. The laboratory experiments were carried out at both the Departments of Plant Science and Chemistry and these were to screen genotypes for bruchid resistance and evaluate the chemical constituents in these genotypes.

3.1 Materials used

The main materials in this study involved bean seeds and beetles (bruchids). Six susceptible and two resistant bean varieties whose characteristics are summarized in Table 1 were used.

The bruchids were collected from bean seeds in the seed store in the Plant Science Department and verified to be *C. maculatus* by an entomologist. These were then cultured and reared and then day old bruchids were used for infestation (Rojas-Rousse *et al*., 1988; Rojas-Rousse, 2006; Tefera *et al*., 2011; Derera *et al*., 2001a).

Table 1: Summary of bean genotypes used

Sample	Source	Bruchid behavior
Carioca 38	UNZA	Resistant
Rab 608	CIAT	Resistant
Kalungu	Misamfu	Susceptible
Lukupa	Misamfu	Susceptible
Lusaka	Misamfu	Susceptible
Lyambai	Misamfu	Susceptible
Chambeshi	Misamfu	Susceptible
Kabulangeti	Misamfu	Susceptible

3.2 Experimental design

The parental genotypes were grown in pots in three replications and soil was mixed with compost in a 2:1 (soil: compost) ratio. Following the days to 50% flowering for each genotype, planting was staggered to synchronize flowering of the male and female plants. Two methods of cross pollination were used: (1) mechanical emasculation of the female parent using tweezers on flower buds one day before the flower opened followed by cross pollination using ripe pollen from open flowers of the male parents, (2) Hooking without emasculation as proposed by Freytag (1977) and Walter *et al*. (1980).

Crosses were made in the green house early morning and evening when temperatures were low (18-24^{o}C) (Walter *et al*., 1980). The crosses made are as shown in Table 2. The two resistant varieties were considered as the males and the six susceptible varieties as females. The temperatures (35-40^{o}C) were high during this period which led to abor-

tion of some flowers during hybridization. All the crosses produced enough F_1 seed to be advanced to the F_2.

North Carolina Mating Design II (Comstock *et al*., 1949) was used as presented in Table 2 to create experimental populations and the resultant populations evaluated as a Complete Randomized Design (CRD). Management practices were followed such as timely weeding, spraying against diseases and pests and irrigation. .

Table 2: 2*6 North Carolina Mating Design II

Males	Carioca 38	Rab 608
Females	Kabulangeti	Kabulangeti
	Lusaka	Lusaka
	Lukupa	Lukupa
	Lyambai	Lyambai
	Chambeshi	Chambeshi
	Kalungu	Kalungu

3.3 Advancement of F_1 to F_2 generation

The F_1 seed was advanced to F_2 generation in order to increase the quantity of seed. The F_1 seed harvested from the greenhouse was treated with a seed dressing containing Imidachloprid and planted in the screen house at the School of Agricultural Sciences to allow for advancement into the F_2 generation. Management practices were followed as indicated in Section 3.2.

Fertilizers used were the D compound (10N:20P:10K) at a rate of 100kg/ha and 0.5 g was applied in each pot as a basal dressing. Foliar fertilizer Omni boost® (containing

boron, manganese, magnesium, molybdenum, nitrogen and calcium) was used at 5 g per litre to counter micro nutrient deficiencies of manganese, magnesium and boron which were observed on the plant samples. Monochrotophos in the form of phoskill, Abamectin and Lambda-cyhalothrin were applied at the rate of 4 ml, 2 ml and 5 ml per litre to control aphids, white fly and leaf miner, red spider mite and pod borers. Mancozeb (Dithane M45) and Chlorothalonil were applied at the rate of 10 g and 15 ml per litre respectively as preventive fungicide. However, when powdery mildew and leaf spots were observed, 10 g, 3 ml, 2 g per litre of Mancozeb, Artea (cyproconazole, propiaconazole) and Benomyl respectively were sprayed consecutively. Watering was done once per day either early in the morning or late afternoon as need arose. Weeding was done manually weekly as the weeds emerged.

3.4 Evaluation of the parental genotypes and the F_2 for bruchid resistance

Some of the parental and F_2 genotypes did not yield adequate seed for inclusion in the North Carolina Design II analysis. There was only one complete set with enough seed (more than 200 seeds) as shown in Table 3, therefore was considered as the test material.

Table 3: The genotypes used in beetle evaluation

Males	Carioca 38	Rab 608
Females	Kalungu	Kalungu
	Lukupa	Lukupa
Cross	Carioca38xKalungu	Rab 608xKalungu
	Carioca38xLukupa	Rab 608xLukupa

The complete set of F_2 generation and the parental genotypes were subjected to laboratory beetle screening at the Department of Plant science (Tefera *et al*., 2011).

Ten seeds from each cross were placed in a jar with a sieve to allow aeration and three replications were used in a Completely Randomised Design (CRD). Where seed was limiting replication was reduced accordingly. Carioca 38 and Rab 608 were used as the controls.

To condition the seed, the following was done as recommended by Tefera *et al*. (2011). The seed was put in a refridgerator at 17^oC for five days to ensure there was no presence of bruchid eggs on the samples to be tested before infestation.

Infestation of day old bruchid pairs (male and female) was done on the test materials using the no choice test as described by Kananji (2007) and also documented in Miller *et al*. (1986); Tefera *et al*. (2011). These were left for five days to ensure mating had occurred. There after the bruchids were removed and the test material was left to stand in the laboratory in the plastic jars at room temperature (24^oC) and after five days the number of eggs laid were observed and counted using a magnifying glass. The materials were then kept in a room with temperatures at 27^oC with 70% relative humidity as described by Kananji (2007). This was to optimise the temperatures for bruchid development for all the test material.

3.5 Thin Layer Chromatography (TLC)

A chemical analysis of the parents and the F_2 was done at the Department of Chemistry in the School of Natural Sciences at the University of Zambia. This was to determine the chemicals that may be linked to bruchid resistance**.** The samples were ground, extracted with a mixture of methanol and dichloromethane, filtered and run through a Thin

Layer Chromatography (TLC) membrane. The details are shown below using one such sample. Five grams sample of the whole beans seed were ground in a domestic grinder for each genotype.

To prepare for TLC analysis, the ground sample of Carioca 38 (five grams) was submerged in 1:1 Methanol/ Dichloromethane (10 ml). The mixture was stirred, covered and kept in a cool shaded place for 24 hours. The mixture was emptied into a filter paper standing on a small beaker. The residue was rinsed twice with 2 ml of Methanol in a 1:1 Methanol/Dichloromethane mixture. Other samples were treated the same way. The clear solution was used for TLC as out lined in the Organic Chemistry III Laboratory Manual (2013).

For TLC, aluminium plates coated with silica gel 60 F_{254} were used. The plates were 5 cm x 4 cm. The spotted TLC plates were developed in 5 ml of different solvent mixtures of distilled (1:1) hexane and distilled ethyl acetate. The developed TLC's were dried and sprayed with 5% vanillin and 5% H_2SO_4 in methanol under a fume hood. The TLC procedure differs on the solvents used but was conducted according to Vogel *et al*. (1989) and the Organic Chemistry III Laboratory Manual (2013).

Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components measured by the Retardation factor (R_f) value can be adjusted (Fair and Kormos, 2008). Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. For instance, if normal phase silica gel is used

as the stationary phase it can be considered polar. Given two compounds which differ in polarity, the more polar compound has a stronger interaction with the silica and is therefore more capable to dispel the mobile phase from the binding places. Consequently, the less polar compound moves higher up the plate (resulting in a higher R_f value) (Harry *et al.*, 1989).

The Kjeldahl procedure was used to determine the protein content (Persson *et al.*, 2008). Two replications for each sample were used as seed was inadequate.

The Susceptibility Index (SI) was used;

Where:

SI = Dobie's index of susceptibility = ($Log_e X$ /MDT) 100

$Log_e X$ = is the natural logarithm of the total number of the F_1 progeny emerged

MDT = Median development time

The Dobies' index (Dobie, 1974) for each genotype was computed by taking the susceptibility of that genotype as a proportion of the susceptibility index of the susceptibility check and multiplied by 10 (Dobie, 1974).The Dobie relative index was then used to classify the genotypes into susceptibility groups following the scales used at CIMMYT in Zimbabwe (Pixley, 1997) which are as follows:

- Dobie relative index of less than or equal to 4 was classified as resistant
- Dobie relative index of 4.1 to 6.0 classified as moderately resistant
- Dobie relative index of 6.1 to 8.0 classified as moderately susceptible
- Dobie relative index of 8.1 to 10 classified as susceptible
- Dobie relative index of more than 10 was classified as highly susceptible

3.6 Data Collection

This section describes the parameters measured and collected and a brief description of how each of these parameters were measured.

Table 4: Parameters measured on the F_1 and F_2 plants and seed

Parameter	Method of measurement
Number of days to flowering	The number of days to 50% flowering were counted from the day of planting. This was done to observe if there any differences in the number of days to flowering after crossing.
100 seed weight(g)	100 seeds of each sample were weighed and then this was converted to the size of each seed (seed size).
Seed coat thickness(mm)	This was measured using a micro meter screw gauge. The bean seed for each sample in three replications was soaked in warm water for a day. Thereafter, the seed coat was then peeled off gently and placed in petri dishes for a day to evaporate the water and dry. The seed coat was then placed between the screw gauge to measure the thickness.
Number of eggs laid	The number of eggs laid were measured using a magnifying glass. Transformation was done using the formula $(x+0.5)^{0.5}$.
Number of days to emergence	The number of days were counted as the adults emerged from the seed from the time of infestation.
Number of adults emerging	The number of adults emerging were counted as they emerged from the seed from the time of infestation.
% Adult emergence	Number of adults emerged/Number of eggs laid*100.
Mean development time	The number of adults that emerged*day of emergence/ number of test days.
Susceptibility Index(SI)	$(\text{Log}_e X / \text{MDT}) 100$
Chemical compounds present	The chemicals present were identified using the Thin Layer Chromatography (TLC) (Harry *et al*, 1989). They were calculated using the retardation factor (R_f), where R_f =distance travelled by compound⁄ distance travelled by solvent.

3.6 Statistical analyses

The collected data on different components were compiled and analyzed statistically using the Genstat 14th Edition. Means were separated using Least Significant Difference (LSD) and Standard Error (SE). General analysis of variance (ANOVA) using Completely Randomized Design (CRD) model was performed for all measured and derived quantitative data which included adult bruchid emergence, mean development time and susceptibility index using Genstat statistical package. Correlation and stepwise regression analyses were carried out between all traits and the susceptibility index (SI).

Data subjected to analysis of variance used the linear model for analysis of North Carolina II for single environment according to Singh and Chaudhary (2004) and Makumbi, (2013).

General combining and specific combing ability effects were calculated (Makumbi, 2013). Test for significance of GCA and SCA effects was done using a t-test, where $t = GCA/SE_{GCA}$ and $t = SCA/SE_{SCA}$ respectively (Kang, 1995). The Baker (1978) coefficient $\{(\sigma^2gca_m + \sigma^2gca_{f)} / (\sigma^2gca_m + \sigma^2gca_f + \sigma^2sca)\}$ was used to determine the type of gene action, the relative importance of GCA to SCA variances. The additive, dominance variances and narrow-sense heritability were estimated according to Singh and Chaudhary (2004).

CHAPTER FOUR

4.0 RESULTS

4.1 General observation

Table 5 presents the ANOVA results for parameters measured and derived. Significant differences ($P \leq 0.001$) were observed among males, among females and among crosses for susceptibility index, the number of insects emerged, the % adult emergence, mean development time, protein content, seed coat thickness 100 seed weight and days to 50% flowering.

4.2 Susceptibility Index (SI)

Overall susceptibility index mean of genotypes evaluated was 3.54 with the highest being for Lukupa with 7.85 and the lowest being for Carioca 38 with 0.0. Males had lower SI values (0.55) as compared to females (6.92). Between the male genotypes, Carioca 38 had lower SI of 0.0 than Rab 608 with a value of 1.1, while Lukupa had lower SI (5.99) than Kalungu (7.85) for the female genotypes. Susceptibility index for crosses ranged from 1.41 for Carioca 38 x Lukupa to 5.08 for Rab 608 x Lukupa. Among the three factors of treatments (males, females and crosses), males had the lowest SI (0.55) with crosses having intermediate (3.35) and females had the highest value of 6.92.

The genotypes were grouped based on feeding behavior of bruchids using the SI and classes varied from resistant, moderately resistant to moderately susceptible and are presented in Table 7 using a relative Dobies' index. Carioca 38 and Rab 608 were resistant with their SI values being 0.0 and 1.1 respectively.

Kalungu and Lukupa were classified as moderately susceptible with Kalungu showing a higher SI (7.85) than Lukupa (5.99). Among the crosses, Carioca 38 x Kalungu and Carioca 38 x Lukupa were in the resistant class with their SI's being 2.45 and 1.41 respectively. The SI for Rab 608 x Kalungu was 5.08 and Rab 608 x Lukupa had 4.45 and both these crosses were classified as moderately resistant.

4.2.1 Number of eggs laid

The mean across the genotypes for the number of eggs laid was 6.94 with Lukupa being highest with 13.0 number of eggs laid, Carioca 38 and Carioca 38 x Kalungu recorded the lowest number (4.0). Males recorded lower number of eggs laid (4.5) as compared to the females (10.5) where Carioca 38 had 4.0 number of eggs laid, Rab 608 had 5.0 and between the females, Kalungu had a lower value (8.0) than Lukupa (13.0). The crosses recorded a range of 9.5 for Rab 608 x Lukupa to 4.0 for Carioca 38 x Kalungu. Among the males, females and crosses, males recorded the least (4.5), an intermediate value was observed with the crosses (6.4) and females had the highest value of 10.5.

4.2.2 Number of insects emerged

Different behavior was observed in the number of insects that emerged from the genotypes with the overall mean at 3.31 and the highest value being observed in Lukupa (10.0) and the least number in Carioca 38 and Carioca 38 x Lukupa (0.0). Males showed reduced number of insects emerging (0.5) as compared to the females (7.25). Carioca 38 showed a lower number (0.0) than Rab 608 (1.0) while Kalungu had a lower value (4.5) than Lukupa (10.0). The mean number of insects that emerged for the crosses ranged from 6.0 for Rab 608 x Lukupa to 0.0 for Carioca 38 x Lukupa.

Among the treatment genotypes used, the number of insects observed were least in the males (0.5), intermediate for crosses (2.75) and highest for females (7.25).

4.2.3 Percentage Adult emergence

The % adult emergence varied among the genotypes with grand mean at 35.91 and the highest emergence being 77% for Lukupa and lowest 0.0 for Carioca 38. Among the males, females and crosses, the females recorded highest percentage (66.5), the crosses were with an intermediate percentage of 33.57 and the males had the least value of 10.0%. The males showed a reduced percentage emergence (10.0) as compared to the females (66.5). Carioca 38 showed significantly less adult emergence (0.0) when compared to Rab 608 (20.0), while Kalungu showed a relatively lower value of 56.0 with regards to Lukupa with 77% insect emergence. Among the crosses Rab 608 x Lukupa was highest with 63.15% while Carioca 38 x Lukupa had the lowest value of 0.0%.

4.2.4 Mean development time (MDT)

The overall mean of the development time was 32.0 days and the longest time to insect emergence was observed in Carioca, as at 44.0 days there was still no emergence and the shortest time was for Kalungu being 22.0 days. There was varied development time for insect emergence among the males, females and the crosses, where the males had the longest time of 40.5 days, crosses recorded an intermediate time of 32.25 days, the females had the shortest time to adult emergence with 23.0 days. Females had significantly lower values as compared to the males with values of 23.0 and 40.75 days respectively. Rab 608 had the shortest mean development time at 37.0 while Carioca 38 had 44.0 days

without any emergence. The mean development time for the crosses varied, ranging from 27.0 for Rab 608 x Lukupa to 44.0 for Carioca 38 x Lukupa.

4.2.5 Protein content

The mean protein content was 16.37% and varied among genotypes ranging from 17.32 for Kalungu and Rab 608 x Kalungu and to 14.98% for Rab 608 and Carioca 38 x Lukupa. Males showed lower protein content (15.69%) as compared to the females (17.05). Rab 608 showed lower protein content than Carioca 38 with values of 14.98 and 16.41% respectively while with the females, Lukupa had lower protein content (16.79%) than Kalungu (17.32%). The crosses showed variation in the protein content with the highest amount being for Rab 608 x Kalungu at 17.32% and the lowest at 14.98% for Carioca 38 x Lukupa. Among the treatment factors, the females had highest protein content (17.05%), the crosses were with an intermediate value of 16.36% and the lowest value was for the males at 15.69%.

4.2.6 Seed coat thickness

The overall mean for the genotypes for seed coat thickness was 0.51 mm. The seed coat thickness varied among the genotypes ranging from a thickness of 0.58 mm for Carioca 38 x Lukupa to 0.45 mm for Kalungu. The females had lower seed coat thickness (0.48 mm) as compared to the males (0.56 mm). Carioca 38 had lower seed coat thickness of 0.54 mm than Rab 608 with 0.57 mm and as regards the females, Kalungu showed lower thickness of 0.44 mm than Lukupa with 0.50 mm. Among the crosses the thickness ranged from 0.58 mm to 0.47 mm for Carioca 38 * Lukupa and Rab 608 * Kalungu respectively.

The males had highest seed coat thickness (0.56 mm), crosses had 0.51 mm and the females had the lowest (0.48 mm).

4.2.7 Seed weight

The 100 seed weight showed variations among genotypes compared to the overall mean of 30.84 g and ranged from 41.49 g for Kalungu to 19.49 g for Carioca 38 x Kalungu. Males had significantly lower seed weight (26.53 g) relative to the females (36.85 g). Carioca 38 had seed weight of 25.44 g while that of Rab 608 was 27.63 g and Lukupa had significantly lower seed weight (32.22 g) than Kalungu (41.49 g). Seed weight for the crosses showed Rab 608 x Lukupa with the highest (36.91 g) while Carioca 38 x Kalungu had the lowest (19.49 g). The seed weight was highest among the females (36.86 g), intermediate for the crosses (29.98 g) and lowest for the males (26.53 g).

4.2.8 Days to 50% flowering

The number of days to 50% flowering showed an overall mean of 33.31 days with the genotypes varying from 41days for Rab 608 to 29 days for Kalungu and Carioca 38 x Kalungu. There were variations between the parental genotypes with the number of days to 50% flowering for the females being 30.5 days compared to the males with 40.25 days. Carioca 38 was at 50% flowering in 39.5 days while that of Rab 608 was 41 days. Kalungu and Lukupa differed in their times of 50% flowering with the days being 29.5 and 31.5 respectively. Among the treatment factors, the males were highest (40.25), crosses were intermediate (31.25) and females were lowest (30.5) for the number of days to 50 % flowering. Among the crosses, Rab 608 x Kalungu recorded the highest number of days (34) while Carioca 38 x Lukupa recorded the least (29.5) days.

Table 5: Mean squares for the eggs laid, insects emerged and days to 50% flowering

	Insect derived parameters						Plant derived parameters			
Source of variation	**d.f**	**SI**	**EL**	**IE**	**%AE**	**MDT**	**P**	**SCT**	**SW**	**FL**
Genotypes	3	19.895***	1.113***	6.177***	1052.42***	7.281**	2.006***	$9.37x10^{-3}$***	$1.01x10^{2}$***	65.45**
Male	1	10.914***	0.764*	2.729***	3089.9*	5.281***	3.892***	$5.94x10^{-3}$***	$2.96x\ 10^{2}$***	4.5
Female	1	0.178	0.3787	0.044	20.4	0.031	1.786***	$5.94x10^{-3}$***	$5.51x\ 10^{1}$***	18**
Male x female	1	3.557**	0.018	0.889**	1101.9	0.551	0.344***	$1.80x10^{-3}$***	$2.54x\ 10^{1}$***	2
Residual	4	0.119	0.077	0.029	201.4	0.069	0.003	$3.75x10^{-6}$	$2.50x\ 10^{-4}$	0.75
CV%		11.1	10.7	11.1	43	20.8	0.4	0.4	0.1	2.8

Key: SI: Susceptibility index, EL: Number of eggs laid, IE: Number of insects emerging, %AE: percentage adult emergence, MDT: mean development time, P: Protein content, Seed coat thickness, SW: 100 Seed weight, FL: days to 50 % Flowering; ***: Significant at P≤ 0.001, **: significant at P≤0.01, *: significant at P≤0.05.

Table 6: Mean performance of the parents and the F_2 crosses evaluated in the study

	Insect derived parameters					Plant derived parameters			
Genotypes	**SI**	**EL**	**IE**	**%AE**	**MDT**	**P**	**SCT**	**SW**	**FL**
Carioca 38	0.0	4	0	0	44	16.415	0.543	25.44	39.5
Rab608	1.1	5	1	20	37	14.98	0.569	27.63	41
Kalungu	7.849	8	4.5	56	22	17.32	0.446	41.495	29.5
Lukupa	5.99	13	10	77	24	16.79	0.506	32.22	31.5
Carioca38 x Kalungu	2.45	4	1	25	30	16.34	0.497	19.495	31.5
Carioca38 x Lukupa	1.41	5.5	0	0	44	14.98	0.582	28.305	29.5
Rab608 x Kalungu	4.45	6.5	4	46.15	28	17.32	0.473	35.215	34
Rab608 x Lukupa	5.08	9.5	6	63.15	27	16.79	0.497	36.905	30
Mean	3.541	6.937	3.312	35.912	32	16.367	0.514	31.609	33.312
CV%	2.6	6	1.3	12.8	16.2	0.4	0.2	0.1	3.9
LSD	0.240	0.204	0.487	9.73	1.192	0.163	0.003	0.065	3.802

Key: SI: Susceptibility index, EL: Number of eggs laid, IE: Number of insects emerging, %AE: percentage adult emergence, MDT: mean development time, P: Protein content, Seed coat thickness SW: 100 Seed weight, FL: days to 50 % Flowering.

Table 7: Classification of bean genotypes for beetle resistance using relative Dobies' index

Genotype	Type	SI	Bruchid behavior
Carioca 38	Parent	0.0	Resistant
Rab 608	Parent	1.1	Resistant
Kalungu	Parent	6.9	Moderately susceptible
Lukupa	Parent	7.85	Moderately susceptible
Carioca 38 x Kalungu	Cross	2.45	Resistant
Carioca 38 x Lukupa	Cross	1.41	Resistant
Rab 608 x Kalungu	Cross	4.45	Moderately resistant
Rab 608 x Lukupa	Cross	5.08	Moderately resistant

Key: SI= susceptibility index

4.3 Simple correlation of the variables measured

Simple correlations measure character associations. A simple linear association between variables such as mean development time, seed coat thickness, seed weight, number of eggs laid, number of adults emerged, % adult emergence, protein content , flowering dates, and the susceptibility index were determined and summarized (Table 8).

It is evident from the correlation coefficients (r) that an inverse relationship existed between the susceptibility index (SI) and mean development time, seed coat thickness, seed weight, number of eggs laid, number of insects emerged, % adult emergence and flowering dates.

The % adult emergence and the number of eggs laid was positively and significantly associated with susceptibility index (SI) at r = 0.884*** and r = 0.677* respectively.

The protein content was negatively correlated with the seed coat thickness (r = -0.9113***). This was observed between Carioca 38 and Rab 608, where Carioca 38 showed protein content at 16.42% with seed coat thickness of 0.54 mm while Rab 608 had protein content of 14.98% and thickness of seed coat at 0.57 mm. In a similar manner Kalungu had protein content of 17.32% with seed coat thickness of 0.45 mm and Lukupa was at 16.79% protein content and seed coat thickness of 0.505 mm.

Among the crosses the highest protein content was observed in Rab 608 x Kalungu at 17.32% and seed coat thickness was the lowest at 0.47 mm while Carioca 38 x Lukupa had a low protein content of 14.98% and the thickness of the seed coat thickness was highest at 0.58 mm.

Table 8: Correlation coefficients of SI and other measured parameters

Parameter	SI	EL	IE	%AE	MDT	P	SCT	SW	FL
SI	1	0.677*	0.424	0.884***	0.101	0.410	0.301	0.588	0.136
EL		1	0.207	0.409	0.743	-0.050	0.099	0.097	-0.194
IE			1	0.315	-0.097	0.521	0.523	0.571	0.547
%AE				1	-0.059	0.441	0.251	0.589	0.213
MDT					1	-0.592	-0.379	-0.490	-0.556
P						1	-0.911***	0.926***	0.932***
SCT							1	0.798	0.912***
SW								1	0.808
FL									1

Key: SI: Susceptibility index, EL: Number of eggs laid, IE: Number of insects emerging, %AE: percentage adult emergence, MDT: mean development time, P: Protein content, Seed coat thickness, SW: 100 Seed weight, FL: days to 50 % Flowering, ***: Correlation significant at P≤ 0.001, *: Correlation significant at P≤0.05.

4.4 Stepwise multiple regression analysis

In order to study the cause and effect relationship between other traits and the SI, a stepwise multiple regression analysis was carried out, regressing all the measured traits on the SI to determine the strength of cause and effect relationships of these traits on SI as a dependent variable and the other traits as independent variables.

The % adult emergence and transformed number of eggs laid had the significant effect on the susceptibility index at $P \leq 0.001$ and $P \leq 0.05$ respectively (Table 9), therefore indicating that these two traits had the most effect on the susceptibility of the bean seed to bruchid attack. Other variables did not amount to significant difference according to the susceptibility index, thus were not included in the model.

The % adult emergence and number of eggs laid explained 74% and 18% effect respectively on the variation in the susceptibility index (Table 9).

Table 9: Stepwise multiple regression of susceptibility index on the components across genotypes.

Variable	Partial Square	R-Model Square	R - F- Value	Pr > F
% AE	.736	.736	25.093	.001
EL	.918	.182	17.827	.003

4.5 Combining ability estimates for adult bruchid emergence

The general combining ability and specific combining ability was determined for the transformed number of eggs laid, the transformed number of insects emerged, % adult emergence, mean development time, susceptibility index, seed coat thickness, seed weight, and flowering dates as shown in Table 10.

There were highly significant differences among genotypes for both GCA and SCA effects. Significant GCA variance effects for the number of insect emerged, % adult emergence and susceptibility index indicated that additive gene action was important in determining bruchid resistance.

Carioca 38 had significant negative GCA effects for susceptibility index (-1.16*), number of insects emerged (-0.584**), % adult emergence (-19.64*), mean development time (-0.812**) and seed weight (-6.08***) while had positive GCA effects for protein content (0.027**) and seed coat thickness (0.027***).

Rab 608 had significant negative protein content (-0.027**) and seed coat thickness (-0.027**). The positive GCA effects for Rab 608 were significant for the susceptibility index (1.16*), number of insects emerged (0.584**), % adult emergence (19.65*), mean development time (0.81*) and seed weight (6.08***).

The SCA effects for the susceptibility index and number of insects emerged for the crosses (Carioca 38 x Lukupa and Rab 608 x Kalungu) were negatively significant with the values of -0.66 (*) and -0.33 (*) for each parameter for both genotypes. Carioca 38 x Lukupa had negative significant effects on protein content (-0.015**) and seed coat thickness (-0.015**).

Carioca x Kalungu had negative GCA effects on the protein content (-1.78**), seed coat thickness (-0.015***) and number of days to 50% flowering (-0.015**) while the effects were positive for the mean development time (0.67*).

Rab 608 had significant positive GCA effects on the protein content (0.015**), seed coat thickness (0.015***) and the seed weight (1.78***). Rab 608 x Lukupa had a negative GCA effect on the seed weight (1.78***).

Carioca 38 and Kalungu showed negative GCA effects for the susceptibility index (1.17, -0.15 respectively) while Lukupa had positive effects (0.15). The progeny showed Carioca 38 x Kalungu with a positive SCA value (0.048) whereas Carioca 38 x Lukupa showed a negative SCA value (-0.67).

Rab 608 showed positive GCA effects with value of 1.17 for SI while the progeny showed Rab 608 x Kalungu and Rab 608 x Lukupa both had negative SCA values of -0.67 each.

4.6 Estimation of genetic parameters

The variance components due to males and females were estimated as shown in Table 11. The additive effects were highly significant compared to the non-additive effects as presented in Table 12.

The additive variance for susceptibility index was higher (7.95) than the non-additive variance (6.87). Additive variance effects for number of eggs laid, and number of insects emerged and mean development time were higher (2.21, 3.68, 8.42) than the non-additive gene effects (-0.117, 1.71 and 0.96 respectively).

The % adult emergence was significantly higher (7952) for the additive gene effects than the non-additive effects (1801). The additive genetic variance was also high in significance for the protein content, seed coat thickness, seed weight and the days to 50 % flowering as compared to the non- additive gene effects. The seed coat thickness showed 0.016 additive genetic variance while the non- additive genetic variance was 0.003 thus significantly lower than the additive variance.

The seed weight showed significantly higher additive genetic variance compared to the non- additive variance with values 0.49 and 0.057 respectively.

The formula used for narrow sense heritability (h^2ns) as shown below (Makumbi, 2013);

$$h^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}^2 + \hat{\sigma}_A^2 + \hat{\sigma}_D^2}$$

The narrow sense heritability was high in the F_2 population all corresponding to the additive genetic action derived. The heritability for susceptibility index was 0.53, number of eggs laid was 0.67, number of insects emerged was 1.00, mean development time 0.89, protein content 0.93, seed coat thickness 0.82 and 0.9 for both the seed weight and days to 50 % flowering.

Table 10: Estimates of GCA and SCA effects on the parameters measured

GCA	SI	EL	IE	%AE	MDT	P	SCT	SW	FL
Carioca 38	-1.167*	-0.309	-0.584**	-19.65*	-0.812*	0.0272**	0.0272***	-6.08***	-0.75
Rab608	1.167*	0.309	0.584**	19.65*	0.812*	-0.0272**	-0.0272***	6.08***	0.75
Kalungu	-0.147	-0.217	-0.074	1.6	-0.062	-0.0272**	-0.0272***	-2.625***	1.5*
Lukupa	0.147	0.217	0.074	-1.6	0.062	0.0272	0.0272	2.625***	-1.5*
SCA									
Carioca 38xKalungu	0.048	0.3335*	11.75	-0.0625	0.667*	-1.78***	-0.015***	-0.5	-0.015**
Rab 608xKalungu	-0.667*	-0.048	-0.333*	-11.75	0.262	0.015**	0.015***	1.78***	0.5
Carioca 38xLukupa	-0.667*	-0.048	-0.333*	-11.75	-0.262	-0.015**	-0.015***	1.78***	0.5
Rab 608xLukupa	0.667	0.048	0.333*	11.75	-0.262	-0.015	-0.015***	-1.78***	-0.5
SE	0.345	0.138	0.086	7.095	0.262	$1.94x10^{-3}$	0.001	0.015	0.433
SE	0.244	0.196	0.122	10.03	0.185	0.00136	0.0013	0.011	0.612

Key: SI: Susceptibility index, EL: Number of eggs laid, IE: Number of insects emerging, %AE: percentage adult emergence, MDT: mean development time, P: Protein content, Seed coat thickness SW: 100 Seed weight, FL: days to 50 % Flowering, ***: Significant at P≤ 0.001, **: significant at P≤0.01,* :significant at P≤0.05, SE: Standard error, GCA: general combining ability, SCA: specific combining ability.

Table 11: Estimation of variance components

Estimation of variance components	SI	EL	IE	%AE	MDT	P	SCT	SW	FL
σ^2GCA_m	7.357**	0.745	2.684***	1988*	4.73	3.5476	0.004***	270.35***	2.5
σ^2GCA_f	-3.378	0.360	-0.844	1988	-0.52	1.441***	0.004***	$-2.53x10^{1}$***	$1.60x10^{1}$
σ^2SCA	1.718*	-0.029	0.429	450.25	0.241	0.170	0.001***	12.674***	0.625
σ^2_E	0.119	0.077	0.029	201.4	0.069	0.003	$3.75x10^{-6}$	$2.50x10^{-4}$	$7.50x10^{-1}$
Bakers ratio	0.698	1.000	0.810	0.898	0.945	0.967	0.902	0.951	0.967

Key: SI: Susceptibility index, Number of eggs laid, IE: Number of insects emerging, %AE: percentage adult emergence, MDT: mean development time, P: Protein content, Seed coat thickness SW: 100 Seed weight, FL: days to 50 % Flowering, ***: Significant at P≤ 0.001, **: significant at P≤0.01, *P≤0.05, GCA: general combining ability, SCA: specific combining ability, m: male, f: female, r: replication.

Table 12: Estimation of the additive and non-additive variance components

Variance	SI	EL	IE	%AE	MDT	P	SCT	SW	FL
σ^2A_m	29.429	2.983	10.736	7952	18.92	14.190	0.016	1081.4	10
σ^2A_f	-13.515	1.441	-3.379	7952	-2.08	5.766	0.016	-101.376	64
σ^2D	6.875	-0.117	1.718	1801	0.965	0.682	0.003	50.7	2.5
σ^2A	7.957	2.212	3.679	7952	8.42	9.978	0.016	490.011	37
h^2ns	0.532	1.00	0.678	0.798	0.890	0.935	0.821	0.906	0.919
Bakers ratio		0.698	1.000	0.810	0.898	0.945	0.966	0.902	0.950

Key: SI: Susceptibility index, EL: Number of eggs laid, IE: Number of insects emerging, %AE: percentage adult emergence, MDT: mean development time, P: Protein content, SCT: Seed coat thickness, SW: 100 Seed weight, FL: days to 50 % Flowering,***: Significant at P≤ 0.001, **: significant at P≤0.01, *P≤0.05, GCA: general combining ability, SCA: specific combining ability: h^2ns: narrow sense heritability

4.7 Chemical Analysis

Thin Layer Chromatography (TLC) plates in Hexane only did not show any elution of compounds (Figure 3). All compounds remained on the base line. In mixtures of Hexane, Ethyl acetate and Methanol, compounds separated (Figure 4). However the good separations were observed in hexane/ethyl acetate mixtures 3:1and 19:1 (Figure 5 and 6).

In Hexane/Ethyl acetate 19:1, besides base material, three compounds were observed with retardation factor (R_f) values in the ranges 0.08 to 0.14, 0.28 to 0.52 and 0.75 to 0.83. The compounds with the R_f values ranging from 0.28 to 0.52 were more intense indicating that this was the major component. In a 3:1 Hexane/Ethyl acetate there were three compounds ranging from 0.472, 0.667 to 0.722 and 0.861 (Table 13 and 14).

Table 13 shows the R_f values of samples in two solvent systems used. In the 19:1 hexane/ ethyl acetate solvent system there were no differences in the R_f values for all the genotypes. Thus in a more polar solvent system the 3:1 Hexane/ Ethyl acetate mixture showed two different compounds with R_f values 0.472 for both sample 2 (Carioca 38 x Lukupa) and 7(Carioca 38 x Lyambai). These two compounds were not appearing in all other genotypes including the parental genotypes (Carioca 38, Lukupa and Lyambai).

Table 13: R_f values of bean samples on thin layer chromatography using two solvent systems with hexane and ethyl acetate.

Sample no.	Samples	Mixtures of hexane and ethyl acetate 19/1			3/1		
4	Carioca38	0.111	0.361	0.778	nil	0.694	0.861
2	Carioca38xLukupa	0.111	0.417	0.778	0.472	0.694	0.861
6	Car 38xKalungu	0.111	0.472	0.778	nil	0.694	0.861
7	Carioca38xLyambai	0.111	0.361	0.722	0.472	0.694	nil
10	Lukupa	0.083	0.361	0.778	nil	0.694	nil
1	Kalungu	0.139	0.556	0.806	nil	0.694	nil
3	Rab608	0.111	0.389	0.778	nil	0.667	nil
5	Rab608xLukupa(R)	0.083	0.361	nil	nil	0.694	nil
13	Rab608xlukupa(B)	0.139	0.472	0.806	nil	0.694	nil
12	Rab608xKalungu	0.111	0.444	0.806	nil	0.722	nil
8	Rab608xKabulangeti(R)	0.111	0.278	0.75	nil	0.722	nil
11	Kabulangeti	0.139	0.5	0.778	nil	0.686	nil
14	Lyambai	0.139	0.528	0.833	nil	0.686	nil
9	Lusaka	0.139	0.361	0.75	nil	0.686	nil
15	Chambeshi	0.139	0.528	0.833	nil	0.686	nil

Table 14: R_f values showing the parental lines and the various crosses

Sample no.	Sample	Solvent system for TLC Hexane/ethyl acetate mixtures					
		19:1 (R_f values)			3:1(R_f values)		
4	Carioca 38	0.11	0.36	0.78	nil	0.69	0.86
10	Lukupa	0.08	0.36	0.78	nil	0.69	nil
2	Carioca38xLukupa	0.11	0.42	0.78	0.47	0.69	0.86
13	Rab608xLukupa	0.08	0.36	0.81	nil	0.69	nil
3	Rab 608	0.11	0.39	0.78	nil	0.69	nil
4	Carioca38	0.11	0.36	0.78	nil	0.69	0.86
1	Kalungu	0.08	0.36	0.78	nil	0.69	nil
6	Carioca38xKalungu	0.11	0.47	0.78	nil	0.69	0.86
12	Rab 608xKalungu	0.11	0.44	0.81	nil	0.72	nil
3	Rab 608	0.11	0.39	0.78	nil	0.67	nil

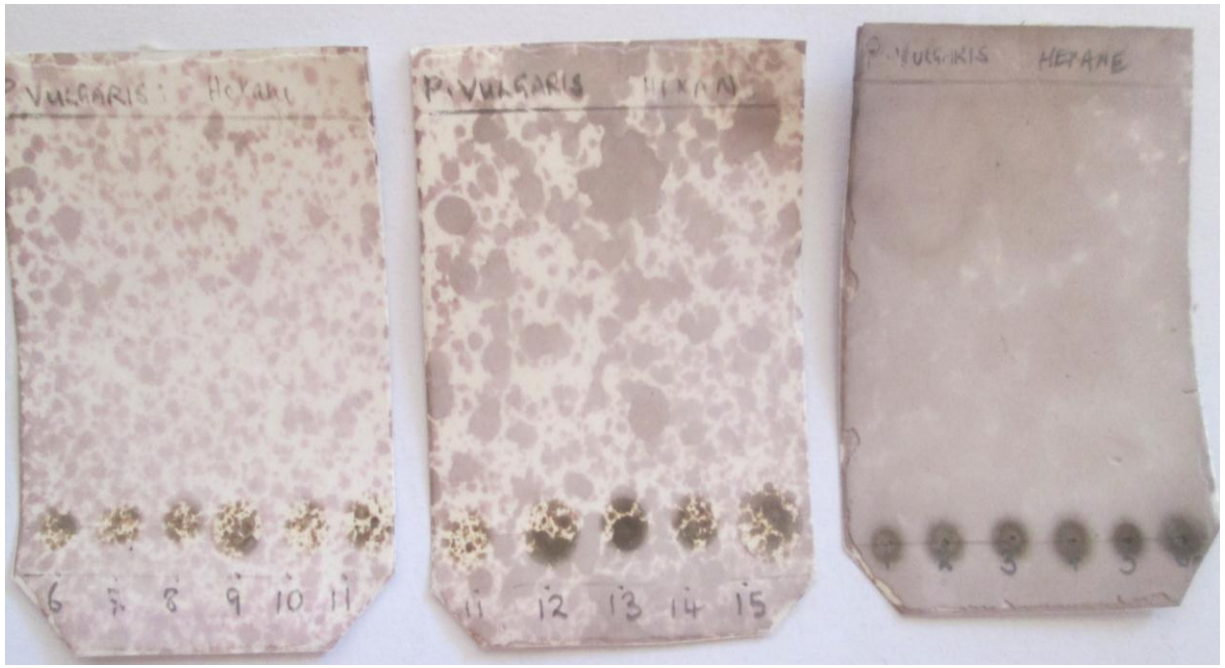


Figure 3: TLC analysis with Hexane only

Key**:** 1: Kalungu, 2: Carioca38xLukupa, 3: Rab608, 4: Carioca38, 5: Rab608xLukupa, 6: Carioca38xKalungu, 7: Carioca38xLyambai, 8: Rab608xKabulangeti, 9: Lusaka, 10: Lukupa, 11: Kabulangeti, 12: Rab608xKalungu, 13: Rab608x Lukupa, 14: Lyambai, 15: Chambeshi

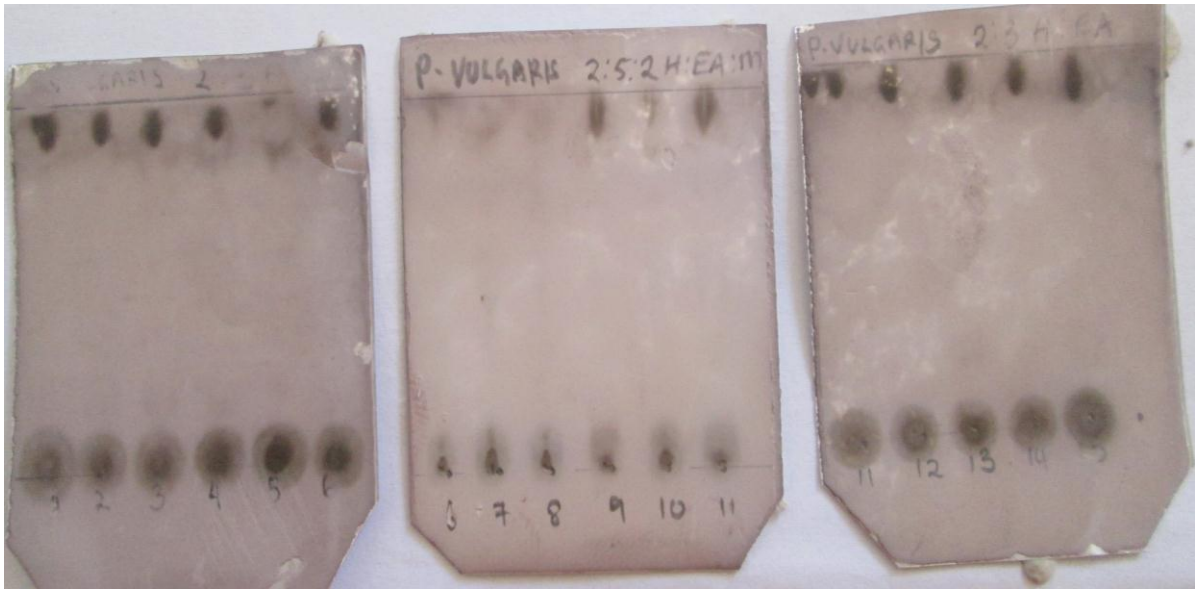


Figure 4: TLC analysis in a 2: 5: 2 Hexane/ Ethyl acetate/Methanol

Key: 1: Kalungu, 2: Carioca38xLukupa, 3: Rab608, 4: Carioca38, 5: Rab608xLukupa, 6: Carioca38xKalungu, 7: Carioca38xLyambai, 8: Rab608xKabulangeti, 9: Lusaka, 10: Lukupa, 11: Kabulangeti, 12: Rab608xKalungu, 13: Rab608x Lukupa, 14: Lyambai, 15: Chambeshi.

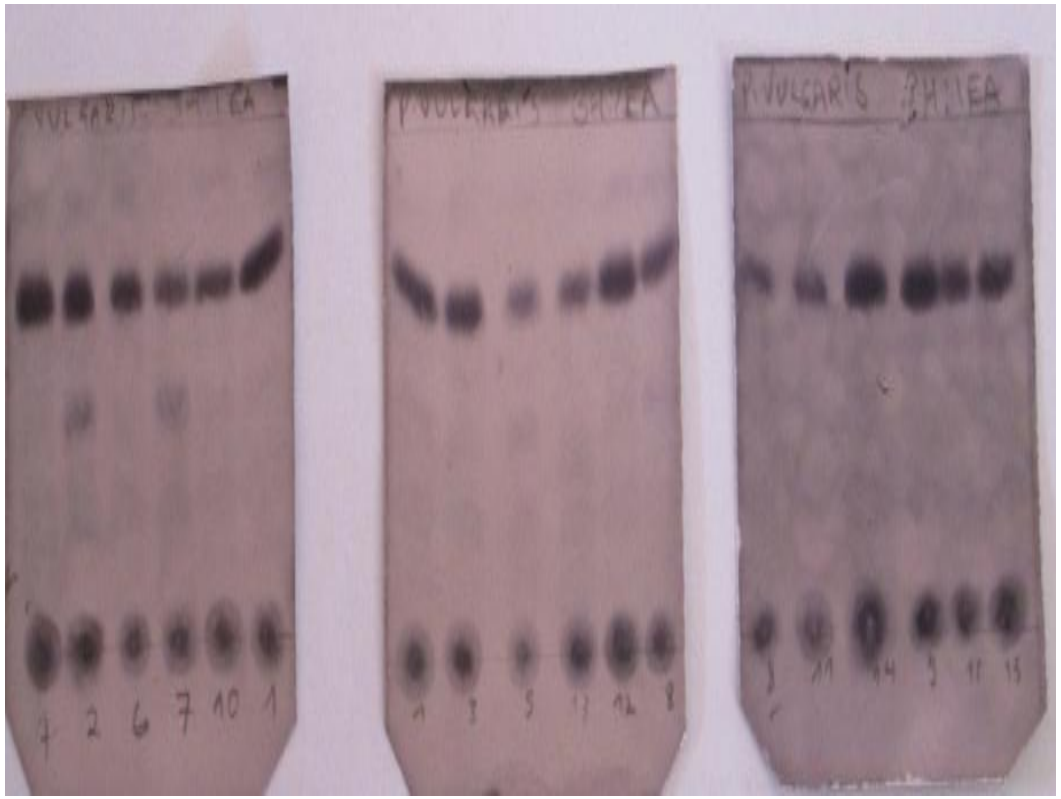
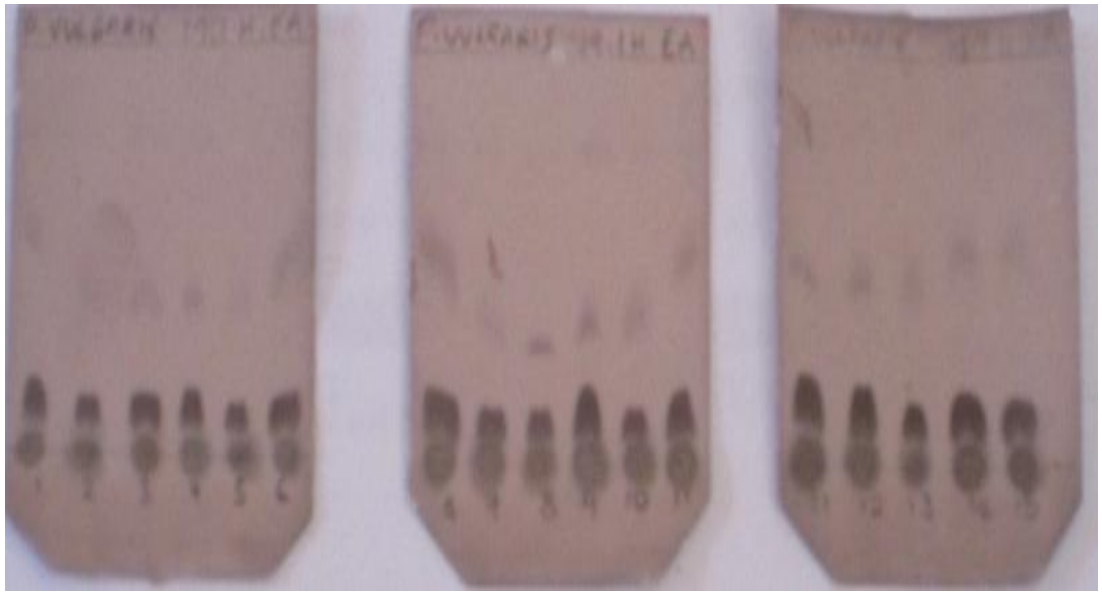


Figure 5: TLC analysis in a 3:1 Hexane/Ethyl acetate

Key: 1: Kalungu, 2: Carioca38xLukupa, 3: Rab608, 4: Carioca38, 5: Rab608xLukupa, 6: Carioca38xKalungu, 7: Cario ca38xLyambai, 8: Rab608xKabulangeti, 9: Lusaka, 10: Lukupa, 11: Kabulangeti, 12: Rab608xKalungu, 13: Rab608x Lukupa, 14: Lyambai, 15: Chambeshi.

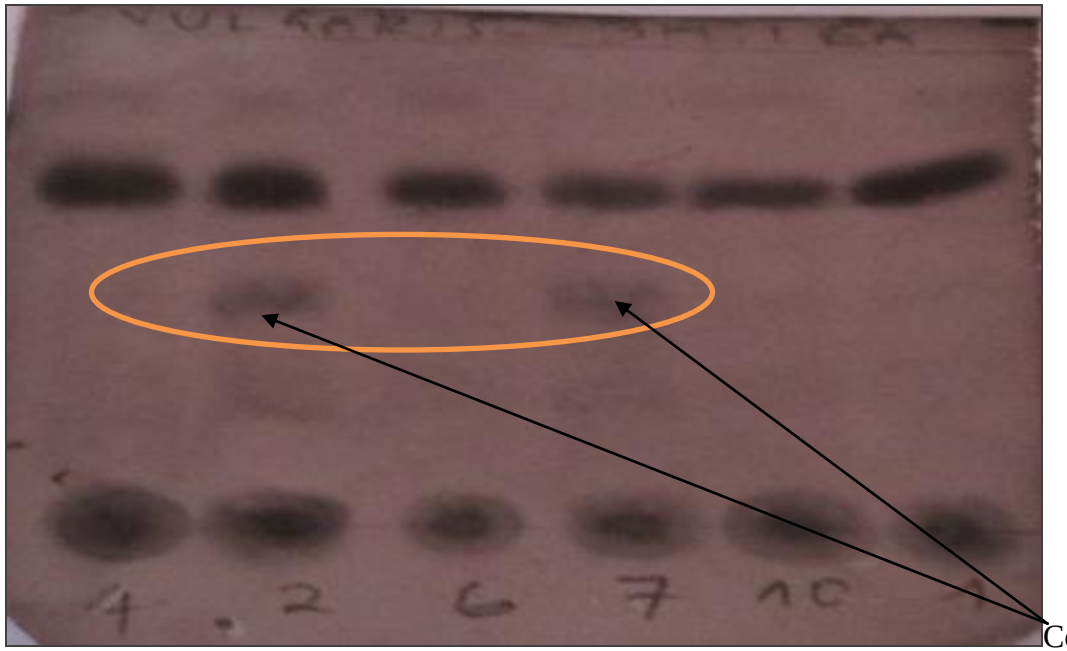


1 2 3 4 5 6 6 7 8 9 10 11 11 12 13 14 15

Figure 6: TLC analysis in 19:1 Hexane/ Ethyl actetate

Key: 1: Kalungu, 2: Carioca38xLukupa, 3: Rab608, 4: Carioca38, 5: Rab608xLukupa, 6: Carioca38xKalungu, 7: Carica38xLyambai, 8: Rab608xKabulangeti, 9: Lusaka, 10: Lukupa, 11: Kabulangeti, 12: Rab608xKalungu, 13: Rab608x Lukupa, 14: Lyambai, 15: Chambeshi.

The TLC in a 3:1 Hexane/ Ethyl acetate solvent showed differences in Carioca 38 x Lukupa and Carioca 38 x Lyambai which are crosses of Carioca with Lukupa and Lyambai respectively. They both show a distinct compound which is significantly different from the parents and other progeny (Figure 7).



Compounds observed different from other samples

4 2 6 7 10 1

Figure 7: TLC analysis in 3:1 Hexane/ Ethyl acetate

Key: 4: Carioca 38, 2: Carioca 38xLukupa, 6: Carioca38xKalungu, 7: Carioca38xLyambai, 10: Lukupa, 1: Kalungu

CHAPTER FIVE

5.0 DISCUSSION

To encourage bean breeding, production and storage, this study endeavored to evaluate selected bean (*P. vulgaris*) genotypes for bruchid (*C. maculatus*) resistance. The objectives of the study therefore, were to evaluate the common bean for resistance to bruchids and determine the gene action controlling bruchid resistance in common bean. In this present study, substantial variation was observed among the common beans genotypes for susceptibility index, protein content, seed coat thickness, seed weight and days to 50% flowering (Tables 5, 6). The SI was used as the measure for beetle damage resistance (Dobie, 1974, Kusolwa, 2007, Kananji, 2007).

The results showed genotypic variations among all the varieties for susceptibility index and other morphological components. Carioca 38 and Rab 608 showed the lowest values of 0.0, and 1.1 respectively (Table 7). This meant for Carioca 38, no adult bruchids emerged even though it recorded a number of eggs laid (4.0) while for Rab 608 it had a lower number of adult insects emerging compared to the number of eggs laid giving a low percentage of adult emergence (20%). Therefore Carioca 38 and Rab 608 were classified as resistant. Among the crosses, Carioca 38 x Kalungu and Carioca 38 x Lukupa were also classified as resistant with values 2.45 and 1.41 respectively while Rab 608 x Kalungu and Rab 608 x Lukupa were moderately resistant with SI values of 4.45 and 5.08 values respectively. Although Lukupa had a relatively higher SI (7.85) than Kalungu (6.9), they were both classified as moderately susceptible.

These results therefore show that eggs are laid on the non-preferred host as a mechanism of survival. The number of eggs laid show a trend of host preference by the bruchids as is shown in the mean performance of the values ranging from 4.0 for resistant Carioca 38 to 13.0 for Lukupa which is moderately susceptible. The mode of resistance between these two parents appears to be different in that one simply inhibits the laying of eggs while the other does not. The one that has more eggs laid equally affects the number of adults emerging resulting in low numbers similar to the one that inhibited laying of eggs.The host preference could be enhanced by the seed coat colour and seed size. These two parents are of different seed coat colors with Carioca being white and smaller in size and Rab 608 being maroon and relatively larger in size. A similar situation was observed for seed coat colour between parents that were susceptible, Kalungu being light colored and Lukupa being dark colored. More eggs were laid on the dark colored one than the light colored one, Lukupa and Kalungu, respectively.

This agrees with what Porca *et al.* (2003) reported that red-seeded bean cultivars were more susceptible to *A. obtectus* than white seeded bean cultivars. Ofuya and Credland (1996) also stated that the seed colours are important for bruchid host selection. Kananji, (2007) however found that though there was a wide range of seed colours in the bean genotypes studied, seed colour was not directly linked to the observed differences in the resistance.

The small seed size however could suggest that it acts as a barrier since mortality, size and fecundity of bruchid progeny are strongly affected by overcrowding within seeds (Schoonhoven *et al*., 1983; Cipollini and Stiles, 1990). It was reported by researchers at CIAT (1985) that most of the developed lines were small-seeded and bruchids showed

preference for the small seeded unlike the large seeds. Misangu (1997) screened and identified some potential lines with resistance to *Z.subfasciatus,* and found the bruchids exhibited a marked preference for large-seeded bean lines.

The dark colored seed coat tended to be thicker than the lighter ones in the current study. The resistant genotypes tended to have a thicker seed coat as compared to the susceptible genotypes as is observed from the results where resistant Carioca 38, Rab 608 and Carioca 38 x Lukupa had thickness of 0.54 mm, 0.57 mm and 0.58 mm respectively while susceptible Lukupa and Kalungu had 0.40 mm and 0.44 mm respectively (Table 6). Therefore the seed coat thickness may inhibit the penetration of newly hatched larva into the seed to allow bruchid development.

The protein content may have an indirect effect on the seed coat thickness and hence the SI as the protein content was significantly negatively correlated to the seed coat thickness ($r = -0.9113^{***}$) and seed coat thickness was found to be significantly influencing the number of insects emerging. It was observed that the genotypes with a high protein content had a thinner seed coat. Carioca 38, with protein content of 16. 41%, had thinner seed coat than Rab 608 which had a protein content of 14.9% and consequently showed some adults emerging unlike Carioca 38. This result suggests that the protein content in the seed coat could indirectly be involved in bruchid resistance resulting in the reduction of the SI. A thin seed coat also entails more adult bruchids will emerge and therefore making the genotypes with this trait more susceptible than those that are not.

Similarly evidence of the seed coat as a possible physical or chemical deterent to seed attack as a mechanism of resistance against C. *maculatus* has been demonstrated (Kemal and Smith, 2001). Silva *et al*. (2004) also concluded that a thick seed coat of *P.vulgaris*

genotypes studied and protein in the seed coat were detrimental to *C. maculatus*. Seed coat thickness was found as one most highly correlated with pod resistance by Kitch *et al*. (2011). These researchers concluded that seed coat thickness was positively influencing bruchid resistance. Luthi *et al*. (2013) found that for *A. obtectus* and *C. chinensis*, adult bruchids were failing to emerge from the seed after successfully completing their development. In contrast, *C. maculatus* larvae frequently failed to perforate the seed coat. In the case of the chickpea seeds, the within seed development (WSD) of the emerging beetles was positively correlated with seed coat thickness and the overall resistance r = 0.795***. Findings of Kashiwaba *et al*. (2003) also indicated that the seed may contain toxins or other substances such as fatty acids or proteins in the cotyledons or its enveloping seed coat that inhibit the larval development. The expression of a *C. maculatus*-detrimental protein in the testa of non-host seeds suggests that the protein may have played a significant role in the evolutionary adaptation of bruchids to legume seeds (Macedo *et al*., 1993). Researchers have reported highly significant and negative correlations between protein content and Dobie index of susceptibility parameters. Maize genotypes were not significantly different (P>0.05) for protein content. However, when the top 5 and least 5 genotypes in protein content in each group (hybrids and OPVs) were considered, it was found that resistant genotypes had a tendency of containing higher levels of protein than susceptible ones i.e. hybrid 19 had protein content of 11.2% and had low SI $\leq$ 4.0 (Siwale *et al*., 2009; Classen *et al*., 1990).

Other findings reported by Beneke (2010) and Kananji (2007) on *Z. subfasciatus* and *A. obtectus* bruchid species showed that seed coat thickness had no effect on bruchid emergence. Similarly Eddie and Amatobi (2003) reported that the seed coat thickness did not

affect resistance to *C. maculatus* in cowpea varieties tested. Cowpea seed with intact seed coats were preffered to decorticated seeds for oviposition of *C. maculatus* and they concluded that seed coat may not be a useful aspect.

The low SI values in the resistant genotypes observed in this study may be linked to chemical constituents in the beans or seed coat that inhibit development. The R_f values of the bean genotypes compared well with literature values (0.10-0.75) reported by in 4/1 cyclohexane and ether, a solvent mixture with similar polarity and were found to be methyl ester mixtures (Molla *et al*., 2007). The values of 0.14-0.47 compared fairly closely to those of methyl myristate and methyl oleate which were esters of fatty acids which were exhibited in crosses of Carioca 38 with Lukupa and Lyambai (Table 13).

Carioca 38 x Lukupa showed significant differences with the parental genotypes and expressed significant amounts of methyl esters of fatty acids. These esters of fatty acids may show an increase in the fatty acid content from the parental genotypes to the progeny. Several classes of methyl esters of fatty acids which are secondary compounds of fatty acids have shown to give resistance to certain pests such as aphids (Schultz *et al*., 1996). The influence of bean seed surface lipids on infestation of seeds by *A. obtectus* was investigated and indicated that bean seed surface lipids are involved in all infestation stages and fatty acids and monoacylglycerols were found to deter bean weevil infestation (Nietupski, 2005). This could suggest the presence of genes in Carioca 38 that trigger biosynthesis of chemical compounds in the progeny that confers resistance.

5.1 Gene action and genetic parameters

In this study general combing ability (GCA) effects were significant for Carioca 38 and negative as regards the insects emerged ($P \leq 0.05$), % adult emergence ($P \leq 0.05$), mean development time ($P \leq 0.05$), susceptibility index and the seed weight ($P \leq 0.05$). Therefore a negative GCA value indicated that the corresponding parent made a positive contribution to resistance (i.e. reduced the number of bruchid emergence). Parents showing high GCA effects (negative values) would directly be useful in a breeding programme to improve bruchid resistance in commercial varieties that are high yielding but lack resistance.

The GCA effects for percent adult emergence were significant for Carioca 38 and Rab 608 with values of -19.65 and 19.65 respectively. The GCA effects for Carioca 38 were negative (lowest GCA effects) showing that as the percent adult emergence reduces it lowers the susceptibility of the bean genotypes and as such could make Carioca 38 useful in the breeding of genotypes to improve bruchid resistance in existing cultivars and commercial varieties that may be high yielding but lack resistance to bruchids. Rab 608 showed positive GCA effects meaning it increase the level of susceptibility in the progeny (i. e. increased the number of insects that emerged).

The general combining ability (GCA) effects accounted for the largest portion of the total variation for resistance, suggesting the predominance of the additive gene action. The additive genetic variance was significantly ($P \leq 0.05$) higher than the non-additive variance for susceptibility index (7.95, 6.87), number of insects emerged (3.69, 1.71) respectively and the % adult emergence (7952, 1802) respectively. Baker (1978), first suggested that the progeny performances could be predicted using the ratio of combining ability

variance components $\{(\sigma^2 gca_{m+}\sigma^2 gca_f /(\sigma^2 gca_m+\sigma^2 gca_f+\sigma^2 sca_{f*m})\}$. The closer the ratio is to unity, the greater the predictability based on GCA alone. In the current study, the ratios ranged from 0.69 for the susceptibility index to 1.00 for the number of eggs laid (Table 11). The number of insects laid, % adult emergence and mean development time had ratios of 0.81, 0.89 and 0.94 respectively. The protein content, seed coat thickness, seed weight and days to 50% flowering all had ratios of ranging from 0.90 to 0.97.

Consequently this suggested that the SI which in this case determines the resistance is highly heritable and can be easily transferred between genotypes and is fixable.

Kang *et al*. (1995) found that additive gene effects were more important than non-additive gene effects in conferring bruchid resistance. In another study to investigate inheritance of resistance to oviposition by maize weevil, Tipping *et al*. (1989) reported that additive gene action was important. Derera *et al*. (2001a, b) investigated gene action for weevil resistance in both free-choice and no-choice tests and found significant additive, non-additive and maternal effects.

The findings of Kananji (2007) showed the non-additive gene action as significant for bruchid resistance in the bean varieties that were studied.

The high heritability in the narrow sense for SI (53%) suggests that the genes of the traits of interest were governed by one or few genes and can be fixed in self-pollinating crops such as beans (Singh, 2009). The genetic components of variations were high compared to the environmental variances for all the traits observed because this was a laboratory experiment performed under controlled conditions and thus the error terms are reduced (Singh and Chaudhary, 2004).

The specific combining effects where significant ($P \leq 0.05$) for the crosses of Carioca 38 especially Carioca 38 x Lukupa with high negative values (-0.67, -0.33) for the SI and the number of insects emerged respectively and these genotypes would be considered as important for use in breeding for resistance. The results for susceptibility index parameters suggest that resistance of these progenies was higher or lower than the expected of their respective parental genotypes and indicates that there could be an increase in the resistance from low resistance genotypes. Significant SCA (Table 10) variations indicates that certain crosses had a higher or lower levels of resistance than expected on the basis of the GCA components of the parents involved according to Gardner and Eberhart, (1966) and Baker, (1978) and significant SCA variations have been reported for resistance to storage insects in maize (Dhliwayo *et al*., 2005). These significant positive SCA variances suggest that the resistance of the progenies was lower than the average resistance of their respective parents implying that resistant genotypes (Carioca 38 x Lukupa) could be produced even from susceptible parents. Significant positive and negative SCA effects were observed in crosses made by Kananji (2007) and the results also suggested that resistance of these progenies was higher or lower than would be expected from the average resistance of their respective parents.

As has been observed the crosses of Carioca 38 and Rab 608 showed differences from their parental genotypes which could indicate transgressive segregation (Kananji, 2007). There are many mechanisms that could be responsible for transgressive segregation in hybrids such as: an elevated mutation rate, reduced developmental stability, epistatic effects between alleles, over dominance caused by heterozygosity at specific loci or chromosome number variation (Xu *et al*., 1998). Rick and Smith (1953) proposed three po-

tential explanations for the occurrence of interspecific transgression including de novo mutation induced by hybridity, complementary action of genes from the two parental species and unmasking of recessive genes normally held heterozygous. Studies of hybrid populations have reported the presence of traits or phenotypes that are extreme, relative to either of the parental lines (Rieseberg *et al*., 1999). The generation of these extreme phenotypes in hybrids (i.e. phenotypes that exceed those of either parental line) is referred to as transgressive segregation (Grant, 1975; De Vicente and Tanksley, 1993). A recent review of phenotypic variation in hybrids indicates that transgressive segregation occurs frequently in segregating plants (Rieseberg *et al*., 1999).

However, genetic studies indicate that transgressive segregation mostly results from the appearance, in individual genotypes, of combinations of alleles from both parents that have effects in the same direction: complementary gene action (De Vicente and Tanksley, 1993; Rieseberg *et al*., 1999).

CHAPTER SIX

6.0 CONCLUSIONS

The study was set out to evaluate the bruchid resistance in common bean and the gene action of the trait that confers resistance. In this study it showed that seed color and seed size were found to have relative effect on host preference selection in bruchid (*C. maculatus)* resistance in common beans.

Carioca 38 and Rab 608 were identified as resistant and could act as good parents in the development of bruchid resistant bean varieties, however, their mode of resistance was different. The mode of resistance for Carioca 38 was observed to be related to the ability to restrict number of eggs laid, which was in turn related to seed coat thickness, presence of methyl esters of fatty acids, seed coat color. Carioca 38 exhibited multi resistance factors including the seed size, seed coat thickness, seed color and lipid content.

Seed coat color and seed size had notable influence on host preference selection by bruchids, while seed coat thickness also played a role in bruchid adult emergence in the resistant genotypes, therefore, contributed to bruchid resistance in common bean.

The gene action conditioning number of eggs laid, number of insects emerging, seed coat thickness and protein content was additive gene action. The heritability of adult emergence (trait showed the most effect on the SI) was 79% thus this trait could be easily be improved through conventional breeding thereby contribute to bruchid resistance.

It can be concluded that given the adequate genotypic variation among common bean genotypes on their resistance to bruchids (*C. maculatus*), resistant genotypes can be de-

veloped via conventional breeding methods targeting low number of eggs laid, low number of insects emerged, high protein content as well as the reduced seed coat. Such varieties could contribute to increased time of storage of common beans and reduce losses experienced in production and storage if adopted by more farmers one as it is governed by several factors.

6.1 Future Research areas

1. This was a one season and location study and it is recommended that the study be repeated in more locations and seasons and increase the number of crosses to be evaluated. It is also recommended that such further studies include more physiological traits and yield components to validate their potential as materials in bean breeding.

2. Future research should be able to find out the different protein and tannin profiles in genotypes such as Carioca 38 but also which protein and/or tannin is predominant in the seed coat and cotyledons and if the bruchids would show a certain affinity for a particular type of protein.

3. Further investigation should identify the gene that triggers the biosynthesis of the chemical formation observed in Carioca 38 x Lukupa.

4. To study the effect of the seed coat texture and the possible chemical constituents in the seed coat which may confer resistance.

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APPENDICES

Appendix I: Brief description of genotypes used

Number	Sample	Description
4	Car 38	39 days to 50% flowering, indeterminate semi climber
2	Car 38 * Lukupa	30 days to flowering, Indeterminate bush type
6	Car 38* Kalungu	31 days to 50% flowering, Indeterminate bush type
7	Car 38 * Lyambai	29 days to 50% flowering, Indeterminate semi climber
10	Lukupa	30 days to flowering, indeterminate bush type
1	Kalungu	29 days to flowering, indeterminate bush type
3	Rab 608	40 days to flowering, Determinate
5	Rab 608 * Lukupa	30 days to 50% flowering, Indeterminate bush
12	Rab 608* Kalungu	33 days to 50% flowering, bush
8	Rab 608* Kabulan-geti	35 days to 50% flowering, indeterminate
11	Kabulangeti	30 days to flowering, Indeterminate semi climber
14	Lyambai	34 days to flowering, determinate bush type
9	Lusaka	30 days to flowering, indeterminate
15	Chambeshi	30 days to flowering, Determinate bush type

Appendix II: ANOVA for Susceptibility Index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
male	1	10.9142	10.9142	91.45	<.001
female	1	0.178	0.178	1.49	0.289
Male x female	1	3.5568	3.5568	29.8	0.005
Residual	4	0.4774	0.1193		
Total	7	15.1263			

Appendix III: ANOVA for Number of Eggs Laid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
male	1	0.76416	0.76416	9.91	0.035
female	1	0.3787	0.3787	4.91	0.091
Male x female	1	0.01836	0.01836	0.24	0.651
Residual	4	0.3085	0.07712		
Total	7	1.46972			

Appendix IV: ANOVA for Number of Insects Emerged

Source of variation	d.f.	m.s.	v.r.	F pr.
male	1	2.72855	91.45	<.001
female	1	0.04449	1.49	0.289
Male x female	1	0.8892	29.8	0.005
Residual	4	0.02984		
Total	7			

Appendix V: ANOVA for % Adult emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
male	1	3089.9	3089.9	15.34	0.017
female	1	20.4	20.4	0.1	0.766
Male x female	1	1101.9	1101.9	5.47	0.079
Residual	4	805.7	201.4		
Total	7	5017.9			

Appendix VI: ANOVA for Mean Development Time

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
male	1	5.28125	5.28125	76.82	<.001
female	1	0.03125	0.03125	0.45	0.537
Male x female	1	0.55125	0.55125	8.02	0.047
Residual	4	0.275	0.06875		
Total	7	6.13875			

Appendix VII: ANOVA for Protein Content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
male	1	3.89205	3.89205	1112.01	<.001
female	1	1.78605	1.78605	510.3	<.001
Male x female	1	0.34445	0.34445	98.41	<.001
Residual	4	0.014	0.0035		
Total	7	6.03655			

Appendix VIII: ANOVA for Seed Coat Thickness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
male	1	5.94E-03	5.94E-03	1584.13	<.001
female	1	5.94E-03	5.94E-03	1584.13	<.001
Male x female	1	1.80E-03	1.80E-03	480	<.001
Residual	4	1.50E-05	3.75E-06		
Total	7	1.37E-02			

Appendix IX: ANOVA for Seed Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
male	1	2.96E+02	2.96E+02	1.18E+06	<.001
female	1	5.51E+01	5.51E+01	2.21E+05	<.001
Male x female	1	2.54E+01	2.54E+01	1.01E+05	<.001
Residual	4	1.00E-03	2.50E-04		
Total	7	3.76E+02			