BEAN BRUCHID RESISTANCE AND GENETIC DIVERSITY OF BRUCHID ECOTYPES FROM BEAN GROWING REGIONS OF TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

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EXTENDED ABSTRACT

Bean bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus* are the serious pests which cause serious damage to the stored beans. Damage is directly related to the time of bean storage, the longer storage time the greater the damage. Host plant resistance is a profitable and a safe alternative to control bruchids in common bean and is associated with biochemical, morphological, and molecular traits. These traits affect insect growth and development and in that way, reduce the losses by the pests. The aim of first objective was to evaluate the damage level of bruchids in resistant (AO-1012-29-3-3A) and susceptible (Njano gololi) bean genotypes associated with biotype variation. Bean bruchids were collected from different bean growing regions Songwe (Vwawa), Kilimanjaro (Mungushi), Karatu (Rhotia), Morogoro (SUA) and Arusha (Kimyaki) in Tanzania, then transferred to the laboratory for inoculation. . Experiment was conducted using a Completely Randomized Design (CRD) with a 2 x 5 factorial scheme, two bean cultivars (AO-1012-29-3-3A and Njano gololi), five bruchids ecotypes for each *A. obtectus* and *Z. subfasciatus* and 3 replications. The results of this study have reported that each bean bruchids from a specific region showed a significant difference at prob. = 0.001 on beans infestation. AO-1012-29-3-3A and Njano showed different results on the resistance to bean bruchids. It was observed that AO-1012-29-3-3A line was resistant to bruchids collected from many regions by experiencing less damage and Njano gololi was observed to have high population of emerged bean bruchids, high percentage weight loss, high Susceptibility index and severity hence susceptible. The purpose of second objective was to determine phenotypic characteristics of the emerged F_1 bruchid ecotypes resulted from crossing resistant (AO-1012-29-3-3A) and susceptible (Njano gololi) bean genotypes. To examine whether these traits are host induced or genetically determined. Resistant genotype was used to assess whether host-race morphological differences are

genetically determined or due to phenotypic plasticity. There was significant difference in size of F_1 bruchids emerging from resistant genotype (AO-1012-29-3-3A) and susceptible genotype (Njano gololi). The results showed that the morphological change of F_1 bruchids from AO-1012-29-3-3A was due to phenotypic plasticity since morphological changes occurred due to feeding on resistant genotypes (environment factor). The purpose of third objective was to identify the genetic diversity of bean bruchid weevils (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) in bean producing regions in Tanzania using molecular taxonomy (*12S rRNA* and *COI* marker). The results obtained did not show genetic diversity (100% identity) of *Acanthoscelides obtectus* present in Tanzania. For *Zabrotes subfasciatus* some variations was observed (80.2% identity). The genetic diversity was observed between *Acanthoscelides obtectus* and *Zabrotes subfasciatus* in which there was difference in some sequence alignment. Better knowledge of bruchids diversity present in Tanzania will help breeders and farmers to propose effective control methods with impact on environmental changes.

DECLARATION

I, EDINA MAYUNGA do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being currently submitted for a degree award in any other institution.

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DEDICATION

This dissertation is dedicated in memory of my late mother Magreth Boniphace, she left a void never to be filled in my life. It is also dedicated to my sister Adija Mayunga, a strong and gentlewoman who taught me to trust in God, believe in hard work and that so much could be done with little.

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LIST OF ABBREVIATIONS

^{0}C	Degree Centigrade
ANOVA	Analysis of Variance
Bp	Base pair
C.V	Coefficient of variation
CIAT	Centro International de Agricultura Tropical
COI	Cytochrome oxidase subunit I
Conc	Concentration
d.f	Degree of freedom
DAE	Days after emergence
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
G	Gram
MEGA7	Molecular Evolutionary Genetics Analysis
Ml	Microlitre
mm	Millimeter
Mn	Minutes
mtDNA	Mitochondria Deoxyribonucleic Acid
NCBI	National Center for Biotechnology Information
NNI	Nearest Neighbour Interchange
PCR	Polymerase Chain Reaction
rRNA	Ribosomal ribonucleic acid
s.d	Standard deviation

s.e	Standard error
s.s	Sum of square
Spp	Species
SSRs	Simple Sequence Reapeats
SUA	Sokoine University of agriculture
TBE	Tris/borate/EDTA (buffer)
U S A	United State of America
UV	Utraviolet
V	Voltage

CHAPTER ONE

1.0 INTRODUCTION

1.1 Importance of common beans

Common bean, *Phaseolus vulgaris* L. is most important grain legume that belongs to the family Fabaceae, subfamily Papilionideae, tribe Phaseoleae, sub tribe Phaseolinae in the section Phaseoli (Debouck, 1991). It is an important component of the crop production systems and a major source of dietary protein for the majority of the people who cannot afford expensive animal protein on a daily basis. Hence plays an important role in improving health of human by controlling human malnutrition (Chacon *et al*., 2005). In many developing countries including Tanzania, beans supply a cheap and high proportion of plant proteins and high minerals content especially Iron (Fe) and Zinc (Zn) for human deity (Singh, 1999; FAO, 2005). Beans have been found to be the second source of dietary protein and third important source of calories after maize and cassava (Hillocks *et al.,* 2006). Bean production also provides farm households with both a source of income and food for nutrition through sales and consumption of part of the produce. Due to its importance as a food crop, every region in Tanzania grows some amount of beans, but more beans are grown in high altitude regions with well-drained soils and high organic matter content (Misangu, 2002).

1.2 Bean production

Worldwide production of common beans is dominated by Brazil, Mexico and United States which produces 50% of the total world production followed by Africa which produce 25% of the total world production (Beebe *et al.*, 2013). Tanzania ranks 5th worldwide in bean production and is the leading producer of beans in Africa which is produced almost entirely under intercropped systems with maize and other crops

(FAOSTAT, 2014; Binagwa *et al.*, 2015). In 2014 Tanzania led with 1.14 million tons of production in East Africa, followed by Uganda 1.02 million tons, Kenya 846 000 tons, Rwanda, Burundi, Congo and then South Sudan (FAOSTAT, 2017). Smallholder farmers who operate 1 to 5 acres on the average produce over 70% of the national bean production in Tanzania for own consumption and for markets, about 40% of the harvests are marketed by households. The main bean production areas in Tanzania are in the northern regions; Arusha, Kilimanjaro and Manyara, great lakes/western; Kagera and Kigoma, the southern highlands; Mbeya, Iringa and Rukwa (Xavery *et al*., 2006; Katungi *et al*., 2010).

1.3 Constraints in beans production

Biotic and abiotic factors are major constraints that reduce beans yield during the growing seasonal. Abiotic factors include poor soil fertility especially phosphorus, post-harvest losses, flood, temperature and drought are the major factors for decline in bean production (Hillocks *et al*., 2006). It has been observed that moderate water deficit during bean production led to a reduction in plant biomass, lower seed number per pods, earlier maturation, lower seed yield and weight, and reduction in nitrogen fixation. Also, Bucheyeki and Mmbaga, (2013) reported that most of farmers especially in Tanzania use unimproved seed varieties which have low yield potential per unit area and reduced bean production.

Several biotic constraints cause yield losses to common bean cultivation. Diseases caused by fungi, virus, bacteria and nematodes are the biotic constraints for reduction in bean production. Examples of such diseases are common bacterial blight (CBB), angular leaf spot (ALS), bean common mosaic virus (BCMV), bean common mosaic necrotic virus (BCMNV), root rots and powdery mildew causes to a severe reduction in yield. It has

been reported by (Tryphone *et al.*, 2015) angular leaf spot causes a yield loss of up to 50-80%.

Apart from disease dry bean seeds are attacked by insect pests. Among the insect pests include bean bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) that contributes to post-harvest losses of beans during storage. Bean bruchids inflict their damage on stored bean produce mainly by direct feeding on the endosperm causing loss of weight quality and viability of bean seed (Reuben *et al*., 2006). The higher grain losses occur between harvesting and consumption period (Nchimbi-Msolla and Misangu, 2002). The average worldwide loss caused by weevil damage ranges from 7-40% of marketable beans. In Mexico, Central America and Panama stored bean damage by bruchids reached up to 35% (McGuire *et al.*, 1967). In eastern Africa, Karel and Autrique, (1989) reported an estimated grain loss between 30% and 73%. Burundi and Rwanda losses to bruchids are commonly about 30% (Nahmana *et al*., 1992). Weight loss up to 40% have been reported by (Kiula and Karel, 1985) in Tanzania. The degree of loss due to bruchid damage depends on the storage period, storage conditions, type of bean weevils, storage containers and bean varieties (Nchimbi-Msolla and Misangu, 2002). The longer the storage period the greater the loss and may reach up to 100% if left untreated.

Other important losses include nutrition loss which involve reduction in the food quality of the grain due to carbohydrate, protein and vitamin degradation (Singh *et al.*, 2011). In addition, bruchid damage also results in significant low market value of edible bean grain (Mshili *et al*., 2011). Farmers limit the time of storage by selling beans normally at low price within 2 to 3 months after harvest so as to avoid total grain losses (Giga *et al*., 1992). Another production strategy that farmers use is to not produce a large quantity during any one season for the fear of such post-harvest losses.

One possible approach to sustain these biotic constraints is the introduction of bruchid resistant cultivars, good storage facilities as well as effective, economical and environment friendly method of pest control that contributes to high productivity and encourage farmers to engage into intensive bean production (Miklas *et al*., 2006). Dry bean cultivars that delay or inhibit growth of the predominant bruchid species will contribute to a stable supply of dry beans in the region, particularly where only a single crop is produced per year. Bruchid resistant bean cultivars should also have a stabilizing influence on market prices during the dry season. But then again environmental condition has an influence on bruchids infestation. Damage caused by insect pest as a result of global climate change may lead to outbreaks in some areas and causing higher losses while in other area loss due to pest may drop (Appleby and Credland, 2004). Biotype's variation in bruchids has rendered some common bean lines susceptible that otherwise would have been resistant to the pest (Giga *et al.,* 1992).

1.4 Genetic diversity of bruchids

Insects respond to climate change in various ways, ranging from changes in phenology and distribution to influencing community dynamics and composition (Menendez, 2007; Moore and Allard, 2008). Temperature has direct or indirect effect on reproduction and growth of an insects since genetic composition of an insects are much more influenced by environmental temperature and moisture (Axmacher *et al*., 2009). *A. obtectus* and *Z. subfasciatus* differ in ecological adaptation and have distinct distribution pattern influenced by temperature (Schoonhoven *et al.,* 1986). *A. obtectus* found in both cool area with high altitude and warmer area of the tropics while *Z. subfasciatus* prefers warmer climates in lower altitudes and thus is more significant in tropical and subtropical regions (Blair *et al*., 2010). Climatic difference and preference were found to be of less important since bruchids strain differ from one geographical location to another (Giga *et al*., 1992).

Management control is difficult against storage pest due to ecotype variation of an insect (Appleby and Credland, 2004)**.** For instance**,** laboratory studies on populations of *Callosobruchus maculatus* was tested in different geographical location and results showed intra-specific variations of performance on resistant cowpeas (Appleby and Credland, 2004).

1.5 General objective

The general objective of this study was to determine genetic diversity of bruchids ecotypes and their effects to beans varieties grown in Tanzania.

1.5.1 Specific objectives

i. To evaluate damage level attributed by bruchid ecotypes in resistant and susceptible bean genotypes.
ii. To determine morphological change of F_1 emerging bruchids in susceptible and resistance bean genotypes.
iii. To determine genetic diversity of bruchid ecotypes *Acanthoscelides obtectus* (bean weevils) and *Zabrotes subfasciatus* (Mexican bean weevil) from bean growing regions of Tanzania.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and distribution of common bean

Common bean originated in Central and South America. Small-seeded and climbing ecotypes are found in the wild in northern Argentina and Central America. Common bean was independently domesticated in both Central America (Mexico and Guatemala) and in the South American (Kambewa, 1997). Common bean became well established as a pulse crop in parts of Africa before the colonial era. Genetic diversity of common bean and its pathogens and linguistic evidence indicate that it became a major crop in Central African highland areas in Rwanda and Burundi earlier than in other parts of Africa (Xavery *et al*., 2005).

2.2 Common bean production in Tanzania

The common bean is the most important food legume in the world (CIAT, 2001). In Tanzania, the common bean crop is cultivated for home consumption as well as for cash income, (Hillocks *et al.,* 2006), and much of East Africa. The East African highlands are a region of important common bean production and high varietal diversity for the crop. Tanzania is the major producer of common bean in East Africa and the importance of common bean as a food crop cannot be over emphasized. Most of the estimated 80% rural communities who depend on agriculture for their livelihood and the urban poor take common beans in their daily diet (Nchimbi, 1989).

In tropical and sub-tropical countries, the per capital consumption of beans may vary from country to country and region to region, within a country. However, generally there is often a high consumption of bean among low-income families in rural and urban areas (Hillocks *et al.,* 2006).

2.3 Ecology of common bean

Common bean is a warm season crop that does not tolerate frost or long period of exposure to near freezing temperatures at any stage of growth. The crop requires moderate amount of rainfall (300 - 600 mm) but adequate amount is essential during and immediately after the flowering stage and grows well at optimum temperatures of 18-24^{o}C (Buruchara, 2007). The maximum temperature during flowering should not exceed 30^{o}C and the optimum pH range is 5.5-6.5.

2.4 History, Origin and Geographical Distribution of bean bruchids

Bean bruchids pose a threat to our agriculture produce and cause serious loss to grain legumes in both fields and stores. Bruchid are Neotropical origin, is a serious pest of kidney bean *Phaseolus Vulgaris* L. (Fabaceae) and various other pulses in Africa (Nahdy, 1994; Abate and Ampofo, 1996; Abate *et al.*, 2000; Msolla-Nchimbi and Misangu, 2002), Australia (Daglish *et al.*, 1993; Keals *et al.*, 2000; Bailey, 2007), Europe (Alvarez *et al.*, 2005, Schmale *et al.*, 2002), America (Johnson, 1970; Johnson, 1990; Martin and Edmund, 1991; Kingsolver, 2004; Napoles, 2010) and various other parts of the world (Southgate, 1979). Both bruchid species are distributed worldwide in all bean-growing areas, but their popularity is highly affected by the ambient temperature. Both *Zabrotes subfasciatus* and *Acanthoscelides obtectus* originated in the tropical and sub-tropical regions of Southern North and Central America, and they are widespread in many other tropical and sub-tropical regions, especially East and Central Africa (Singh 1979; Abate and Ampofo 1996; Wortman *et al.*, 1998; Alvarez *et al.*, 2005).

In Africa the pest has caused widespread damage in Kenya, Lesotho, Malawi and Nigeria is also present in Angola, Burundi, DRC, Rwanda, Tanzania, Uganda, Zambia, and Zimbabwe (Songa and Rono, 1998).

Table 2.1: Geographical distribution of *Acanthoscelides obtectus* in Africa

Country	Distribution	Reference
Angola	Present	CABI and EPPO (2002); EPPO (2020)
Burundi	Present	Garaud (1984); CABI and EPPO (2002); EPPO (2020)
Congo, Democratic Republic	Present	CABI and EPPO (2002); EPPO (2020)
Egypt	Present	NHM (1912); CABI and EPPO (2002); EPPO (2020)
Eswatini	Present, Widespread	Haines (1981); CABI and EPPO (2002); EPPO (2020)
Kenya	Present, Widespread	Haines (1981); CABI and EPPO (2002); EPPO (2020)
Lesotho	Present, Widespread	Haines (1981); CABI and EPPO (2002); EPPO (2020)
Malawi	Present, Widespread	Haines (1981); CABI and EPPO (2002); EPPO (2020)
Mauritius	Present	Facknath (1993); CABI and EPPO (2002); EPPO (2020)
Morocco	Present	CABI and EPPO (2002); EPPO (2020)
Nigeria	Present, Widespread	Aitken (1975); CABI and EPPO (2002); EPPO (2020)
Réunion	Present	CABI and EPPO (2002); EPPO (2020)
Rwanda	Present	Biemont et al. (1987); CABI and EPPO (2002); EPPO (2020)
Saint Helena	Present	CABI and EPPO (2002); EPPO (2020); CABI (Undated)
Senegal	Present	CABI and EPPO (2002); EPPO (2020)
South Africa	Present	NHM (1921); CABI and EPPO (2002); EPPO (2020)
Tanzania	Present	Haines (1981); CABI and EPPO (2002); EPPO (2020)
Uganda	Present	NHM (1913); CABI and EPPO (2002); EPPO (2020)
Zambia	Present	Haines (1981); CABI and EPPO (2002); EPPO (2020)
Zimbabwe	Present	CABI and EPPO (2002); EPPO (2020); CABI (Undated);

Source: EPPO 2020. Last updated: 23 April 2020

2.5 Bean Bruchids Classification

Bean bruchids belong to the order Coleoptera in the family Bruchidae. Bruchidae has got 56 genera which are divided into 5 subfamilies namely bruchinae, cubaptinae, kytorhininae, amblycerinae and pachynaemerinae (Howe and Curie, 1964). The family consists of about 1300 species of seed weevils out of which 20 species are recognized as being pests in stored legume seeds especially in developing countries (Southgate, 1979). Four species are of cosmopolitan importance namely *Callosobruchus maculatus C. chinensis*, *A. obtectus* and *Z. subfasciatus* (Southgate *et al.*, 1978). *Z. subfasciatus* and *A. obtectus* are the main bean bruchid species distributed in nearly all bean producing areas.

2.6 Description of common bean bruchids

- **Common bean weevil (*Acanthoscelides obtectus*)**

Compact and oval in shape, with small heads somewhat bent under. Adults have 3.2 – 4.0 mm conical prothorax, posterior femora bearing a strong notched tooth. Reddish legs, orangey red abdomen and pygidium. Reddish brown or greenish grey pronotum and elytra except the posterior border which is reddish (Nadir *at el.*, 2006).

Source: Patrick R Marquez, USDA APHIS PPQ, Bugwood.org. Last updated 22 October 2013.

Mexican been weevil (*Zabrotes subfasciatus)*

Elytra are short, relatively broad and a bit square in shape. An adult Mexican bean weevil has 1.8 – 2.8 mm body length. The male has slightly uniform light grey-brown pubescence over a dark-grey cuticle. Female elytra are strongly marked with a line of white and pale grey setae on a dark background. There are two movable spurs, called calcaria on the apex of the tibia of each hind leg; these are reddish in color and equal in length (CABI, 2019).

Source: Pest and diseases image library Bugwood. Org. Last updated 3 December 2021.

2.7 Bruchids developmental biology

The two major species *A. obtectus* and *Z. subfasciatus* have comparable developmental biology except *Z. subfasciatus* firmly glues its eggs to the bean surface while *A. obtectus* scatters its eggs among stored bean seeds (Dendy and Credland, 1991). The adult *Z subfasciatus* commonly lays and attaches eggs in groups of 2 or more eggs to the bean surface in an apparently random manner (UTIDA, 1967). The adult *A. obtectus* deposit eggs (milky white 0.60 mm x 0.25 mm) 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 (Henderson, 2007). The larva that hatches from the egg penetrate the seed coat. Once the larva burrows into the bean, the remaining eggshell becomes mottled as it fills with feces from the larva. The larva burrows and feeds on the

bean endosperm and embryo. In the seed the larvae of both species molt four times before pupating. During the last larvae instar, a circular window will be visible on the seed at the location where the beetle is pupating. After pupation the adults push their way out of the window and emerge from the bean seed, soon thereafter mate (Schoonhoven and Cardona, 1986). The adults are fully mature 24 to 36 hours after emergence. *A. obtectus* female lay an average of 45-60 eggs and their eggs lasts 6-7 days. Larvae and pupa stage take 23 days and adult lives 14 days. *Z. subfasciatus* female lay an average of 36 to 56 eggs and the eggs lasts for 5-6 days. Different larvae instar takes 14 days, pupa stage 6-7 days and adults live 10-13 days (Cardona, 1989).

2.8 Host plants attacked by bean bruchids

Bean bruchids have a widely host range, may affect *Cajanus cajan* (pigeon pea), *Cicer arietinum* (chickpea), *Phaseolus vulgaris* (common bean) and other legumes. Also, have ability to infest flower buds, flowers, pods and seeds. The most destructive critical stages of growth are flowering and seed production therefore constitutes a significant constraint to the productivity of grain beans (Abate and Ampofo, 1996).

2.9 Types of damages caused by bean bruchids

Bean bruchids develops at the expense of the seeds. Eggs are laid on the flowers, young pods or seed of bean crops, boring into the young pod and seed. Larvae pupate and hatch into adults that bore out of the seed leaving characteristic exit holes that ruin their appearance. This typical feeding habit protects the larvae from natural enemies and other adverse factors, including insecticides. Bruchid damage can reduce seed germination, encouraging soil fungi and damping off disease and damage is to reduce the quality of beans grown for human consumption (Karel *et al.,* 1999).

2.10 Mechanism of Resistance to Bruchids

Host plant resistance is related to inherent ability of crop plants or varieties to restrict, retard or overcome pest infestations (Koona and Dom, 2005). Available literature reveals that resistance to bruchids could be attributed to either general defense substances or host specific pest resistance. Resistance phenomenon in insect pests consists a specific number of resistance mechanisms namely antixenosis (non-preference), antibiosis, tolerance, and escape (Talekar and Lin, 1992; Edwards and Singh, 2006). Tolerance and escape are resistance mechanisms applicable for field infestations but not for storage insect pests of grain crops pests and the other two mechanisms are effective for storage pests (Keneni *et al.,* 2011; Mwila, 2013). Antibiosis is associated by plants producing secondary metabolites that offers defense against pests as repellents, feeding inhibitors and anti-nutritional factors (Panda and Kush, 1995; Kusolwa, 2007; Kusolwa *et al*., 2016).

The resistance mechanism may involve morphological adaptations as direct defense and physiological and/or biochemical mechanisms affecting the insects' cellular processes, growth and development (Edwards and Singh, 2006). Common bean contains defensive compounds with insecticidal effects that protect their seeds against widely different herbivores. These includes tannins, non- protein amino acids and proteins such as protease, α-amylase inhibitors, lectins, chitinases, β-1, 3-glucanases and arcelins (Sales *et al*., 2000; Kusolwa, 2007).

2.10.1 Lectins

Lectins are one of the greatest important secondary metabolites in plants which are used as a defense tool against pathogens and insect pests that have harmful effects to plants. According to Peumans and Van Damme, (1995) definition "Lectins are a class of proteins of non-immune origin that possess at least one non-catalytic domain that specifically and

reversibly bind to mono-or oligosaccharides". They are similar to antibodies in their ability to agglutinate red blood cells in animals; however, lectins are not the product of immune system. They may bind to a soluble carbohydrate or to a carbohydrate moiety that is a part of a glycoprotein or glycolipid. These glycoproteins or glycolipid is multivalent and possess more than one sugar binding site (Lis and Sharon, 1998; Rudiger and Gabius, 2001). These sugar binding proteins are found in bean seeds, with small amounts also found in roots, leaves and sometimes in seed testa (Sales *et al*., 2000). The principal function of lectins act as recognition molecules within the immune system, storage proteins and cell surface adhesion and they have been implicated in defense mechanisms of plants against invading pathogens and pests (Sauvion *et al.,* 1996; Van Damme *et al*., 1998; Rudiger and Gabius, 2001; Trigueros *et al*., 2003). Various lectins from different sources have already been found to be toxic towards important members of insect orders, including Lepidoptera (Czapla and Lang, 1990), Coleoptera (Gatehouse *et al*., 1984; Czapla and Lang, 1990) and Homoptera (Powell *et al*., 1993; Sauvion *et al*., 1996). The types of plant lectins are Phytohaemagglutinin (PHA), α-amylase inhibitors (α-AI) and Arcelins as described below.

2.10.1.1 Phytohaemagglutinin (PHA)

PHA, is a type of plant lectin especially in legumes, which are carbohydrate-binding proteins that can interact with membrane receptors that agglutinates cells (Banwell *et al*., 1983; Imran, *et al.,* 2013). It is a major lectin of common bean made up from two polypeptides sub-units that occurs in many accessions of beans. The two polypeptide sub-units are called leucoagglutinin (PHA-L) and erythroagglutinin (PHA-E) and are closely related proteins formed by tandemly linked gene.

Leucoagglutinin (PHA-L) is responsible for agglutination of leucocytes (white blood cells) and erythroagglutinin (PHA-E) for agglutination of erythrocytes (red blood cells) (Mirkov *et al*., 1994). Phytohaemagglutinin can exert anti-nutritive effects (Bardocz *et al*., 1996) and affect parameters related to animal growth and metabolism such as binds to glycoprotein in the intestinal mucosa of mammals and insects whereby it inhibits nutrient absorption across the walls and its toxicity results from its initial binding properties (Jones, 1999). By enhancing the activities of lectin-like proteins, phytohaemagglutinin contributes to resistance of common bean to bruchids (Goosens *et al*., 2000).

2 10.1.2 α-amylase inhibitors (α-AI)

Seeds of the common bean (*Phaseolus vulgaris* L.) contain a plant defense protein that inhibits the α-amylases of mammals and insects. α-amylase inhibitor is a lectin-like protein that inhibits bruchids and other insect species infestation in common bean due to its porcine pancreatic α-amylase inhibitory activities that prevent feeding, block starch digestion, retard instar development and even cause larvae mortality (Ishimoto *et al*., 1995). Furthermore, α-AI from kidney bean inhibits a-amylase from three species of bruchids that are seed storage pests (Ishimoto and Kitamura, 1989). Because α-AI inhibits the development of bruchid beetles we consider the gene that encodes α-AI as potentially useful for crop protection via plant genetic engineering (Huesing *et al*., 1991).

α-amylase inhibitors are found in both cultivated and wild accessions of common bean. Electrophoretic mobility and pancreatic α-amylase inhibitory activities led to identification of eight α-amylase inhibitors. Among the eight α-amylase inhibitors identified, α-amylase inhibitor-1 (αAI- 1) and α-amylase inhibitor-2 (αAI- 2) which are allelic, are found to have adverse effects on bruchid development. αAI-1 is closely linked to PHA and exists in both wild and cultivated common bean accessions whereas αAI-2 is

found only in wild accessions with various arcelin variant (Suzuki *et al*., 1995). These two variants of α-amylase inhibitors provide different levels of resistance to bruchids. αAI- 1 found in genotypes that lacks Arcelin and has inhibitory effects to *C. maculatus* and *C. chinensis* but offers weak resistance to some bruchid species and do not inhibit α-amylase of *Z. subfasciatus*. αAI- 2 is contained in genotypes with Arcelin that inhibit α-amylase of *Z. subfasciatus* and offers high resistance to bruchids (Kusolwa, 2007).

2.10.1.3 Arcelins

Arcelin (*Arcs*) belong to the lectin-like family described in eight allelic variants in various accessions of the wild common bean (*P. vulgaris*) which exhibit different levels of resistance to bruchid insect pests. A comparison of the amino acid sequences of arcelins with other seed proteins shows that they belong to the bean lectin family that includes PHA and α-amylase inhibitor. Arcelins are not strong lectins, but may have weak lectin activity (Osborn *et al.,* 1988b; Zaugg *et al.,* 2013). Afterward, arcelins were shown to have inhibitory effects on the larval growth of *Z. subfasciatus* and to account for the resistance of the seeds of these wild accessions (Osborn *et al.,* 1988a; Cardona *et al*., 1990). Some wild bean accessions containing arcelin are also highly resistant to *A. obtectus* (Schoonhoven *et al*., 1983; Cardona *et al*., 1989; Kornegay and Cardona, 1991).

Kusolwa and Myers, (2008) developed population of backcross lines expressing *arcelin*-2 (*Arl-2* ^{pv}) from *P. vulgaris* and the ARLs from tepary beans accession G40199 in a phaseolin null background. More important bruchid screening of these materials indicated a high degree of resistance to *A. obtectus* in a homozygous and heterozygous state. Arcelin variant ARL-^{pa} found in G40199 and its hybrid as cDNA demonstrate close nucleotide sequence identity with the present Lectin like proteins (LLPs) published in

tepary bean and may represent evolution of a new arcelin variant (Kusolwa and Myer, 2008).

The antibiosis properties of arcelins may be related to their ability to recognize and interact with glycoproteins and other constituents of the digestive tract membrane, as well as to their capacity to directly bind to the intestinal cells of insects (Minney *et al*., 1990; Fabre *et al*., 1998; Paes *et al*., 2000). In efforts to develop bruchid-resistant beans, researchers have identified and introgressed different genetic resistance factors from wild relatives into common bean. The principal resistance factors in mature bean seeds are arcelins (Osborn *et al.,* 1988a; Osbon *et al*., 1988b; Zaugg *et al*., 2013). Six electrophoretic variants of arcelin, designated arcelin-1 to 6, have been reported (Osborn *et al*., 1986; Santino *et al.,* 1991) and among them, arcelin-5 and arcelin-1 exhibit the highest level of bruchid resistance (Cardona *et al*., 1990; Fory *et al*., 1996).

2.10.2 Antixenosis (non-preference)

Antixenosis refers to the absence of the host plant attractiveness for insects to laying eggs and feeding because of their texture, seed coat thickness seed size**,** color, odor or taste, (Nwanze and Horber, 1976; Oyetunji *et al*., 2014)**.** Non preference by an insect is caused by unsuitability of the plant host for oviposition, growth and/or survival due to some morphological or biochemical factors in the host plant. Morphologically, varieties with smooth, soft and thin seed coats may be more preferable for oviposition than those with rough, hard, wrinkled and somewhat spiny seed coats (Keneni, 2011). It has been confirmed 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 in

oviposition 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**).**

2.11 Bruchids management strategies

2.11.1 Cultural control

Cultural control involves the manipulation of conducive host environment to eliminate the occurrence, growth and multiplication of storage pests (bean bruchids). These practices include use of clean certified seed, removal of egg shells and dead larvae, remove and destroy all infested crop residues immediately after harvest, air-dry the beans to a moisture level of 12% or lower before storage (Keneni *et al*., 2011). Bean bruchids infest beans in the field only when the pods are almost dry therefore timely harvesting can ensure that the weevils are not carried into the store along with the beans hence beans should be harvested as soon as they are mature to reduce the risk of heavy infestation (Stoll, 1988). It is very important to clean tracks, trailers, combine harvester machine and the bins during harvesting period, also clean the storage facility prior to storage, using a disinfectant if necessary. Removal of infested grains before storage of new grains and old beans should not be stored together with newly harvested beans. Store beans in air-tight containers if possible, such as in plastic sealable bags, drums, or clay pots. These practices make the host environment less attractive and unfavorable for the survival, dispersal, growth and reproduction of the bruchid pests (Casida and Quistad, 1998).

Cultural methods provide cheapest and most reliable ways of post-harvest handling of the bean crops, especially for marginal farmers, as they require standard post-harvest practices without the need for extensive labor.

2.11.2 Chemical control

Chemical insecticides are classified into four chemical groups organochlorines, organophosphates, carbamates and pyrethroids. These groups of insecticides have been used for over five decades to control insect pests both at the field and in storage conditions (Dent, 1991; Megerssa, 2010). Researchers have reported the effectiveness of using chemical pesticides such as dusts, fumigants and sprays for the prevention of bruchid pests (Harberd, 2004). Despite of its effectiveness chemical control is associated with some constraints for example it is toxic to the user and can kill none target organisms, easy for crop to adopt pesticide resistance, health hazards and environmental effects and it is expensive thus mainly practiced by large scale farmers (Khan and Selman, 1987).

2.11.3 Physical control

These methods involve the treatment of seeds and insects using physical agents, such as temperature, pressure, moisture content and heat (Sahadia and Aziz, 2011). Each insect species requires an optimal environment comprising temperature, humidity and photoperiod for its growth and perpetuation (Evans, 1987), which can vary depending upon the stages of growth and reproduction. The amount of bruchids development can be minimized by either lowering or raising the temperature and altering relative humidity from its optimal range (Benz, 1987). Cold storage rooms with a minimum temperature of 4 to 5°C can be used to slow down the development and reproductive activities of bean weevils. Though, this technique is not practicable for small-scale bean producers in tropical regions due to high cost of investment and unstable power supplies in Tanzania. The use of solar heating techniques has also been recommended for controlling bruchid beetles in mung bean and cowpea seeds (Murdock and Shade, 1991). This method can

also be applicable in controlling bean bruchids. Exposure of bean seeds to extreme warm and dry conditions is unfavorable to bruchid development and can effectively kill all life stages of stored grain insects including bruchids in cowpea, mung bean, pigeon pea, common bean and moth bean (Sahadia and Aziz, 2011; Upadhyay and Ahmad, 2011).

2.11.4 Botanical control

These methods involve the use of vegetable oils and botanical herbs (Schoonhoven, 1978; Mazzonetto and Vendramim, 2003) that can be mixed with bean seeds before beans are stocked in the storage warehouses. These plant products include extracts from leaves of *Eucalyptus citriodora*, neem leaves or seeds (*Azadirachta indica* L.), tobacco leaves, leaves of coriander, *Chenopodium ambrosioides*, citrus rinds, and tephrosia (*Tephrosia spp.)* which act as insecticides and red or black peppers used as irritants. Maize oil, soy oil concentrated juice from banana plants appear to suffocate various insect stages and penetrate into eggs reducing eggs' fertility thus protecting beans from *A. obtectus* and *Z. subfasciatus* (Schoonhoven and Cardona, 1986; Baier and Webster 1990; Slumpa and Ampofo 1991).

2.11.6 Biological control

Biological control of bruchids involves use of living organisms (biological control agents/ natural enemies) to maintain the pest populations below damaging level. The natural enemies include predators, parasitoids and pathogens (Altieri *et al*., 2005; Mahr *et al*., 2008). Predators such as spiders, ladybirds, lacewings or predatory mites, usually feed on a range of different insects. The parasitoid *Horismenus ashmeadii* (Dalla Torre) (Hymenoptera -Eulophidae) have been suggested to reduce *A. obtectus* infestation in the field that feeds on the larvae of *A. obtectus* (Schmale *et al*., 2002) but the parasitoid was

not effective in storage. Pathogens may be bacteria, fungi, viruses, nematodes or protozoa.

2.12 Impact of climate change on insect pests

Insects are good indicators of current climate change. They have responded to warming in all predicted ways from changes in phenology and distribution to undergoing evolutionary changes. Insects are among the groups of organisms most likely to be affected by climate change because climate has a strong direct influence on their development, reproduction and survival. Additionally, insects have short generation's times and high reproductive rates, so they can more like to respond quicker to climate change than long-lived organisms, such as plants and vertebrates (Menendez, 2007).

Climate change such as global warming may result on breakdown of resistance to certain insect pests. For example, Sorghum varieties exhibiting resistance to sorghum midge, *Stenodiplosis sorghicola* (Coq.) in India became susceptible to the pest under high humidity and moderate temperatures near the equator in Kenya (Sharma, *et al.*, 1999). Climatic change may also change the flowering times in temperate regions, leading to ecological consequences such as introduction of new insect pests and attaining of a pest status by non- insect pests (Willis, *et al.*, 2008)**.**

Climate change resulting in increased temperature could impact crop pest populations in several ways. Most researchers seem to agree that warmer temperatures in temperate climates will results in more types and higher populations of insects. Researchers have shown that increased temperature can potentially affects insect survival, development, geographic range and population size. It can impact on physiology and development directly or indirectly through the physiology or existence of hosts. Some insects that take

several years to complete one life-cycle- (cicadas) will tend to moderate temperature variability over course of their life history.

Some pests are ‘stop and go’ developers in relation to temperature. They develop more rapidly during periods of time with suitable temperatures. Increased temperature will accelerate the development of these types of insects-possibly resulting in more generations and crop damage per year.

This concludes that higher temperature in the future may thus increase the damage on crops, by increasing the number of generations of the pest (Abolmaaty *et al.*, 2011).

2.13 Molecular markers in phylogenetic studies of Coleoptera

2.13.1 Mitochondrial DNA (mtDNA)

Possibly the most commonly used molecule in phylogenetic analyses of insects and animals is mitochondrial DNA (mtDNA). Mitochondrial DNA data can be very powerful in resolving species-level phylogenies. The order of genes in the mitochondrion is variable, and they are separated by large regions of noncoding DNA. Animal mtDNA is compact, lack introns, intergenic regions are usually small or absent, few duplications and heteroplasmy (such as the coexistence of more than one type of mtDNA within a cell or individual) is considered to be very rare in natural populations. All these features make mtDNA an ideal candidate for phylogenetic investigations on different taxonomic levels. The mitochondrial genome rearranges itself frequently so that many rearranged forms can occur in the same cell. The use of *mt*DNA has become increasingly popular and proven useful in studies of population structure, geographic variation and phylogeny (Alberts *et al.,* 2002).

2.13.2 Cytochrome oxidase I/II (COI/II)

The enzyme cytochrome oxidase is a very well-known protein of electron transport chain and is found in both bacteria and mitochondria. The COI and COII genes code for two of seven polypeptide subunits in the cytochrome oxidase complex. The COI gene consists of approximately 894 bp. COI and/or COII sequences have been applied to phylogenetic problems at a wide range of hierarchical levels in insects, from closely related species to genera and subfamilies, families, and even orders. The COI gene is slowly evolving compared to other protein coding mitochondrial genes and is widely used for estimating molecular phylogenies (Russo *et al*., 1996) and is a good performer in recovering an expected tree (Zardoya and Meyer, 1996). COI and COII have been used for species and population analyses of parasitoids and COI has recently been suggested as a potential 'barcode' for insect identification in general.). It has been reported the COI sequence of mitochondrial data had higher sequence divergence than four other nuclear genes, in beetles (Zhang and Sota, 2007).

2.13.3 Control region for replication of mitochondrial DNA

The only major non-coding area of the mtDNA is the control region, typically 1kb, involved in the regulation and initiation of mtDNA replication also transcription and is responsible for the regulation of heavy (H) and light (L) strand transcription and of H-strand replication. The approximate mutation rate in mtDNA is 10-8/site/year compared to 10-9/site/year in nuclear genes. Most differences between mtDNA sequences are point mutations, with a strong bias for transitions over trans versions (Brown *et al*., 1982). It has been reported by (Rogaev *et al.,* 2006) the presence of variable number of tandem repeats (VNTR) in the control region which are characterized by high somatic hyper variability in some mammoth. The evolution of the control region of mammalian mtDNA

shows some features such as strong rate heterogeneity among sites, the presence of tandem repeated elements, a high frequency of nucleotides insertion/ deletion, and lineage specificity (Pesole *et al*., 1999).

2.13.4 Mitochondrial *12S rRNA*

Mitochondrial 12S rRNA gene a ribosomal RNAs encoded by the mitochondrial genome necessary for the translation of messenger RNAs into mitochondrial protein. Mitochondrial 12S rRNA gene sequence analysis is extensively used in molecular taxonomy and phylogeny. Earlier, mitochondrial 12S rRNA gene sequence was used for species determination in wild-life forensic biology. It has been hypothesized earlier that 12S gene sequences are useful for the determination of moderate to long species divergence times. The length of this gene is about 450 bp and it can be amplified by universal primers. It is mostly used in phylogenetic studies among families (Allard and Honeycutt, 1992).

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CHAPTER THREE

3.0 DAMAGE LEVELS ATTRIBUTED BY DIFFERENT BRUCHID ECOTYPES IN RESISTANT AND SUSCEPTIBLE BEAN GENOTYPES.

3.1 Abstract

The bean bruchid species *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Bohem.) are major storage pests of common beans (*Phaseolus vulgaris* L) in Tanzania and are the main constraint to small-holder bean producers. The degree of loss depends on storage period such as the longer the storage period the greater the loss and losses may reach up to 100% if not managed (Misangu *et al*., 2000). Host plant resistance is a profitable and a safe alternative to manage bruchids in common bean and is associated with biochemical, morphological, and molecular traits. These traits affect insect growth and development and in that way, reduce the losses by the pests. The aim of this research study was to evaluate the damage level caused by bruchid ecotypes in resistant (AO-1012-29-3-3A) and susceptible (Njano gololi) bean genotypes. Bean bruchids were collected from different bean growing villages in Tanzania; Songwe (Vwawa), Kilimanjaro (Mungushi), Karatu (Rhotia), Morogoro (SUA) and Arusha (Kimyaki), then taken to the laboratory at SUA for experimentation. Experiment was conducted using a Completely Randomized Design (CRD) with a 2 x 5 or 2^5 factorial scheme, two bean cultivars (AO-1012-29-3-3A and Njano gololi), five bruchids ecotypes for each *A. obtectus* and *Z. subfasciatus* and 3 replications. The results showed that; significant differences (P = 0.001) exist between bruchid ecotypes on infestation rate. Again species differences among cultivars was also observed. It was observed that cultivar AO-1012-29-3-3A was resistant and Njano gololi was susceptible for all ecotypes tested. This was expressed by Njano gololi having high population of emerged bruchids, high percentage

weight loss, high susceptibility index and high severity score. This shows that bruchids from different geographical locations may develop different virulence.

3.2 Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the major staple crops in eastern and southern Africa. It is an important source of protein as well as source of income by most farmers in developing countries (Broughton *et al*., 2003). Disparate other legumes, such as groundnut or pigeon pea, the dry bean plant is a source of food throughout the life cycle of the crop as its leaves, green beans and dry beans can be consumed. The production of beans (*Phaseolus vulgaris* L.) is restricted by a number of biotic and abiotic factors both in the field and in the store.

Among the constraining biotic factors are insect pests (bean bruchids). Storage of beans grain over long periods, especially at small scale farming levels, is limited due to bean bruchids (*Acanthoscelides obtectus*) and (*Zabrotes subfasciatus*). Their damage causes loss of weight, nutritional value and viability of stored grains. Larvae developing within the grain cause the largest damage.

Control of bean bruchids in the field and store has to be considered in relation to the economic importance of the crop, since it is evident that these weevils are capable of attacking beans both in the field and in storage. The use of synthetic insecticides is one of the methods used to control bruchids (Opolot *et al.,* 2006). However, continuous use of chemical insecticides may lead to serious problems such as insecticide resistance and negative effects towards health of the consumers. Non-chemical methods of bruchid control offer the best alternative because they neither leave chemical residues in the commodity nor could their use give rise to resistance in the pest. Most of small-scale farmers use botanicals as their indigenous bruchid control (Mbongo *et al*., 2013) although

this method is less efficient in most cases due to shortage supply of botanicals. The use of improved storage structures can be effective way of controlling these bruchid species but this method is unaffordable to small scale farmers as a result less practically used, therefore one of the best and sustainable ways of controlling bean bruchids is by genetic resistance methods.

Most of cultivated common bean genotypes are susceptible to bean bruchids. It is only the wild genotypes of bean that were originally found from central highlands of Mexico known to be resistant to bean weevils infestation (Singh and Schwartz, 2011). It has been reported that, CIAT screened and used wild accessions to develop cultivated bean lines known as RAZ lines that are resistant to bruchid infestation (Valencia *et al*., 1986). Different researchers used these accessions from CIAT and promising levels of resistance has been obtained. In Tanzania, resistance transfer from the wild accessions to cultivated genotypes has been done and only one Arcelin derived bruchids resistant germplasm AO-29-3-3A have been officially registered as a bean line resistant to *Acanthoscelides obtectus* (Kusolwa *et al.,* 2016).

However, cultivar resistant to one biotype may be susceptible to another due to genetic variability of the pest population. Both genetic and environmental factors would affect the resistance and/or susceptibility of the bean cultivars against bruchids (Appleby and Credland, 2004). This research aimed at identifying damage levels attributed by different bruchid ecotypes in resistant and susceptible bean genotypes.

3.3 Material and Methods

3.3.1 Location of study

The experiment was conducted in African seed health laboratory in the Department of Crop Science and Horticulture of Sokoine University of Agriculture, Morogoro, Tanzania in 2020.

3.3.2 Experimental Materials used

The main material used in this study involved bean bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*), susceptible bean cultivar a yellow seeded bean landrace (Njano gololi) and bruchid resistant bean genotype AO-1012-29-3-3A line from the cross (Rojo)*3///SMARC-2-PN-1// (ICA Pijao)*2/G40199 that sers as useful source of resistance to the bean weevil, BCMV and some path groups of BCMNV.

3.4 Methods

Bean bruchids samples were collected from different bean growing villages. *Acanthoscelides obtectus* were collected from Vwawa, Rhotia and SUA. *Zabrotes subfasciatus* were collected from Tengeru and Mungushi in Tanzania. Then they were brought to SUA laboratory for experimentation. Samples of AO-1012-29-3-3A bean genotypes were obtained from Bean Breeding Project at Sokoine University of Agriculture while the yellow seeded Njano gololi were collected from local farmers. Bruchids feeding trial was laid out under Completely Randomized Design (CRD) with a 2 x 5 or 2^5 factorial scheme, two bean cultivars (AO-1012-29-3-3A and Njano gololi), five bruchids ecotypes for each *A. obtectus* and *Z. subfasciatus* and 3 replications. Twenty adult bruchids (*A. obtectus* and *Z. subfasciatus*) from each locations were inoculated in the plastic containers containing thirty bean seeds of Njano gololi and AO-1012-29-3-3A separately. The trial

was left undisturbed for 14 days to allow bruchids to lay eggs. Then all adults were removed in each plastic container leaving only eggs for reproduction. Observations and counting of newly emergence of adult F_1 bruchids commenced on the 20^{th} day from inoculation. Counting of emerging adult was then done daily by checking each experimental unit for a duration of 90 days in both susceptible and resistant bean genotypes. The insects were maintained at a temperature of 26^0C ± 3^0C and 70 ± 5% Relative Humidity throughout the experiment.

3.5 Data collection

Data collected were; total number of bruchids emergence every day, number of holes per lines this included seed with 1 hole, 2-3 holes, 4 and above holes, weight of susceptible and resistant bean sample, number of days for first adult emergence and susceptibility index (%).

Percentage weight loss of grains was calculated as follows;

$$Grain\ weight\ loss\ (\%) = \frac{(IGW - FGW)}{IGW} \times 100$$

Where;

FGW = final grain weight

IGW = initial grain weight for the sample.

Then susceptibility index was calculated as follows;

$$\text{SI (\%)} = \frac{(\log \#\ \text{of emerged Adults})}{\text{DAE}} \text{ x } 100\%$$

Where;

SI – Susceptibility index

DAE – number of days for first adult emergence

3.5.1 Data analysis

These parameters were analyzed in the analysis of variance (ANOVA) set at 5% significance level. The analysis done by using GenStat software. The mean separation test conducted by the use of Tukey's test at $p \leq 0.05$.

3.6 Results

3.6.1 Number of total bruchids emerged

Higher emergence of *Z. subfasciatus* from Kilimanjaro and Arusha regions was observed in both susceptible and resistant bean seeds compared to *A. obtectus* from other regions. Emergence of *A. obtectus* in resistant genotype was high in Songwe, Karatu, and Morogoro respectively. Emergence *A. obtectus* in susceptible genotype was higher in Karatu, Songwe and Morogoro respectively as shown in (Fig. 3.1) below. There was significant difference at Prob. = 0.001 in mean number of F_1 adults emerged in all bean genotypes (susceptible and resistant). In addition, there was significant difference in interaction between regions and bean genotype at p = 0.006 (Appendix 3.1).

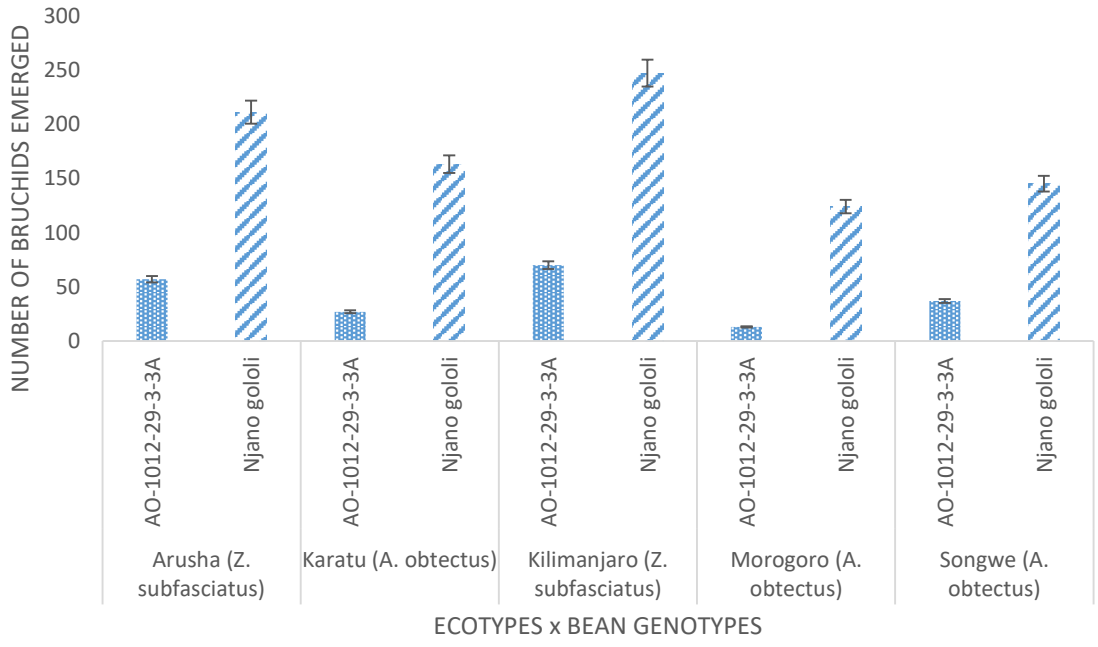

Figure 3.1 Mean number of total F_1 bruchids (*Z. subfasciatus* and *A. obtectus*) from different regions emerged in resistant and susceptible bean genotypes.

3.6.2 Number of days for first adult emergence (DAE)

Significant delay of *Z. subfasciatus* and *A. obtectus* emergence was observed in resistant bean genotype while early emergence was observed in susceptible bean genotypes (Fig.3.2).The results in mean days of first F_1 adults emergence in all bean genotypes (susceptible and resistant) showed the significant difference Prob. = 0.001. There was no significant difference in interaction between regions and bean genotypes at Prob. = 0.115 (Appendix 3.2).

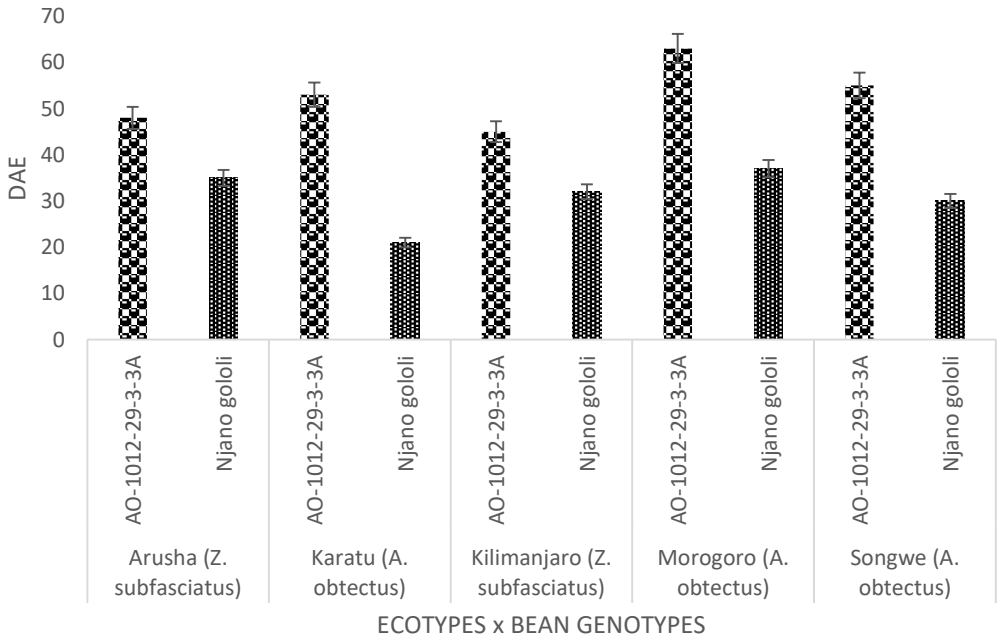

Figure 3.2: Mean number of days for first F_1 bruchids emerged in resistant and susceptible bean genotype.

3.6.3 Susceptibility index (%)

For susceptibility index, Njano gololi was highly infested by all bruchids ecotypes compared to AO-1012-29-3-3A genotype. High susceptibility of bean genotypes was observed in Kilimanjaro and Arusha by bruchid specie *Z. subfasciatus***.** For *A. obtectus* high susceptibility index of bean genotypes was observed in Karatu followed by Songwe and Morogoro respectively (Fig. 3.4). Bruchids from different regions showed a

significant difference on bean infestation at p = 0.001. Furthermore, there was significant difference among susceptible and resistant bean genotypes at p = 0.001 (Appendix 3.3).

Figure 3.3: **Picture A; Njano gololi seeds with severe damage caused by *Acanthoscelides obtectus* from Songwe (Vwawa), Picture B; AO-1012-29-3-3A seeds with mild damage caused by *Acanthoscelides obtectus* 90 days after inoculation, Picture C; AO-1012-29-3-3A seeds with less damage caused by *Acanthoscelides obtectus* from Morogoro 90 days after inoculation.**

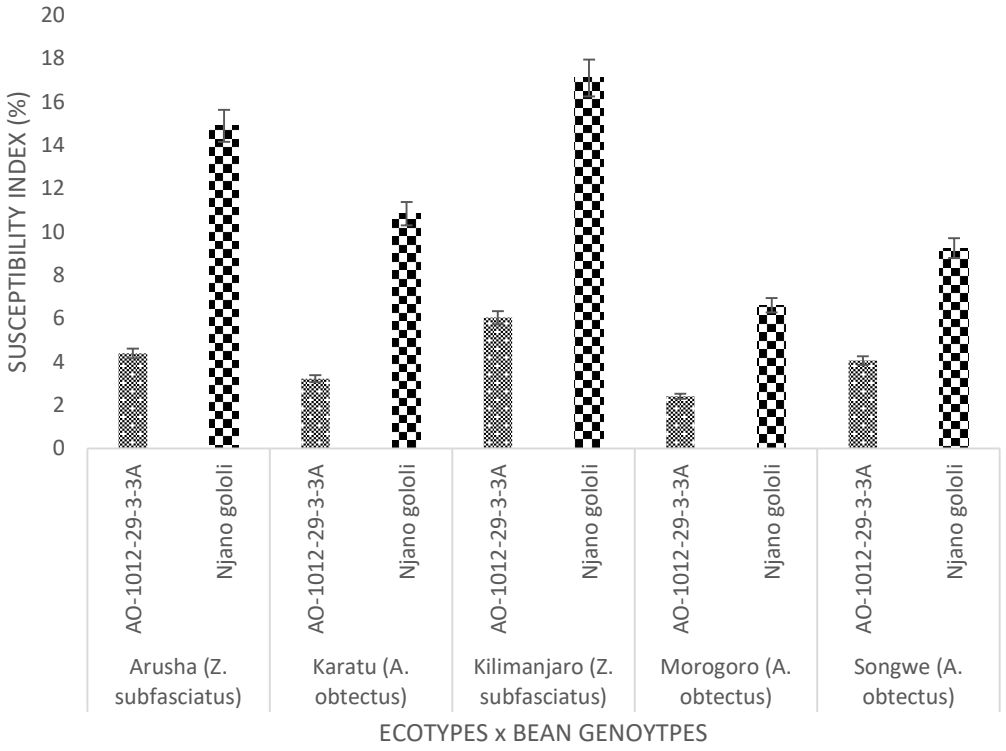

Figure 3.4: **Mean susceptibility index of F_1 bruchids (*Z. subfasciatus* and *A. obtectus*) emerged from susceptible and resistant bean genotypes.**

3.6.4 Percentage weight loss (%)

Bean bruchids from different regions showed significant difference on weight loss of resistant and susceptible bean genotypes (Fig. 3.5). Significant reduction in seed weight of resistant and susceptible bean genotypes were observed at Prob. = 0.001 (Appendix 3.4).

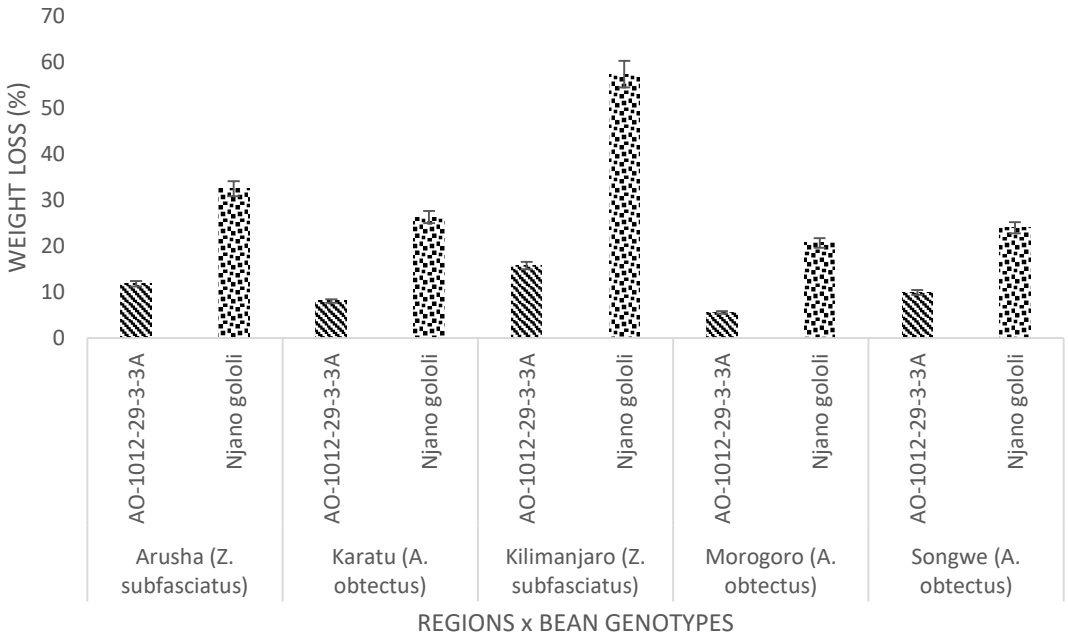

Figure 3.5: Mean percentage weight loss of resistant and susceptible bean genotypes infested by *Z. subfasciatus* and *A. obtectus* from different regions.

3.6.5 Severity

Damaged bean seeds with 10 or more holes demonstrated high weight loss, high bruchids emergence as a result of severity. The mean number of Njano gololi with severe perforation was observed in Kilimanjaro and Arusha by bruchid species *Z subfasciatus* where all seeds in each replicate had more than 10 holes. Mild seed perforations in AO-1012-29-3-3A genotype was observed in Kilimanjaro and Arusha region by bruchid

species *Z subfasciatus*. Njano gololi with severe seed perforation was observed in Karatu by bruchid specie *A. obtectus* where all seeds in each replicate had more than 10 holes then followed by Songwe and Morogoro respectively. Less seed perforations in AO-1012-29-3-3A bean genotype was observed in Songwe, Karatu, and Morogoro respectively by bruchid specie *A. obtectus* as shown in Fig. 3.7 below. Bean genotypes (Njano gololi and AO-1012-29-3-3A) showed significant differences prob. = 0.001 (Appendix 3.5).

Figure 3.6: Picture A shows Njano gololi seeds that are highly damaged by *Zabrotes subfasciatus* from Kilimanjaro 90 days after inoculation; all bean seeds had more than 10 holes. Picture B shows AO-1012-29-3-3A seeds with less damage compared to Njano gololi caused by *Zabrotes subfasciatus* 90 days after inoculation.

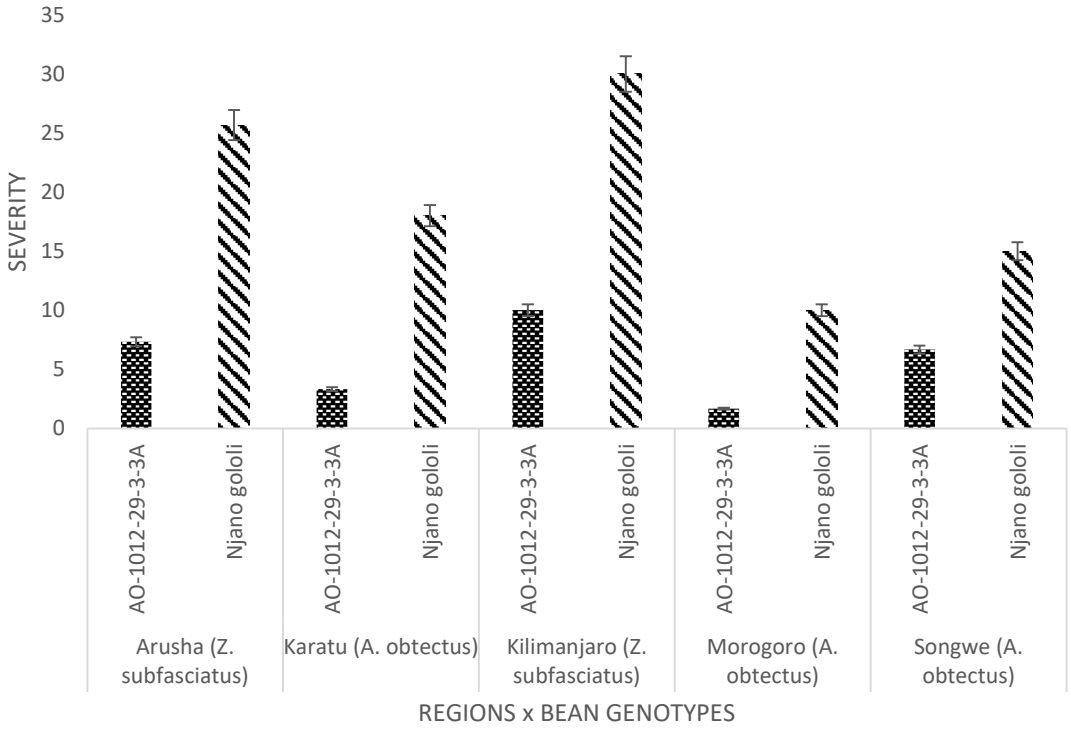

Figure 3.7: Mean number of AO-1012-29-3-3A and Njano gololi seeds with severe, mild and less perforation infested by *Z. subfasciatus* and *A. obtectus* from different bean growing regions.

3.7 Discussion

The findings of this study have provided information that the responses of the insect population's growth depend on the environment condition and seed variety in which their development had occurred. Examination of the independent and interactive effects of bruchids population and insect performance on seed genotypes was confirmed by revealing that seed genotypes had a significant effect on various variables associated with performance of insects. This includes development time, number of bruchids emerged, first day of adult emergence. The interaction is imperative because it suggests that the effect of resistant seeds in lowering the performance of bean bruchids populations is dependent to a significant extent on the nature of the environmental conditions during development. (Appleby and Credland, 2004) suggested that the performance of resistant or susceptible seeds cannot necessarily be predicted reliably without taking account of both the effect the seeds themselves on controlling population growth and also on the environmental conditions in which the seeds will be grown and stored.

The study done by (Kenneth and Credland, 1986; Shade *et al.,* 1999; Appleby and Credland, 2004) on *C. maculatus* showed that the response in terms of performance is dependent to a significant extent on the particular *C. maculatus* population by its origin in the field, and the particular seed variety. Additional to this, environmental differences of an order relevant to common bean storage across different area of Tanzania and probably other areas along the same latitude, are sufficient to produce differences in infestation.

Furthermore, significant interactions between environmental conditions, seed variety and insect population have been revealed in terms of their effects on bean bruchids performance on resistant seeds, and again these effects are likely to alter the effective

level of resistance that a given variety will confer in a given location under given conditions. The implication is therefore, that the only reliable way to determine the effectiveness of resistant common bean varieties in controlling *A. obtectus* and *Z. subfasciatus* in the field would be to test the varieties against bean bruchids in the areas targeted for release, under local environmental and storage conditions. Testing resistant varieties against specific populations in specific locations might then enable varieties to be targeted against those populations in those areas where they had proved during testing to be most effective (Shade *et al.,* 1999) recommended this method.

3.8 Conclusion and Recommendation

Results suggest that, AO-1012-29-3-3A bean genotype is resistant to *A. obtectus* hence can be grown and stored in a wider range of environment such as Morogoro, Songwe and Karatu without applying insecticides. However, AO-1012-29-3-3A is observed to be susceptible to *Z. subfasciatus* from Kilimanjaro and Arusha region, hence it is very essential for the farmer incorporates both cultural and chemical control to reduce the damage of the bean bruchids during storage.

Availability of this resistant bean genotype provides Tanzanian bean farmers with varieties that can be stored longer with less post-harvest loss. Bean bruchids resistance cultivar can be kept from weevil damage for at least 60-90 days or longer after harvest. A mean delay of first F_1 bruchids adult emergence of 60-65 days after inoculation in AO-1012-29-3-3A is a significant factor for long bean storage.

In the absence of resistance and any control measures, weevil damage starts within 20-38 days of harvest. With extended time of safe storage, farmers will have greater flexibility of when to sell their beans at the best market price during the year. This will stabilize bean supply and minimize seasonal price fluctuation in the market and increased seed

availability for the following production season. Breeding of bruchids resistant cultivars may also reduce the use of expensive pesticides used to protect large stocks of dry beans in storage and ensure good health of bean consumers, since no chemical residues will be found.

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CHAPTER FOUR

4.0 MORPHOLOGICAL STUDY OF F_1 EMERGING BEAN BRUCHIDS FROM SUSCEPTIBLE AND RESISTANT BEAN GENOTYPES.

4.1 Abstract

A lot of members of family Bruchidae are major pests of cultivated legumes because they develop within and consume legume seeds which are meant for human consumption. The purpose of this study was to determine phenotypic characteristics of the emerging F_1 bruchids from resistant (AO-1012-29-3-3A) and susceptible (Njano gololi) bean genotypes and whether these traits are host induced or genetically determined. Resistant genotype was used to assess whether host-race morphological differences are genetically determined or due to phenotypic plasticity. There was significant difference in size of F_1 bruchids emerging from resistant genotype (AO-1012-29-3-3A) and susceptible genotype (Njano gololi). F_1 bruchids emerged for both *A. obtectus* and *Z. subfasciatus* from susceptible Njano gololi were consistently larger in size than the emerging adults from resistant AO-1012-29-3-3A genotype. The mean weight of F_1 adults emerging from resistant genotype was significantly reduced than adults emerging from susceptible bean genotype.

4.2 Introduction

Bruchids, commonly known as pulse beetles are a serious threat to grain legumes worldwide. Many of the bruchid species have crossed the geographical boundaries and have become cosmopolitan in distribution through human-mediated migrations and import/export of food grain (Thakur, 2012). These cosmopolitan insects belong to the bruchid family (Coleoptera: Bruchidae) and cause damage by reducing grain quality and

marketability as well as seed germination (Paul *et al*., 2009; Thakur, 2012). Climate change may affect the food reserves and the availability of seeds for growing plants in the near future. Post-harvest loss caused by pests may exceed 20% in poorly developed and tropical countries due to inadequate management practices and environmental conditions that allow rapid reproduction of pests, especially in developing countries (Broughton *et al*., 2003). Ensuring global food security goal requires a range of strategies including improvement of staple food crops and the reduction of post-harvest loss of foods.

The present study explores morphological differentiation among bean bruchids *A. obtectus* and *Z. subfasciatus* after being inoculated in resistant (AO-1012-29-3-3A) and susceptible (Njano gololi) bean genotypes. Changing diet is known to have a large effect on the phenotypes of adults in many insects, including effects on size, shape, internal anatomy, attractiveness to conspecifics, and mating behaviors (Miller, 2008; Gillespie *et al.,* 2014; Somjee *et al.,* 2015; Sasson *et al.,* 2016). A differential host chemistry or phenology can result in corresponding physiological adaptations on the side of the insects (Feder *et al.,* 1997; Carrol *et al.,* 1998). Morphological level changes in size and weight of bean bruchids emerged in resistant bean genotypes evolved in order to adapt to the new host.).

However, host-related differentiation in morphological traits does not necessarily mean that these have been formed by natural selection. Instead, they might be an expression of non-adaptive phenotypic plasticity. Host plants differ, for example, in nutritional value (Häggström and Larsson, 1995) and number of secondary metabolites (Roitberg and Isman, 1992). This can translate into body-size differences of an insects or parasite (Leclaire and Brandl, 1994; Stoyenoff *et al.,* 1994; Lill and Marquis, 2001)). If host races differ when developing in their original host but not when developing in the same environment, differences can clearly be assigned to phenotypic plasticity. On the other

hand, a genetic basis is supported if morphological differentiation between host races is independent of the environment (original host or hybrid plant). Hence for this study morphological differentiation is due to phenotypic plasticity.

4.3 Material and Methods

4.3.1 Location of study

The experiment was conducted in bean bruchids laboratory in the Department of Crop Science and Horticulture of Sokoine University of Agriculture, Morogoro, Tanzania in 2020. Located at latitude 06’50’S, longitude 37^{o} 40'E and altitude of 526 m above sea level (TMA, 2011).

4.3.2 Experimental Materials used

The main material used in this study involved bean bruchids, susceptible bean varieties (Njano gololi) and resistant bean genotype (AO-1012-29-3-3A).

4.3.3 Methods

Bean bruchids samples *A. obtectus* and *Z. subfasciatus* were obtained from SUA field. Then brought at bruchid laboratory for inoculation. Samples of AO-1012-29-3-3A bean genotype were obtained African Seeds Health Centre Sokoine University of Agriculture and Njano gololi were collected from farmers in Mgeta village, Morogoro. A total of 20 bruchids sample (Parents) in each replicated for both *A. obtectus* and *Z. subfasciatus* were weighed and measured before inoculation. 20 adult bruchids for both *A. obtectus* and *Z. subfasciatus* were inoculated in the plastic containers containing 30 susceptible and resistant bean seeds separately. The trial was left undisturbed for 14 days to allow bruchids to lay eggs. Then all adults were removed in each plastic container leaving only

eggs for reproduction. The experiment was conducted in a completely Randomize Design with two source of variation which were susceptible and resistant bean genotypes, two treatments *A. obtectus* and *Z. subfasciatus* were used in this experiment. Observations of emergence of adult F_1 bruchids commenced on the 24^{th} day from inoculation. After emergence F_1 bruchids were taken from containers for observation and measurement. The insects were maintained at a temperature of $26^0C \pm 3^0C$ and ambient Relative Humidity throughout the experiment.

4.4 Data collection

The diameter and length of emerged F_1 were measured by using Vernier caliper and ruler respectively. Twenty bruchids sample from each trial were weighed by using digital weight scale.

4.4.1 Data analysis

These variables were analyzed in the analysis of variance (ANOVA) set at 5% significance level. The analysis done by using GenStat software. The mean separation test conducted by the use of Tukey's test. All means were compared at $p < 0.05$.

4.5 Results

4.5.1 Length of Parents and F_1 adults (*A. obtectus*).

There was significant difference at $p = 0.001$ (Appendix 4.1) in the mean length of emerging F_1 bruchids emerged from resistant and susceptible bean genotypes (Fig. 4.1). F_1 adults emerged from Njano gololi had almost the same length with parents. The finding showed that F_1 adults emerged from AO 1012-29-3-3A had short length compared to parents and F_1 from susceptible Njano gololi.

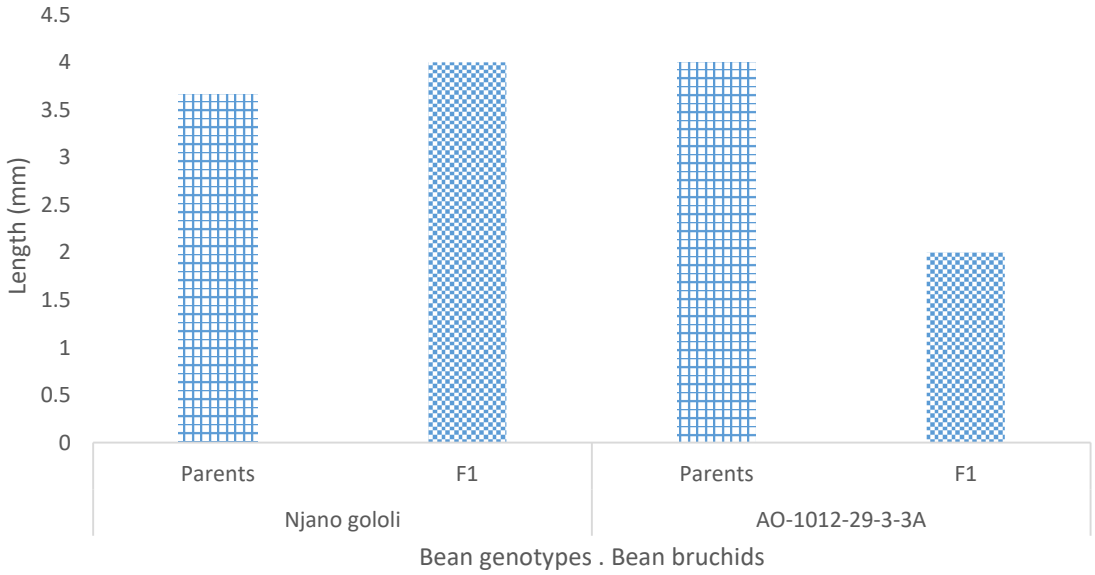

Figure 4.1: Mean number of Length of parents and emerged F_1 (*A. obtectus*) from susceptible and resistant bean genotypes.

4.5.1.2 Diameter of Parents and F_1 adults bruchids (*A. obtectus*).

There was significant difference at p = 0.001 (

Appendix 4.2) in tdiameters of F_1 adults between susceptible and resistant genotype (Fig 4.3).

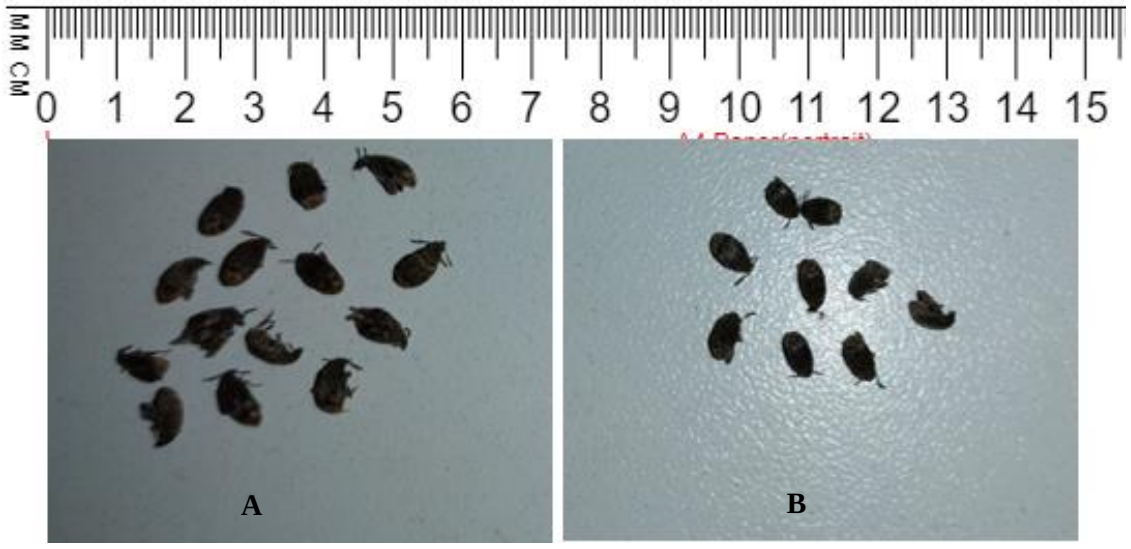

Figure 4.2: Picture A large sized F_1 *Acanthoscelides obtectus* emerged from susceptible genotype (Njano gololi). Picture B: small sized

***Acanthoscelides obtectus* emerged from resistant bean genotype (AO 10-12-29-3-3A).**

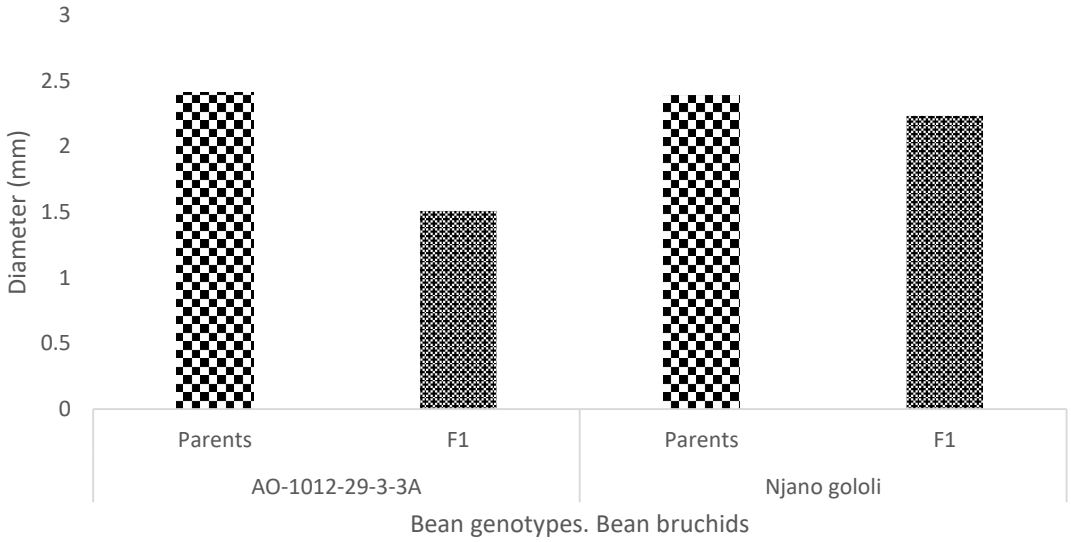

Figure 4.3: Mean number of Diameter of Parents and F_1 (*A. obtectus*) emerged from susceptible and resistant bean genotypes.

4.5.1.3 Weight of Parents and F_1 adult bruchids (*A. obtectus*).

Significant reduction in weight of F_1 adults emerged from resistant and susceptible bean genotype was observed at Prob. = 0.001 (Appendix 4.3). The results showed equivalent weight of F_1 adults emerged from Njano gololi and weight of parents (bean bruchids inoculated in resistant and susceptible bean genotypes separately) Fig. 4.4.

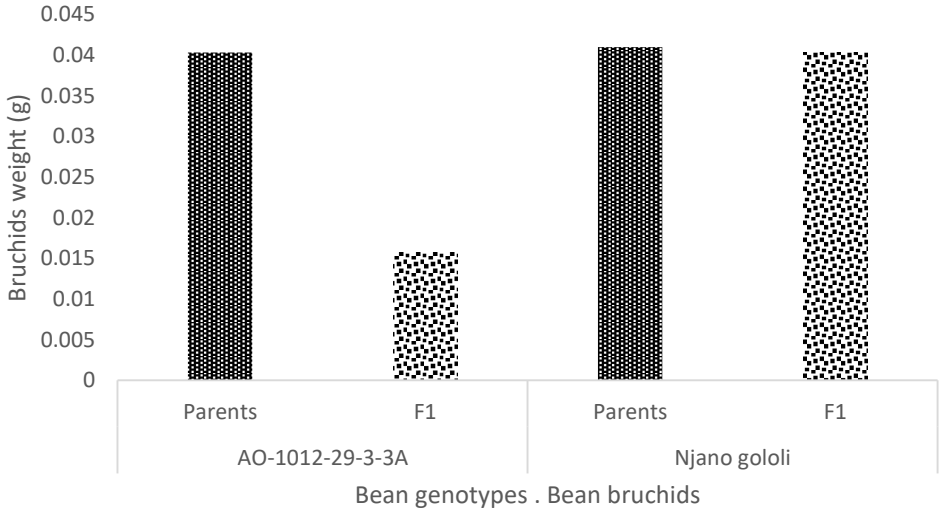

Figure 4.4: Mean weight of Parents and emerged F_1 *A. obtectus* from resistant and susceptible bean genotypes.

4.5.2 Length of Parents and F_1 adults (*Z. subfasciatus*)

There was significant difference at p = 0.001 (Appendix 4.1) in the mean length of emerging F_1 *Z. subfasciatus* from resistant and susceptible bean genotypes (Fig. 4.6).

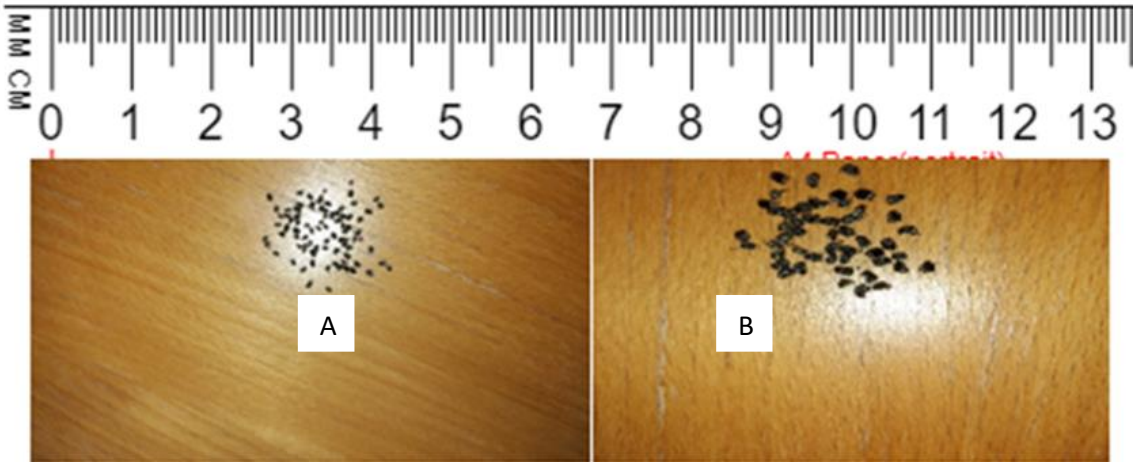

Figure 4. 5: Picture A: Small sized F_1 *Zabrotes subfasciatus* emerged from resistant genotype (AO 10-12-29-3-3A) Picture B: Large sized *Zabrotes subfasciatus* emerged from susceptible bean genotype (Njano gololi).

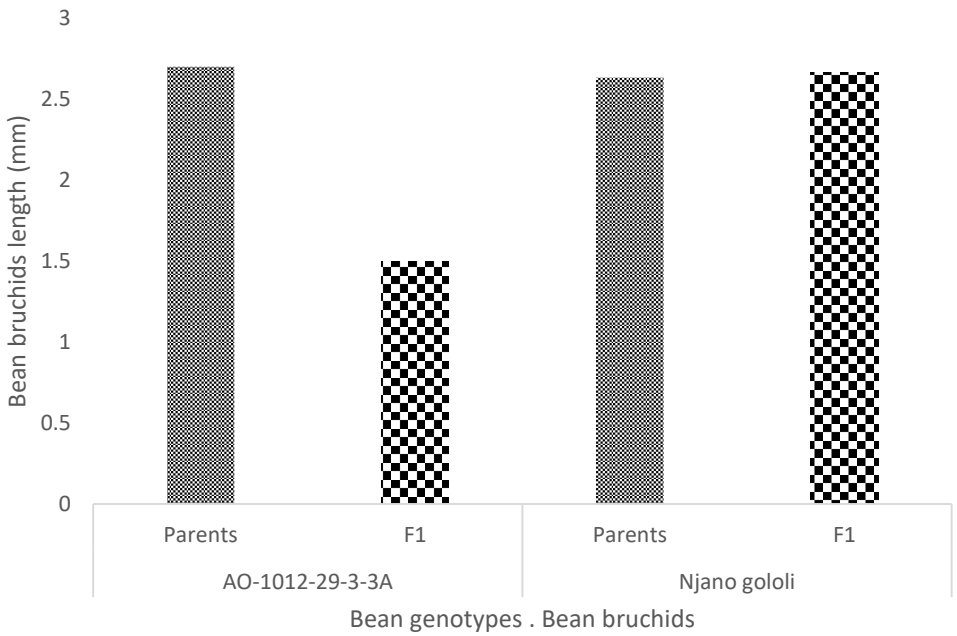

Figure 4.6: Length of Parents and F_1 (*Z. subfasciatus*) emerged from susceptible and resistant bean genotypes.

4.5.2.1 Diameter of Parents and F_1 (*Z. subfasciatus*)

F_1 bruchids emerged from Njano gololi had the same diameter with parents. Significant difference at p = 0.001 (Appendix 4.2) was observed in the mean number of diameters of F_1 adults emerged from susceptible and resistant genotype (Fig 4.7).

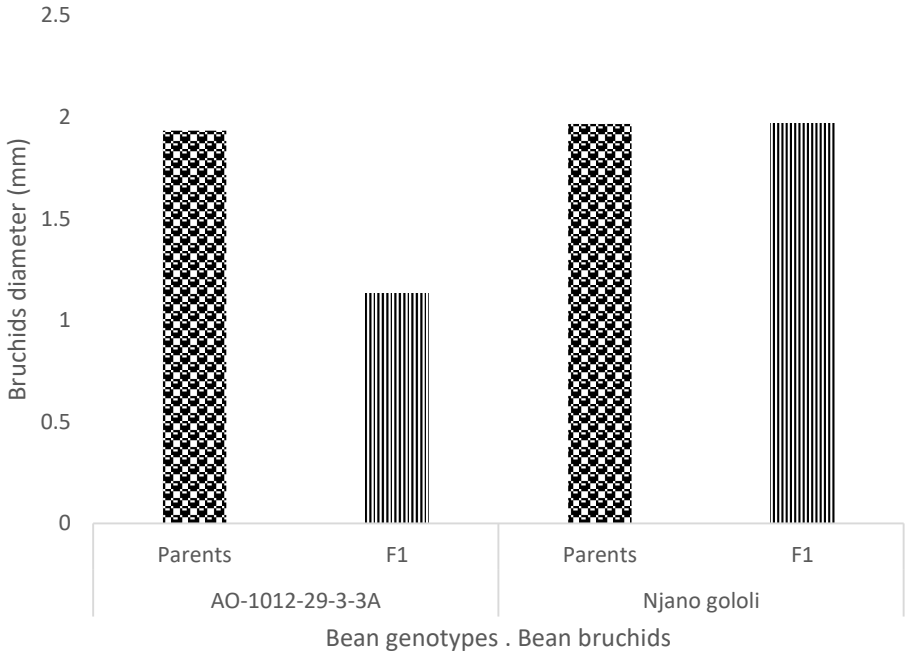

Figure 4.7: Mean number of Diameter of Parents and emerged F_1 (*Z. subfasciatus*) from susceptible and resistant bean genotypes.

4.5.2.2 Weight of F_1 *Z. subfasciatus* emerged from resistant and susceptible bean genotypes.

Significant reduction in weight of F_1 adults emerged from resistant and susceptible bean genotype was observed at Prob. =0.001 (Appendix 4.3). The results showed equivalent weight of F_1 adults emerged from Njano gololi and weight of parents (bean bruchids inoculated in resistant and susceptible bean genotypes separately) (Fig.4.8).

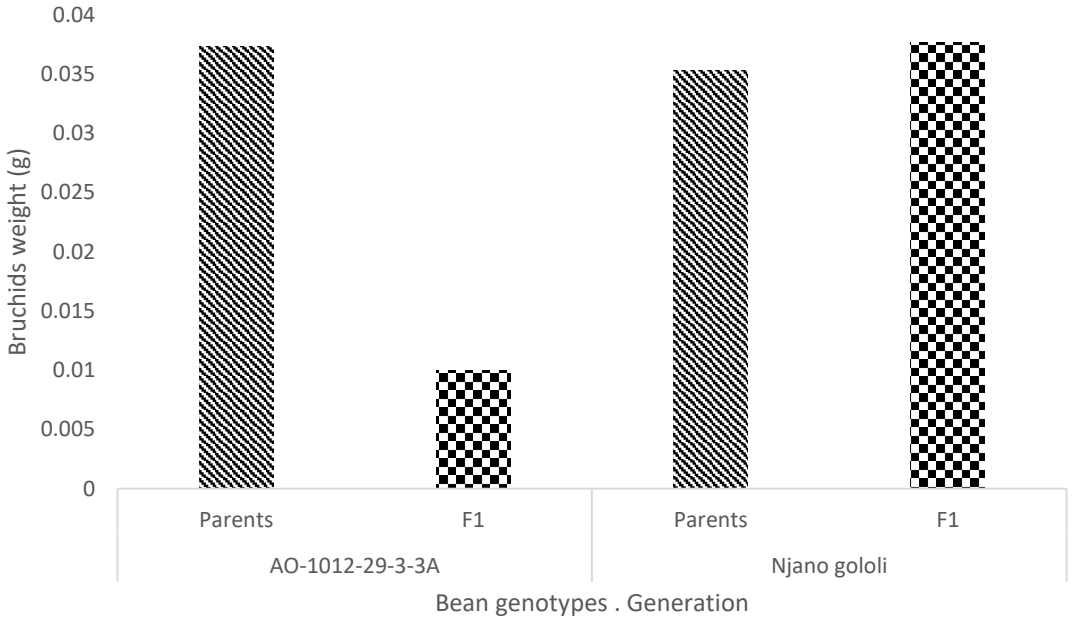

Figure 4.8: Mean weight of Parents and F_1 *Z. subfasciatus* emerged from resistant and susceptible bean genotypes.

4.6 Discussion

In tropical and subtropical countries, the common bean suffers important post-harvest losses caused mainly by the bruchid pests. Bruchids are the most serious pests attacking food legume seeds during storage. Extensive amounts of stored common bean, *Phaseolus vulgaris* (L.), are lost due to the damage caused by these two main storage pests. High levels of resistance against bean bruchids were found in a number of wild *P. vulgaris* populations of Mexican origins whereas cultivated genotypes exhibit no or very low level of resistance (Schoonhoven *et al*., 1983).

The findings from this study showed that resistant genotype had significant negative effects on adult bruchid weights and sizes. This have been reported by Appleby and Credland, (2004) who did a research on *Callosobruchus maculatus* and reported an interactive effects of resistant variety on size, performance and growth of bean bruchids.

The analyses of variance revealed that the mean fresh weight of F_1 adult bruchids emerged from resistant bean genotype (AO-1012-29-3-3A) was significantly reduced than those emerged from susceptible bean genotype (Njano gololi). The reduction of fresh weight in F_1 bruchids emerged from AO-1012-29-3-3A genotype was an important confirmation that the larvae of bean bruchids experienced an obstacle on feeding bean cotyledon this could be possibly contributed by the Arcelin protein contained in AO-1012-29-3-3A bean genotype. (Minney *et al*., 1990) reported poor digestibility of Arcelin protein by insect larval enzymes in common bean containing Arcelin. The emerged F_1 bruchids may have suffered physiological disabilities that contributed to reduction in weight, size, poor reproduction as well as delay of bruchids emergence. Poor reproduction was associated with fewer number of bruchids emerged from AO-1012-29-3-3A compared to Njano gololi.

Previous reports have shown host-based intraspecific morphological variation in sap-feeding insects (Kennedy, 1986; Moran, 1986; Heie, 1987; Carroll and Boyd, 1992). Confirmation for such morphological adaptations are provided by a number of studies such as, host-related differences in beak length (Carroll and Boyd, 1992), but also into host-plant induced shape variation of leafhopper (Gillham and Claridge, 1994). The differences in size of F_1 adults bruchids emerged from resistant genotypes may have little genetic basis and are probably host plant induced.

In addition, small size, weight as well as the lowest number of emerging adults F_1 observed in AO-1012-29-3-3A genotype was a significant factor for bruchid resistance. This was the same findings reported by (Kusolwa *et al.*, 2007) delayed F_1 adult emergence, slow the rate of emergence, reduced number of F_1 adults, reduction in size

and weight of adult bruchids are an important factor for bruchid resistance, conditioned by the presence of Arcelin proteins.

4.7 Recommendation and Conclusion

From results obtained, AO-1012-29-3-3A genotype presented reduced number of F_1 adults, reduction in size and weight of adult bruchids; these are important factors for bruchid resistance. In these findings the resistant (AO-1012-29-3-3A is likely to bring significant reduction in post-harvest losses towards bean farmers. Hence, further efforts may be of importance to ensure a constant availability of this resistant bean genotype to farmers in all bean-growing villages of Tanzania.

Since the study was only based on morphological study of bean bruchids emerged in resistant (AO-1012-29-3-3A) and susceptible (Njano gololi) cultivars. Hence, further studies have to be conducted to observe more genetic change of the F_1 emerged. Would they reproduce or the generation will fade away, would bruchid size continue to decrease or remain the same over an extended period of time.

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CHAPTER FIVE

5.0 GENETIC DIVERSITY OF COMMON BEAN BRUCHIDS (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) FROM DIFFERENT BEAN GROWING REGIONS OF TANZANIA.

5.1 Abstract

Bruchid beetles (Bruchidae, Coleoptera) are seed-eating insects, most species of which feed on legume. Common bean around the world are favorably attacked by the common bean weevil (*Acanthoscelides obtectus* (Say)) and Mexican bean weevil (*Zabrotes subfasciatus* (Boheman). These pests are present in almost all bean-producing localities in Tanzania. The purpose of this study was to identify the genetic diversity of bean bruchids weevil (*A. obtectus* and *Z. subfasciatus*) in bean producing regions of Tanzania using molecular taxonomy (*12S rRNA* and *COI* marker). The results obtained did not show genetic diversity of *A. obtectus* present in Tanzania, they showed 100% identity. For *Z. subfasciatus* showed 80.2% identity. The genetic diversity was observed between the two species *A. obtectus* and *Z. subfasciatus* was explained by difference in some sequence alignment. Better knowledge of bruchids diversity present in Tanzania will help breeders and farmers to propose effective control methods with impact on environmental changes and human health.

5.2 Introduction

Common bean *Phaseolus vulgaris* (L.) is an important legume crop. It serves as a food and cash crop for farmers and is a good source of protein, vitamins, minerals, and fiber (Broughton *et al*., 2003; Castro-Guerrero *et al.*, 2016). It is consumed as dry seeds and as bean sprouts. It is grown in tropical and sub-tropical regions mainly as part of cereal-based cropping systems. Common bean also plays an important role in many agronomic systems, its popular among farmers for its short life cycle, improve soil fertility mainly through incorporation of nitrogen from the atmosphere in the biological nitrogen fixation process, larger leaf area index (Percentage of soil surface covered by vegetation in cultivated fields) enabling soil conservation by reduced erosion, (Germaine and Henry, 2008).

Acanthoscelides obtectus and *Zabrotes subfasciatus* are the important pests of common bean and cause damage in the field and in storage (Hill, 1989; Paul, 2007). Bruchid infestation reduces the nutritional and market value of the grain and renders seeds unfit for human consumption, agricultural and commercial uses. These pests are controlled mainly by fumigation with highly toxic chemicals such as carbon disulfide, phosphine, and methyl bromide, or by dusting with several other insecticides, which leave residues on the grain, thus, threatening food safety s (Koona and Dom, 2005; Swella and Mushobozy, 2007).

Some plant-based extracts have been found useful in controlling bruchids, but are not fully successful due to their short-term activity, rapid degradability, and potentially negative effect on seed germination (Yusuf *et al*., 2011). Host plant resistance is a cost-effective and a safe alternative to control bruchids in common bean and is associated with morphological, biochemical, and molecular traits.

The use of resistant variety is of economic importance although ecotype variation in bruchids has rendered some common bean lines susceptible to bruchids that would have been resistant to the pest in other geographical area. Genetic variability of the pest population is one more challenge for the breeders. Development of ecotypes has led to the breakdown of resistance in common bean against bruchids (Fox *et al*., 2010). A cultivar resistant to one insect ecotype may be susceptible to another, and the development of a cultivar with resistance against multiple biotypes is a complicated process (Appleby and Credland, 2004).

Understanding phylogeographic patterns in the species is of particular agronomic concern, because knowledge of the innate range of a species may give new data on the climatic conditions in which the species originally evolved, and can also otherwise guide the search for agents of biological control of *A. obtectus* and *Z. subfasciatus* in accordance of environmental variation. In developing countries, farmers who produce *Phaseolus* beans experience significant losses because of the high reproductive rate of bean bruchids for their ability to reproduce in a broad range of ecological conditions (Labeyrie, 1981). This modern expansion of the geographical and host range of common bean bruchids thus threatens production of other crops, and there is an urgent need to control bruchid populations by other means than chemical method. This research aimed at determining genetic diversity of common bean bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) from different bean growing regions of Tanzania.

5.3 Materials and Methods

Specimens of *Acanthoscelides obtectus* were collected in December 2020 from farmer's sites in Songwe, Morogoro and Karatu and *Zabrotes subfasciatus* were collected from Kilimanjaro and Arusha in Tanzania. Then brought in African seed health laboratory at

SUA for inoculation. The parent samples from each location were inoculated in bean genotypes AO-1012-29-3-3A and Njano gololi in a plastic container separately. The trial left undisturbed for 14 days to allow bruchids to lay eggs. Then all parents removed in each plastic container were taken for DNA extraction. And the newly emerged adult F_1 bruchids from AO-1012-29-3-3A and Njano gololi were taken for DNA extraction. Only 15 parents and 15 F_1 bruchids from each location made a sample for DNA extraction.

5.3.1 DNA extraction

The total genomic DNA was extracted using Quick-DNA Tissue/Insect kit (from ZYMO research). 750µL of **bashing bead™ buffer** was added to bruchid sample then grinded after grinding the mixture was put onto Centrifuge at 13000rpm for 2min. Then 400µL supernatant was transferred to a **Zymo-Spin™ III-F** filter in a collection tube and centrifuge at 8000rpm for 1min. The cap was removed and 1200µL of genomic lysis buffer was added to the filtrate in the collection tube containing supernatant liquid and mixed well. After mixing 800µL were transferred to **Zymo-Spin™ IICR Column[1]** in a collection tube and centrifuge at 10000 for 1min. After centrifuge the flow was discarded from the collection tube and the step was repeated for the remained 800 µL. 200µL of **DNA pre wash buffer** were added to the **Zymo-Spin™ IICR Column** in a new collection tube and centrifuge at 10,000 × g for 1minute. After centrifuge 500µL of **g-DNA Wash Buffer** was added to the **Zymo-Spin™ IICR Column** and centrifuge at 10,000 × g for 1minute. Final step, the **Zymo-Spin™ IICR Column** was transferred to a clean 1.5 ml microcentrifuge tube and 100 µL of **DNA Elution Buffer** was added directly to the column matrix and centrifuge at 10,000 × g for 1minute to elute the DNA. The extracted DNA was stored at -20 °C until required for amplification.

5.3.2 DNA amplification

Polymerase chain reaction (PCR) amplifications were performed in a final volume of 25 µL which contained 1µL of extracted DNA, 12.5µL of 2x Taq-Master mix, 9.5 of Nuclease free water and 1µL of each forward and reverse primers. PCR condition were set corresponding to particular primers requirement in term of number of cycles and temperature. Sample for *A. obtectus* and *Z. subfasciatus* were amplified using *12Sbi* and *12Sai* for 12s *rRNA*; *C1-J-2183* and Modified *TL2-N-3014* for *COI* obtained from Inqaba Biotech with their specific PCR conditions as shown in Table 2 below.

Table 5. 1: Polymerase chain reaction description of the primer used (Simon *et al*., 1994)

Primer	Primer sequences	Primer base pair
12Sbi-F;*12Sai*-R	F-5´- AAGAGCGACGGGCGATGTGT-3´; R-5´- AAACTAGGATTAGATACCCTATTAT-3´	379
C1-J-2183-F;Modified *TL2-N-3014* f	F-5´- CAACATTTATTTTGATTTTTTGG -3´;R-5´- TCCATTGCACTAATCTGCCATATTA -3´	736

PCR reaction were performed for each primer pair on a Gene Amp® PCR System 9700 using the following cycling conditions: initial denaturation at 92°C for 1min 30 s; 30 cycles of 92°C for 30 s, annealing temperature for 45 s, 55°C for 1 min 30 s; and final elongation at 72°C for 10 min. Annealing temperatures were, 55°C for both *12s rRNA*, and *COI*.

5.3.3 DNA visualization of Amplicons

To visualize the amplicons, 8µl of the PCR product was separated by electrophoresis in 1% agarose gel (1g of agarose dissolved in 100ml of 1 x TAE) with subsequent staining by 3µl of ethidium bromide. Then electrophoresed at 100V for 50min until dye markers have migrated to an appropriate distance. The gel were photographed using a smart phone.

5.4 Preparation of sample and Mitochondrial DNA sequencing

The integrity of the PCR amplicons was visualized on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye. In order to identify nucleotide sequences from PCR products, single bands of PCR amplified DNA fragments generated by each of the 12*Sbi* forward primer and *12Sai* reverse primer for *12s rRNA* and *C1-J-2183* forward primer and *TL2-N-3014* reverse primer for COI were excised from the agarose gels and recovered into Agarose purification column using ExoSAP Protocol. The Fragments were sequenced using the Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, and BRD3-100/1000 according to manufacturer's instructions. The products were labelled and then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053). Purified PCR products were used for mitochondrial DNA sequencing at Inqaba bio technology in South Africa using an applied Biosystems ABI 3730XL Genetic Analyser with a 50cm array, using POP7. Gene specific nucleotide sequences were generated in separate reactions using the forward and reverse primers. Mitochondrial DNA sequences from parents and F_1 bean bruchids emerged from Njano gololi and AO-1012-29-3-3A bean lines were compared and subjected to a BLAST search for sequence difference or identity. The NCBI database and sequences were aligned by Clustal-W program to determine sequence identity.

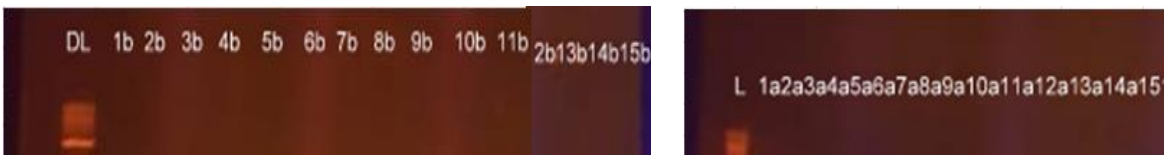
5.5 Sequence alignment and Phylogenetic analysis

The sequences were aligned using the CLUSTAL W multiple alignment procedure in Macvector version 18.0.2 (Thompson *et al.,* 1994) and a phylogenetic analysis was performed in MEGA v.7 (Kumar et al. 2016) using maximum likelihood (ML) model. For *12s RNA* and COI there were a total of 366 and 749 nucleotide positions respectively in the final dataset. Finally, the distance matrices for 12S rRNA and COI were constructed using a General Time Reversible model (Tavare, 1986). A Nearest-Neighbour-Interchange analysis was used to draw a tree with bootstrap analysis of 1000 replicates/ bootstraps (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances, computed using General Time Reversible model (Tavare, 1986). Are expressed as the number of uniform rates per site. Only bruchid sequences were included in the analysis in General Time Reversible method (Tavare, 1986). A sequence of the species KP682959 *Spermophagus* ssp and KP682946 *Spermophagus decellei* (plant host *Porana racemose*) were added as an outgroup species. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2016). For *Acanthoscelides obtectus* out group were HQ178007 *Bruchidius lutescens* and HQ178006 *Bruchidius kiliwaensis*

5.6 Results

5.6.1 PCR amplicons

Data presented from DNA analysis of bruchids species based on Mitochondria DNA have demonstrated similarity of bruchid ecotypes. There were no polymorphic bands of bean bruchids observed in the progenies emerged from resistant and susceptible bean genotype and their parents (Fig. 5.1).



B

Figure 5.1: PCR amplicons of the bean bruchid samples collected from different bean growing region. Picture A: PCR product amplifying the *COI* region. Picture B: PCR product amplifying the *12s rRNA*. Note: DL= DNA Ladder (1kb; N3232S), 1b to 15b and 1a to 15a=bean bruchids mtRNA samples

5.6.2 Mitochondrial DNA sequencing *12S rRNA*

Mitochondrial DNA sequences were obtained from PCR amplified products. The nucleotides were compared to published sequences in the data base for the genes originally used to design primers. DNA sequences for parents in Morogoro, Songwe and Karatu as well as F_1 bean bruchids emerged from Njano gololi of Morogoro and Karatu showed a 100% identity to the published KF157282 *Acanthoscelides obtectus* sequence from Brazil, MF925724 from Sweden and AY676676 from Switzerland. The published nucleotide sequence KX825864 from China, AY945998 from France and MN420800 from Republic of the Congo exhibited 99.7% nucleotide sequence similarities. Bean bruchids from Kilimanjaro and Arusha were *Zabrotes subfasciatus*. Parents bruchids from Arusha, F_1 emerged from Njano gololi and F_1 emerged from A0 29-3-3A of Arusha region exhibited 82.8%, 82.8% and 82.2% sequence identity respectively. Arusha parents, F_1 from Njano gololi and Kilimanjaro F_1 emerged from Njano gololi showed 82.8% similarity to the published AY945994 *Zabrotes subfasciatus* from France. Emerged F_1 in AO 10-12-29-3-3A from Arusha showed 82.2% identity to AY945993 Zabrotes *obliteratus* from France.

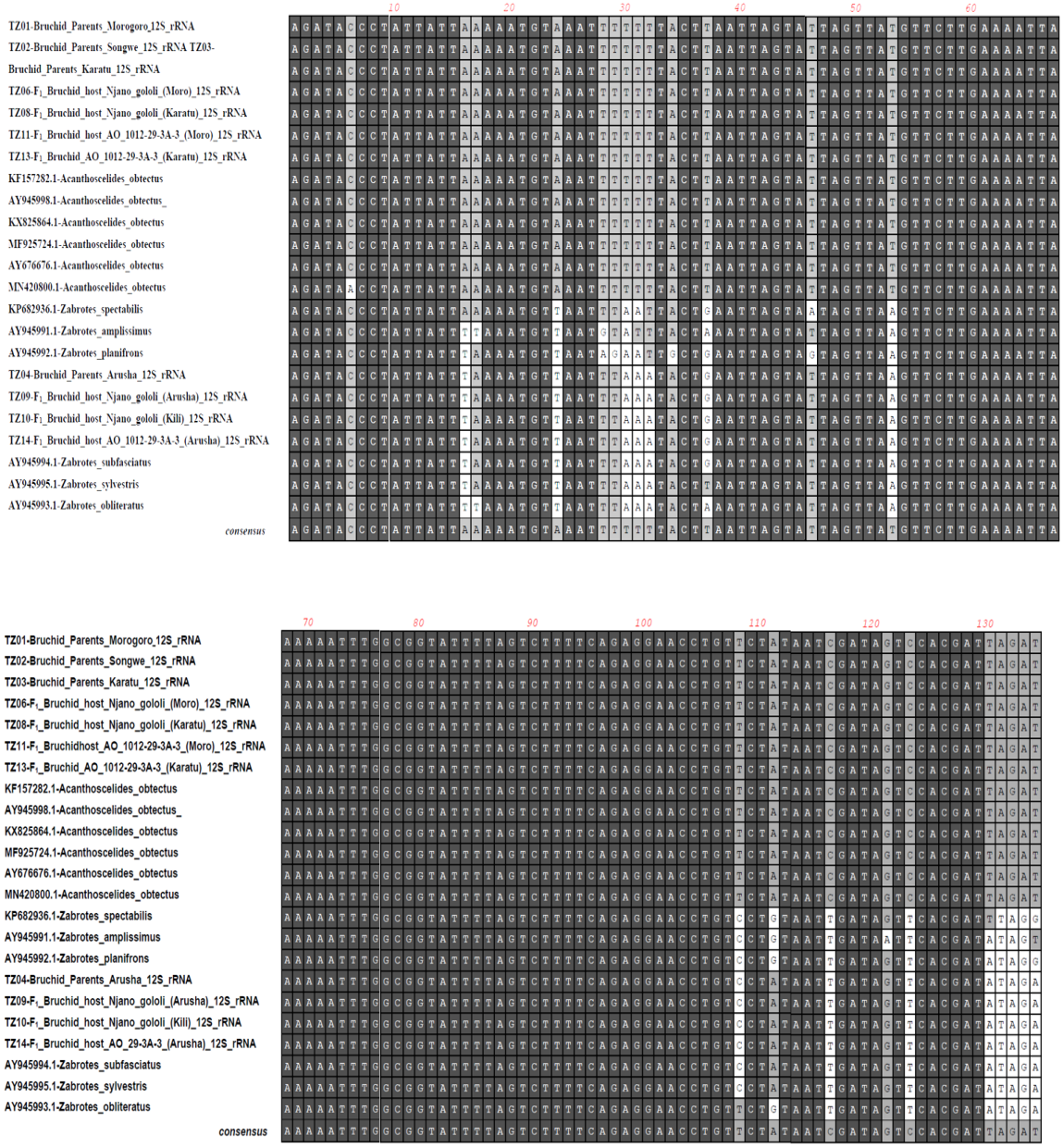


Figure 5.2: Sequence alignment for mitochondrial gene (12S rRNA) of bean bruchids and the out groups.

Multiple sequence alignment

23 Sequences Aligned Alignment Score = 10472862
Gaps Inserted = 14 Conserved Identities = 275

** Identity Scores (%) **

	TZ01-Br uchid_P arents_ Morogor o_12S_	TZ02-Br uchid_P arents_ Songwe_ 12S_rRN	TZ03-Br uchid_P arents_ Karatu_ 12S_rRN	TZ06-F1 _Bruchi d_host_ Njano_g ololi_(	TZ08-F1 _Bruchi d_host_ Njano_g ololi_(	TZ11-F1 _Bruchi d_AO_29 A-3_(Mo	TZ13-F1 _Bruchi 2.1-Aca -3A-3_(Karatu)	KF18728 nthosce lides_o btectus	AY94599 nthosce lides_o btectus	KX82886 nthosce lides_o btectus	MF92572 nthosce lides_o btectus	AY67667 nthosce lides_o btectus	MN42080 nthosce lides_o btectus	KP68293 rotes_s pectabi lis	AY94599 rotes_a mplissi mus	AY94599 rotes_p lanifro ns	TZ04-Br uchid_P arents_ Arusha_ 12S_rRN	TZ09-F1 _Bruchi d_host_ Njano_g ololi_(	TZ10-F1 _Bruchi d_host_ Njano_g ololi_(	TZ14-F1 _Bruchi d_host_ AO_29-3 A-3_(Ar atus is tus	AY94599 4.1-Zab rotes_s ubfasci	AY94599 5.1-Zab rotes_s ylvestr	AY94599 3.1-Zab rotes_o blitera
TZ01-Bruchid_Parents_Morogoro_12S_rRNA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
TZ02-Bruchid_Parents_Songwe_12S_rRNA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
TZ03-Bruchid_Parents_Karatu_12S_rRNA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
TZ06-F1_Bruchid_host_Njano_gololi_(Moro)_12S_rRNA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
TZ08-F1_Bruchid_host_Njano_gololi_(Karatu)_12S_rRNA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
TZ11-F1_Bruchid_host_AO-1012-29-3A-3_(Moro)_12S_rRNA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
TZ13-F1_Bruchid_AO_29-3A-3_(Karatu)_12S_rRNA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
NF157282.1-Acanthoscelides_obtectus	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
AY945998.1-Acanthoscelides_obtectus_	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	100.0	100.0	99.7	99.7	99.5	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
KX825864.1-Acanthoscelides_obtectus	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	100.0	100.0	99.7	99.7	99.5	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
MF925724.1-Acanthoscelides_obtectus	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
AY676676.1-Acanthoscelides_obtectus	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	100.0	100.0	99.7	85.8	86.1	84.4	82.8	82.8	82.8	82.2	82.8	82.5	82.2
MN420800.1-Acanthoscelides_obtectus	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.5	99.5	99.7	99.7	100.0	85.5	85.8	84.2	82.5	82.5	82.5	82.0	82.5	82.2	82.0
NP682936.1-Zabrotes_spectabilis	85.8	85.8	85.8	85.8	85.8	85.8	85.8	85.8	85.8	85.8	85.8	85.8	85.5	100.0	90.1	92.0	91.2	91.2	91.2	90.7	90.7	90.4	90.4
AY945991.1-Zabrotes_amplissimus	86.1	86.1	86.1	86.1	86.1	86.1	86.1	86.1	86.1	86.1	86.1	86.1	85.8	90.1	100.0	92.3	92.1	92.1	92.1	91.5	91.5	91.5	90.7
AY945992.1-Zabrotes_planifrons	84.4	84.4	84.4	84.4	84.4	84.4	84.4	84.4	84.4	84.4	84.4	84.4	84.2	92.0	92.3	100.0	91.0	91.0	91.0	90.1	90.4	90.1	89.9
TZ04-Bruchid_Parents_Arusha_12S_rRNA	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.5	91.2	92.1	91.0	100.0	100.0	99.7	98.9	99.5	99.2	92.3
TZ09-F1_Bruchid_host_Njano_gololi_(Arusha)_12S_rRNA	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.5	91.2	92.1	91.0	100.0	100.0	99.7	98.9	99.5	99.2	92.3
TZ10-F1_Bruchid_host_Njano_gololi_(Kili)_12S_rRNA	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.5	91.2	92.1	91.0	99.7	99.7	100.0	99.2	99.2	98.9	92.3
TZ14-F1_Bruchid_host_AO-1012-29-3A-3_(Arusha)_12S_rRNA	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.0	90.7	91.5	90.1	98.9	98.9	99.2	100.0	98.4	98.1	91.8
AY945994.1-Zabrotes_subfasciatus	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.8	82.5	90.7	91.5	90.4	99.5	99.5	99.2	98.4	100.0	98.6	91.8
AY945995.1-Zabrotes_sylvestris	82.5	82.5	82.5	82.5	82.5	82.5	82.5	82.5	82.5	82.5	82.5	82.5	82.2	90.4	91.5	90.1	99.2	99.2	98.9	98.1	98.6	100.0	92.3
AY945993.1-Zabrotes_obliteratus	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.2	82.0	90.4	90.7	89.9	92.3	92.3	92.3	91.8	91.8	92.3	100.0

** Similarity Scores (%) **

Similarity Scores(s) are shown below the diagonal (x) with Identity Scores(I) above

	a	b	c	d	e
a	x	i	i	i	i
b	s	x	i	i	i
c	s	s	x	i	i
d	s	s	s	x	i
e	s	s	s	s	x

Figure 5.3: **Identity matrix of Bean bruchids (*Acanthoscelides obtectus and Zabrotes subfasciatus*) from mitochondrial Gene *12s rRNA***

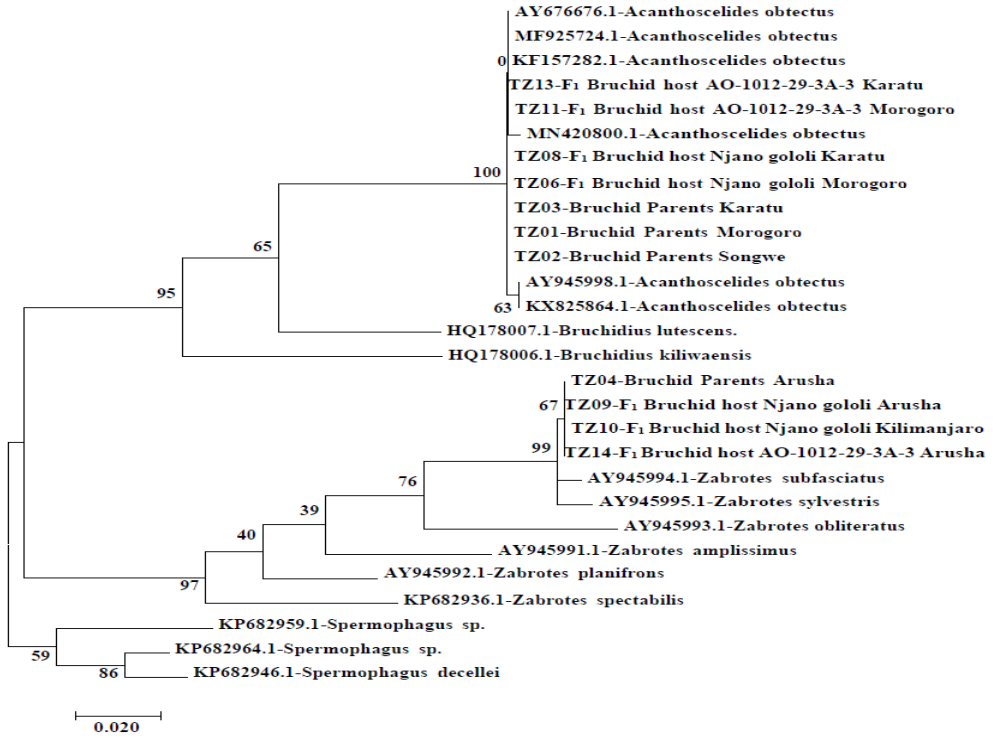
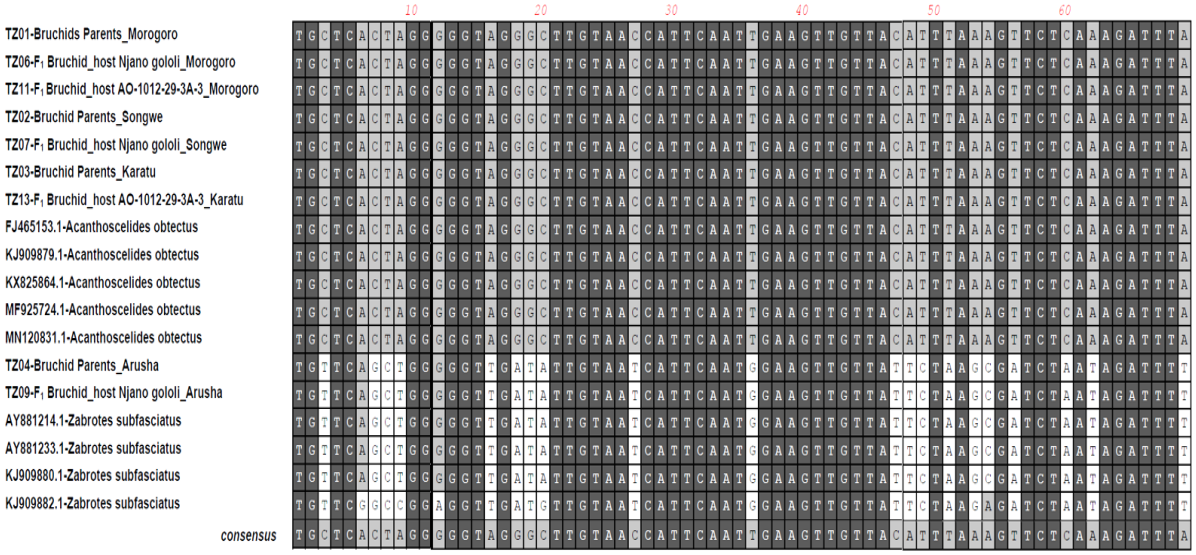


Figure 5.4: **Phylogenetic tree developed from PAUP program with bootstrap values calculated based on the nucleotide sequences of mitochondrial genes from bean bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*). HQ178007 *Bruchidius lutescens* and HQ178006 *Bruchidius kiliwaensis* were used as an out-group for *Acanthoscelides obtectus* and AY945992 *Zabrotes planifrons*, KP682936 *Zabrotes spectabilis*, AY945991 *Zabrotes amplissimus*, KP682959 *Spermophagus sp*, KP682964 *Spermophagus sp* and KP682946 *Spermophagus decellei* were used as an out-group for *Zabrotes subfasciatus*. Sequence genes for out-group were taken from NCBI database.**

5.6.3 Mitochondrial DNA sequencing *COI*

Mitochondrial DNA sequences were obtained from PCR amplified products. The nucleotides were compared to published sequences in the data base for the genes originally used to design primers. DNA sequences for parents bruchids in Morogoro, F_1 bean bruchids emerged from Njano gololi in Morogoro region and F_1 adults emerged from AO 29−3A−3 IN Morogoro, Songwe parents, Songwe F_1 adults emerged from Njano gololi, Karatu parents and Karatu F_1 adults bruchids emerged from A0 29 3A 3 showed 100% nucleotide sequence similarities to the published KJ909879 *Acanthoscelides obtectus* sequence from China and KX825864 *Acanthoscelides obtectus* sequence from China. Also there was 99.7% and 99.5% similarity to the published MF925724 from Sweden and MN120831 from India respectively.

For Zabrotes subfasciatus species in Arusha region parents and F_1 adults emerged from emerged from Njano gololi showed 80.2% identity to the published KJ909880 *Zabrotes subfasciatus* from China.



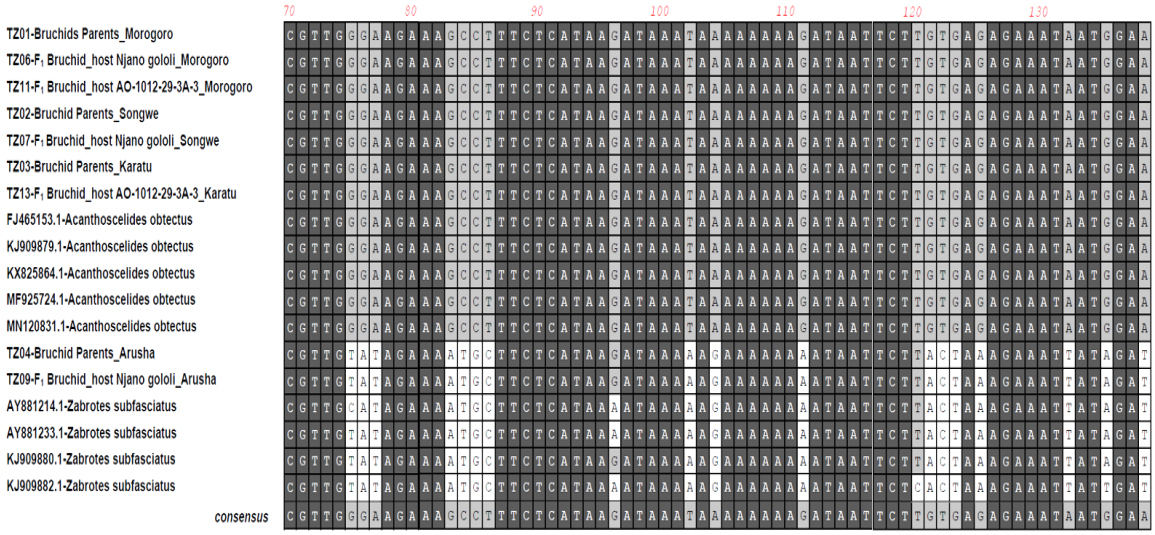


Figure 5.5: Sequence alignment for mitochondrial gene (*COI*) of bean bruchids and the out groups.

```
ClustalW multiple sequence alignment

18 Sequences Aligned          Processing time: 3.6 seconds
Gaps Inserted = 0             Conserved Identities = 570
score = 6902704

Pairwise Alignment Mode: Slow
Pairwise Alignment Parameters:
    Open Gap Penalty = 15.0 E x t e n d  Gap Penalty = 6.7

Multiple Alignment Parameters:
    Open Gap Penalty = 15.0  Extend Gap Penalty = 6.7
    Delay Divergent = 40%     Transitions: Weighted
```

** Identity Scores (%) **

	TZ01-Bruchids Parents_Morogoro	TZ06-F1 Bruchid_host Njano gololi_M	TZ11-F1 Bruchid_host AO-1012-29-3A-3_Mor	TZ02-Bruchid Parents_Songwe	TZ07-F1 Bruchid_host Njano gololi_S	TZ03-Bruchid Parents_Karatu	TZ13-F1 Bruchid_host AO-1012-29-3A-3_Kar	FJ46515 3.1-Acanthoscelides obtectus	KJ90987 9.1-Acanthoscelides obtectus	KX82586 4.1-Acanthoscelides obtectus	MF92572 4.1-Acanthoscelides obtectus	MN12083 1.1-Acanthoscelides obtectus	TZ04-Bruchid Parents_Arusha	TZ09-F1 Bruchid_host Njano gololi_A	AY88121 4.1-Zabrotes subfasciatus	AY88123 3.1-Zabrotes subfasciatus	KJ90988 0.1-Zabrotes subfasciatus	KJ90988 2.1-Zabrotes subfasciatus
TZ01-Bruchids Parents_Morogoro	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	99.7	99.5	80.2	80.2	80.1	80.0	80.2	78.8
TZ06-F$_1$ Bruchid_host Njano gololi_Morogoro	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	99.7	99.5	80.2	80.2	80.1	80.0	80.2	78.8
TZ11-F$_1$ Bruchid_host AO-1012-29-3A-3_Morogoro	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	99.7	99.5	80.2	80.2	80.1	80.0	80.2	78.8
TZ02-Bruchid Parents_Songwe	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	99.7	99.5	80.2	80.2	80.1	80.0	80.2	78.8
TZ07-F$_1$ Bruchid_host Njano gololi_Songwe	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	99.7	99.5	80.2	80.2	80.1	80.0	80.2	78.8
TZ03-Bruchid Parents_Karatu	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	99.7	99.5	80.2	80.2	80.1	80.0	80.2	78.8
TZ13-F$_1$ Bruchid_host AO-1012-29-3A-3_Karatu	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	99.7	99.5	80.2	80.2	80.1	80.0	80.2	78.8
FJ465153.1-Acanthoscelides obtectus	99.5	99.5	99.5	99.5	99.5	99.5	99.5	100.0	99.5	99.5	99.7	100.0	80.6	80.6	80.5	80.4	80.6	79.2
KJ909879.1-Acanthoscelides obtectus	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	99.7	99.5	80.2	80.2	80.1	80.0	80.2	78.8
KX825864.1-Acanthoscelides obtectus	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	99.7	99.5	80.2	80.2	80.1	80.0	80.2	78.8
MF925724.1-Acanthoscelides obtectus	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	100.0	99.7	80.5	80.5	80.4	80.2	80.5	79.0
MN120831.1-Acanthoscelides obtectus	99.5	99.5	99.5	99.5	99.5	99.5	99.5	100.0	99.5	99.5	99.7	100.0	80.6	80.6	80.5	80.4	80.6	79.2
TZ04-Bruchid Parents_Arusha	80.2	80.2	80.2	80.2	80.2	80.2	80.2	80.6	80.2	80.2	80.5	80.6	100.0	100.0	99.2	99.5	100.0	91.6
TZ09-F$_1$ Bruchid_host Njano gololi_Arusha	80.2	80.2	80.2	80.2	80.2	80.2	80.2	80.6	80.2	80.2	80.5	80.6	100.0	100.0	99.2	99.5	100.0	91.6
AY881214.1-Zabrotes subfasciatus	80.1	80.1	80.1	80.1	80.1	80.1	80.1	80.5	80.1	80.1	80.4	80.5	99.2	99.2	100.0	99.7	99.2	91.7
AY881233.1-Zabrotes subfasciatus	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.4	80.0	80.0	80.2	80.4	99.5	99.5	99.7	100.0	99.5	92.0
KJ909880.1-Zabrotes subfasciatus	80.2	80.2	80.2	80.2	80.2	80.2	80.2	80.6	80.2	80.2	80.5	80.6	100.0	100.0	99.2	99.5	100.0	91.6
KJ909882.1-Zabrotes subfasciatus	78.8	78.8	78.8	78.8	78.8	78.8	78.8	79.2	78.8	78.8	79.0	79.2	91.6	91.6	91.7	92.0	91.6	100.0

** Similarity Scores (%) **

```
**Similarity Scores(s) are shown below the diagonal (x) with Identity Scores(I) above**
  a b c d e
a x i i i i
b s x i i i
c s s x i i
d s s s x i
e s s s s x
```

Figure 5.6: Identity matrix of Bean bruchids (*Acanthoscelides obtectus and Zabrotes subfasciatus*) from mitochondrial gene *COI*

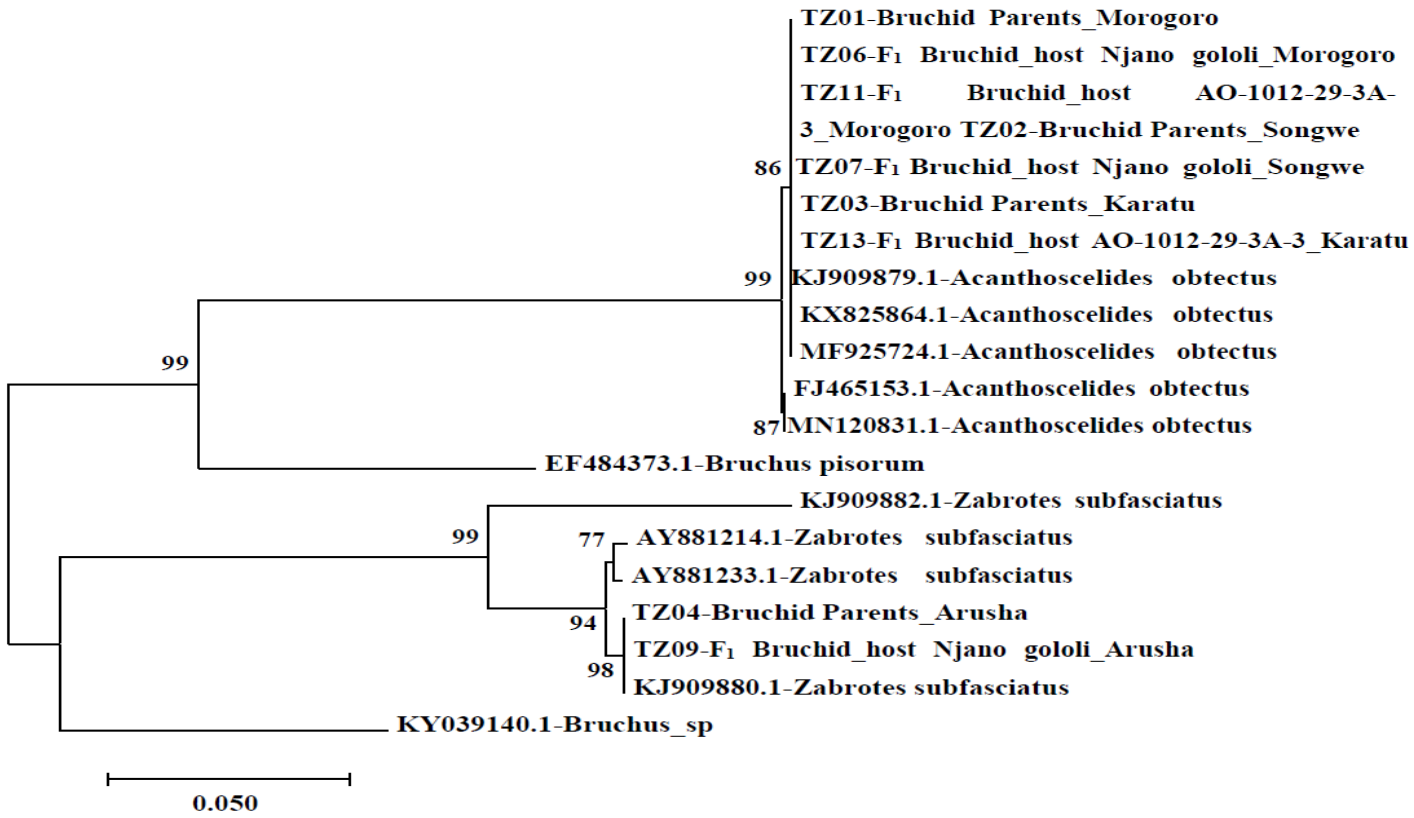


Figure 5.7: **Phylogenetic tree developed from PAUP program with bootstrap values calculated based on the nucleotide sequences of mitochondrial genes from bean bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*). EF484373 *Bruchus pisorum* was used as an out-group for *Acanthoscelides obtectus* and KY039140 *Bruchus* sp was used as an out-group for *Zabrotes subfasciatus*. Sequence genes for out-group were taken from NCBI database.**

5.7 Discussion

Bean bruchids are destructive storage pests that have a strong adaptability to many environmental conditions. In Tanzania bean bruchids are found in all bean growing regions. If remain uncontrolled populations of *A. obtectus* and *Z. subfasciatus* can grow exponentially and causing significant losses of bean seeds (Southgate, 1979). Genetic diversity analysis is useful for understanding genetic structure, differentiation and relationship among bean bruchids populations which make sense when developing pest management approaches as well as enacting resistance-screening and resistance breeding strategies.

The findings from this study showed that there were no intra specific genetic variation observed in *A. obtectus* species despite of geographical location and feeding effect of resistant bean genotype. The findings are in agreement with studies of Ong'amo (2012) who did a study on genetic diversity and population structure of *Busseola segeta* and confirmed the *B. segeta* moths did not exhibit genetic variation in terms of host use. *B. segeta* presence in a wide range of hosts in different fields without genetic variation strongly suggests the existence of host use plasticity. Some insects have ability to overcome plant defense without undergoing genetic variation.

Similarities of nucleotide sequence of *Acanthoscelides obtectus* in geographical location of Tanzania may be due to human trade of host bean. Since bean weevils move with bean seeds the sequence identity of *Acanthoscelides obtectus* may be due to transfer and exchange of common beans from one region to another for marketing within the country. This is in agreement with (Duan *et al.*, 2017) who reported that the absence or very low

genetic variation between bean weevils is apt to communicate and diffuse along with bean seed by means of egg, larva, pupa or adult which likely result in transfer and expansion of this pest between regions and identical host selection pressures in both population.

A very low genetic variation of 0.6% observed in *Zabrotes subfasciatus* emerged from resistant bean genotypes may be due to feeding effects of AO 10-12-29-3-3A bean genotypes to bean bruchids. This was the same as Gaete- Eastman *et al.*, 2004 who did a study on phenotypical and genetic variation of *Neuquenaphis edwardsi* and *Neuquenaphis staryi* and reported a slightly intraspecific genetic variation of *N. edwardsi* species could be the outcome of environmental factors and/or host features affecting aphid morphology.

In this study inter specific genetic diversity was observed between *Acanthoscelides obtectus* and *Zabrotes subfasciatus*. These two species were observed to have different nucleotide sequencing. In phylogenetic tree clustering plot based on genetic distance between population of *Acanthoscelides obtectus* from different regions were clustered into one genetic group and the population of *Zabrotes subfasciatus* into another group. This suggesting that there is existence of distinct genetic differentiation among two bruchid species.

5.8 Conclusion and Recommendation

The results did not show within/intra species genetic diversity of bean bruchids and the F_1 progeny emerged due to feeding on resistant or susceptible bean genotypes. This concludes that environment has nothing to do with genetic change of an insects perhaps for a very long period of time since mutation is a gradually change. Hence virulence of an

insect on attacking beans can change according to environment conditions without undergoing genetic variations.

Variation between *A. obtectus* and *Z. subfasciatus* have been validated in this experiment confirming their genetic variability and virulence of each to bean during storage. Of more importance is that bruchid resistance should essentially focus on addressing resistance to two bruchid species and not resistance to both *A. obtectus* and *Z. subfasciatus.*

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APPENDICES

Appendix 5.1: Analysis of variance of total number of bruchids emerged

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Regions	4	27510.7	6877.7	15.21	<.001
Bean genotypes	1	142003.2	142003.2	314.07	<.001
Regions x Bean genotypes	4	9486.1	2371.5	5.25	0.006
Residual	18	8138.4	452.1		
Total	29	189234.7			

Appendix 5.2: Analysis of variance days for first F_1 adults emergence (DAE)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Bean genotypes	1	4177.2	4177.2	127.87	<.001
Regions	4	746.47	186.62	5.71	0.003
Genotypes x Regions	4	278.47	69.62	2.13	0.115
Residual	20	653.33	32.67		
Total	29	5855.47			

Appendix 5.3: Analysis of variance Susceptibility index (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	12.885	6.442	1.69	
Regions	4	176.113	44.028	11.55	<.001
Bean genotypes	1	366.575	366.575	96.14	<.001
Regions x Bean genotypes	4	52.870	13.217	3.47	0.029
Residual	18	68.635	3.813		
Total	29	677.07			

Appendix 5.4: Analysis of variance Percentage weight loss%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	73.978	36.989	5.60	
Regions	4	2251.393	562.848	85.27	<.001
Bean genotypes	1	3716.591	3716.591	563.04	<.001
Regions x Bean genotypes	4	742.969	185.742	28.14	<.001
Residual	18	118.817	6.601		
Total	29	6903.747			

Appendix 5.5: Analysis of variance of seed with 5 and above holes (Severity)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	11.267	5.633	0.68	
Regions	4	492.000	123.000	14.89	<.001
Bean genotypes	1	1484.033	1484.033	179.60	<.001
Regions x Bean genotypes	4	392.133	98.033	11.86	<.001
Residual	18	148.733	8.263		
Total	29	2528.167			

Appendix 5.6: Analysis of variance of bruchids Length (mm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Bean genotypes	1	2.87042	2.87042	57.48	<.001
Generation	3	9.61458	3.20486	64.17	<.001
Bean genotypes. Generation	3	5.34458	1.78153	35.67	<.001
Residual	14	0.69917	0.04994		
Total	23	18.58958			

Appendix 5.7: Analysis of variance of bruchids Diameter (mm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Bean genotypes	1	0.924337	0.924337	94.53	<.001
Generation	3	2.210613	0.736871	75.36	<.001
Bean genotypes. Generation	3	0.904412	0.301471	30.83	<.001
Residual	14	0.136900	0.009779		
Total	23	4.182296			

Appendix 5.8: Analysis of variance of bruchids Weight (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Bean genotypes	1	0.00078204	0.00078204	29.22	<.001
Generation	3	0.00122512	0.00040837	15.26	<.001
Bean genotypes. Generation	3	0.00093346	0.00031115	11.62	<.001
Residual	14	0.00037475	0.00002677		
Total	23	0.00331596			