GENETIC RESISTANCE TO ADZUKI BEAN BRUCHID (*Callosobruchus chinensis*) IN SOYBEAN

BY

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DECLARATION

The work presented in this thesis is my own research and to my knowledge it has not been presented for the award of degree or diploma in any other University.

Signed..... Date....12 Aug 2019

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DEDICATION

This work is dedicated to my husband, Dr. Mavuto Denis Tembo and our children, Uchizi na Mtende Tembo, Ungweru Walumbike-Letitia Tembo and Urunji Denise Tembo for their unceasing prayers, love, support and patience during the entire period of my studies.

To my father Maxwell Chibwatiko Msiska for always believing in me and protecting me.

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Msiska U.M., T.L. Odong, M. Hailay, B. Miesho, S. Kyamanywa, P.R. Rubaihayo And P. Tukamuhabwa. 2018. Resistance of Uganda Soybean Germplasm to Adzuki Bean Bruchid. *African Crop Science Journal* 26 (3): 399-415. http://dx.doi.org/10.4314/acsj.v26i3.2

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ABSTRACT

Soybean [*Glycine max* (L.) Merrill], an annual legume that belongs to the family Fabaceae is grown in every continent for its high protein (40%) and oil (20%) content. Soybean was introduced to Uganda in the 1900s. However, for the first time in Uganda, soybean is threatened by a storage pest *Callosobruchus chinensis*. *C. chinensis* causes tremendous losses because of its high fertility, ability to re-infest, short generation times and irreversible damage which is direct on the grain. *C. chinensis* causes overall seed weight loss, loss of seed viability and altered nutritional quality. Utilization of resistant varieties is the most effective, economical and environmentally sustainable method but it is obstructed by lack of sources of resistance and information on genetics of inheritance.

Consequently, studies were undertaken to establish sources, basis and inheritance of resistance to *C. chinensis* in soybean. The specific objectives of the study were to:- (i) Identify sources of resistance to *C. chinensis* in the available germplasm in Uganda, (ii) determine the biochemicals associated with *C. chinensis* resistance in soybean and (iii) determine the mode of inheritance of resistance to *C. chinensis* in soybean. The studies were carried out at Makerere University Agricultural Research Institute–Kabanyolo (MUARIK) and National Crops Resources Research Institute (NaCCRI)- Namulonge between 2015 and 2018. Four hundred and ninety eight genotypes from Uganda, Zimbabwe, USA and Taiwan were infested with 1-3 day old unsexed bruchids under a no choice test in a randomized complete block design with three replicates. Genotypes showed variations in response to *C. chinensis* indicating differences in resistance levels implying that they contain different amounts of intrinsic and extrinsic factors responsible for resistance. The highest resistance was observed in genotypes AVRDC G8527 and PI G89 while AVRDC G 2043 was the most susceptible. Therefore, AVRDC G8527 and PI G89 were the identified sources of resistance.

Based on the results of the no choice test, eight genotypes with varying resistance levels were assessed for biochemical concentrations. The biochemical concentrations of soybean indicated that high tannins, total antioxidants, peroxidase activity and low flavonoids were associated with resistance to *C. chinensis.* The study established that secondary and not primary metabolites were associated with resistance to *C. chinensis* in soybean. The study also identified that in some genotypes, *C. chinensis* was probably detoxifying the

biochemicals associated with resistance possibly through sequestering, increased secretions or and altered biochemical composition.

To comprehend the mode of gene action and inheritance patterns of resistance of *C. chinensis*, nine soybean genotypes were crossed under full diallel mating design. Subsequently, genetic analysis was conducted on the F_2 progenies and parents to generate the general and specific combining abilities, maternal effects and heritability values. Significant differences in the GCAs and SCA amongst genotypes indicated the presence of both additive and non-additive gene action. The study identified SREB-15C, S-Line 9.2 and S-Line 13.2A as useful parents in breeding for resistance to *C. chinensis* based on general combining abilities. The presence of maternal effects signified the importance of direction of the cross during hybridization. The Baker's ratio of seed weight loss was unity indicating greater predictability of progeny performance based on the GCA alone and better transmission of trait to the progenies. Crosses with significant negative SCA effects such as SREB-15C x S-Line 13.2A, SREB-15C x Maksoy 3N would be very beneficial in the development of *C. chinensis* resistant varieties and therefore were recommended as start up material for the bruchids breeding programme**.**

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

1.1.1 Soybean origin and distribution

Soybean [*Glycine max* (L.) Merrill] is an annual legume that belongs to the family Fabaceae and belongs to the genus *Glycine* Willd (Singh, 2009). Soybean grows in tropical, subtropical, and temperate climates (Maphosa *et al.,* 2012). It is a strictly self-pollinating legume with 2n = 40 chromosomes (Tefera, 2015). Soybean was domesticated in the 11th century BC around northeast of China. It suffices to note that soybean is classified as an oil seed rather than a pulse by the UN Food and Agriculture Organization (AMIS, 2018).

The genus *Glycine* includes two sub-genera: *glycine* and *soja*. Subgenus *soja* contains the wild soybean (*Glycine soja*) and the cultivated soybean (*Glycine max*). Evidence from morphological, cytogenetic and molecular analyses has indicated that soybean was domesticated from wild ancestor (*G. soja*) in China (Acquaah, 2007; Guo *et al.,* 2010). Consequently, soybean is indigenous to Manchuria, China and is considered one of the five oldest cultivated crops utilized by the Chinese as a source of food before 2500 BC (Gibson *et al.,* 2005). However, it was only discovered by the western world as a source of oil and protein in the 19th century. In the past 33 years, world production of soybeans increased to more than 167 million metric tonnes. Of this, 34% is produced in the USA, 33% in Brazil, 16% in Argentina and 4% in China (Cook, 2018.) More than 216 million tonnes of soybeans were produced worldwide in 2007, of which 1.5 million were in Africa. Africa imports nearly as much soybean as it produces. Africa exports about 20,000 tonnes annually (Tefera, 2015).

Soybean was introduced to Africa in the 19th century by Chinese traders along the east coast of Africa (Mohamedkheir *et al.,* 2018). Nigeria is the largest producer of soybean in sub-Saharan Africa, followed by South Africa (Tefera, 2015). Commercial soybean production on large farms takes place in Zambia, Zimbabwe and South Africa (Tefera, 2015). However, it is mostly cultivated by small-scale farmers in other parts of Africa where it is planted as an intercrop with sorghum, maize, or cassava (Tefera, 2015).

Soybean is said to have been introduced in Uganda from both the United States and South Africa in 1938 (Shurtleff and Aoyagi, 2007) however Rubaihayo and Leakey (1970),

Bashaasha (1992) and Tukamuhabwa and Maphosa (2010) report date of between 1908 and 1913.

Soybean in Uganda is grown throughout the country with the Northern region being the highest (66.6%) producer followed by the Eastern region (24.6%) and the Central region being the least 0.9% (UBOS, 2010). UBOS (2010) further reports that at district level in Uganda the largest soybean producers are Oyam (7841 tons); Apac (3100 tons); Tororo (2200 tons) and Lira (2000 tons). Tukamuhabwa *et al.* (2012) reported that there was a steady increase in soybean production since 1990; but notable increase was reported from 2004 following the release of new varieties.

1.1.2 Importance of soybean

Soybean is among the major industrial and food crops grown in every continent (Dugje *et al.*, 2009). Two traits namely protein (40%) and oil (20%), all derived from processed seed make it a hugely popular crop in the world. In fact soybean produces the highest amount of protein per unit area among crops Tukamuhabwa and Maphosa (2010).

Soybean is eaten as roasted whole beans and the flour used as ingredients of confectionery products and snacks. Immature whole green soybeans are also consumed as a vegetable. Soybean is also eaten as germinated sprouts (Goldsmith, 2008). Products such as soymilk, soybean curd (tofu), soybean paste, soysauce are produced from soybean for human consumption (Agarwal *et al.*, 2013). Roasted soybean seeds are used as a coffee substitute (Shurtleff, 2012). Seeds yield edible, semi-drying oil, used as salad oil and for manufacturing margarine and shortening. Whole soybeans can be processed into full-fat flour with about 20% oil, mechanically pressed meal provides low-fat flour with 5% to 6% oil, and solvent-extracted meal gives defatted flour with about 1% oil. The flour is used in bakery and other food products and as additives and extenders for cereal flour and meat products, as well as health foods (Gibson *et al*., 2005).

Soybean oil is used industrially in the manufacturing of paints, oil cloth, printing inks, soap, insecticides and disinfectants (Christou, 1992). Lecithin phospholipids are obtained as a by-product of soybean from the oil industry and are used as a wetting and stabilizing agent in the food, cosmetic, pharmaceutical, leather, paint, plastic, soap and detergent industries (Gibson

et al., 2005). Soybean meal and protein are used in the manufacturing of synthetic fibre, adhesives, textile sizing, water proofing, fire-fighting foam and for many other purposes. The straw can be used to make paper stiffer than that made from wheat straw.

Additionally, owing to the rich protein soybean is used as feed for livestock (Willis, 2003). The vegetative portions of plants are used for silage, hay, pasture or fodder, or could be ploughed back into the soil as a green manure (Gibson *et al.*, 2005).

Soybean improves soil fertility by fixing atmospheric nitrogen (Njeru *et al.*, 2013). This is a major benefit in African farming systems, where soils have become exhausted by the need to produce more food for increasing populations, and where fertilizers are hardly available and are expensive for farmers (Tukamuhabwa and Maphosa, 2010).
In Africa, Uganda is the second largest consumer of soybean following Nigeria (IITA, 2015).

1.1.3 Constraints to soybean production and storage

Soybean is grown on an estimated 6% of the world's arable land. In 1960 soybean production was 17 million metric tons (MMT) (IITA, 2015) and 346 MMT in 2018 (Cook, 2018). Recent increases in production coincide with increases in demand for soybean meal and oil. Along with increased production comes increased importance of abiotic and biotic constraints that threaten soybean production by directly reducing seed yields and/or seed quality (Hellal and Abdelhamid, 2013). The most limiting abiotic constraints for soybean include phosphorus, magnesium and sulphur deficiencies (Keino *et al.*, 2015). In fact, phosphorus levels of less than 30 kg ha^{-1} will lower soybean yield by 29-45% (Nandini, 2012). The magnitude of soybean yield losses due to nutrient deficiency vary among nutrients (Balboa *et al.*, 2018). For example, deficiencies of N, Fe, B and S may cause soybean yield losses of up to 10% (Hellal and Abdelhamid, 2013), 22-90% (Zahoor *et al.*, 2013), 100% (Zahoor *et al.*, 2013) and 16-30% (Anil, 2014) respectively, depending on soil fertility (Hellal and Abdelhamid, 2013), climate and plant factors (Xiang *et al.*, 2012). However, toxicities are also reported to limit yield especially with the micronutrients (Yasari and Vahedi, 2012). Work done by Abdelhamid *et al.* (2010) show that soil salinity is one of the major limiting factors of soybean production in semi arid regions and chloride salinity has a more depressive effect on the yield than sulphate salinity (Hellal and Abdelhamid, 2013). Nutrient deficiencies reduce production directly but also indirectly, through increases in pathogens

and pests (Shanker *et al.*, 2013). Despite these losses due to abiotic factors, soybean which is a hardy crop as reported by Hellal and Abdelhamid (2013) has the ability to grow well even in marginal soils.

The biotic constraints on the other hand which contribute to economic damage in terms of loss of quality and quantity of soybeans include pests and diseases such as soybean rust (Maphosa *et al.*, 2012), red leaf blotch, frog-eye leaf spot, bacterial pustule, bacterial blight, sclerotina stem, soybean mosaic virus, nematodes, semi loopers, cut worms, groundnut leaf miner, aphids, beetles, mites, and stinkbugs (Tukamuhabwa and Oloka, 2016). The extent of economic plant damage depends on the type of pathogen or pest, the plant tissue being attacked, the number of plants affected, the severity of the attack, environmental conditions, host plant susceptibility, plant stress level, and stage of plant development (Singh, 2009).

In storage, all legumes suffer damage inflicted by bruchids with degree of damage varying according to insect species and legume type (Singh, 2009). The most important pests are of the genera *Callosobruchus*, *Acanthoscelides* and *Zabrotes* (Credland, 2000). Unlike field pests, damage caused by pests on stored products (Plate 1.1) is completely irreversible (Dent, 2000; Kananji, 2007).

a (Eggs) b (Exit Holes) c (Presence of adults)

Plate 1.1 Damage caused by *Callosobruchus chinensis* on soybeans a) Cosmetic b) Loss of market value c) Magnitude of infestation

In developing countries, bruchids become a big issue because most subsistence farmers rely on traditional storage structures under the same roof, which are highly vulnerable to bruchid

attack and lead to cross infestations among stored products which are sharing a common pest (Naito, 1999).

A review of literature indicates that soybean was rarely attacked by storage pests and as such storage pests are not part of many breeders priorities. For example, reports by Srinives *et al.* (2007), Dugje *et al.* (2009), Tefera (2015) and Tukamuhabwa and Oloka (2016) do not report any storage pest of soybean. Furthermore, IITA (2015) which does great research on soybean in Africa, does not have soybean storage pest on the priority research areas suggesting that damage on soybean by bruchids has previously been considered negligible. Nevertheless, recent reports by Rees (2010) in Australia; Sharma and Thakur (2014c) in Palampur-India indicate that soybean is attacked by soybean bruchid (*Bruchidius mackenziei* Kingsolver) and cowpea bruchid (*Callosobruchus maculatus).* In Uganda, while bruchids were a serious problem on most legumes, soybean in general was not known to be attacked by bruchids (Tukamuhabwa and Oloka, 2016). Dispersal and distributional changes in crop pests pose a threat to both native and agricultural systems. Although much pest spread is human-mediated, latitudinal shifts in pest distributions have been documented for a wide variety of groups suggesting that climate change coupled with other environmental factors play critical roles on pest distribution (Syfert *et al.*, 2017).

To avoid the storage losses that invariably develop in untreated grains, farmers in Uganda sell their legumes after harvest when prices are low; those that store grain for future use usually experience a rapid decline in legume quality, the longer the grain is stored (Ebinu, 2014). Moreover, metabolic activity of bruchids generates heat and moisture, which can favor the growth of mycotoxin-producing fungi. Ultimately, bruchid damage results in significant grain price discounts (Mishili *et al.,* 2011) and thus forcing farmers to sell legumes within 2-3 months after harvest, so as not to incur total grain losses (Ebinu *et al.,* 2016).

In terms of control, use of storage pesticides is recommended; however, the method is expensive for resource-poor farmers, toxic to environment and often leads development of pesticide resistance (Mulungu *et al.,* 2007). For this reason, use of resistant varieties is most preferable as part of an integrated pest management strategy to reduce losses and maintain grain quality. From the farmer's perspective, the use of resistant cultivars may represent one of the simplest and most convenient methods of bruchid control (Kananji, 2007).

1.1.4 Host Plant resistance to bruchids

There are two types of host plant resistance categories applicable to stored products;

antibiosis and antixenosis (Smith, 1994). Often, there is an overlap between antibiosis and antixenosis resistance in plants. The basis for antibiosis and antixenosis in legumes to bruchid attack is mainly due to the morphological and/or physiochemical characteristics of the seed (Venugopal *et al.,* 2000). The morphological traits in legumes include seed colour, texture, hardness and size, while physiological and/or biochemical traits include secondary metabolites and anti-nutritional compounds affecting the metabolic activity of bruchids (War *et al.,* 2017).

Host plant resistance to bruchids is measurable and can thus be tested in laboratory and/or field condition (Smith, 1994). However, different experimental procedures are followed to evaluate bruchid resistance depending on whether antixenosis or antibiosis resistance is being evaluated (Mendesil *et al.,* 2016). Antixenosis resistance is evaluated either in choice or no-choice situations and is expressed in terms of oviposition (Jager *et al.,* 1995) and/or number of emigrating insect in a test cultivars (Jackai, 1991) both in the laboratory and the field (Babarinde *et al.,* 2008; Dent, 2000). The rationale behind this approach is that insects that have located a susceptible plant will be less inclined to leave it than an insect on a resistant plant (Parrott *et al.,*1998); hence the numbers leaving susceptible and resistant plants should differ. Assessment of antixenotic resistance can also be done by comparing the behaviour of the insect on plants having a range of susceptibilities (Dent, 2000; Souframanien *et al.,* 2010). However, the initial temptation to devise behavioural experiments testing for resistance must be avoided until the insects and their interactions with plants in question are well understood (Dent, 2000).

Antibiosis resistance is tested under no-choice tests with the insects confined on plants or plant materials inside a cage (Dent, 2000). Parameters of interest when evaluating plants for antibiosis resistance are mainly the biology of insect, such as insect development, reproduction, survival, mortality and plant damage scores (Mendesil, 2014). Insect development can either be measured as a rate or expressed in terms of insect size or weight (Dent, 2000). The development rate is usually considered in terms of the length of time taken between stages on resistant and susceptible cultivars. For instance, the larval and pupal periods were shorter and adult longevity longer for individuals of *Chilo partellus* reared on susceptible maize than on resistant maize (Sekhon and Sajjan, 1987). Indeed, in this example the more resistant varieties reduced larval weight by 51 to 60 mg per larva and pupal weight by 49 to 52 mg per pupa.

Even though antibiosis and antixenosis can be assayed separately, in practice there are often overlaps between morphological and biochemical bases of resistance (Rector *et al.,* 2000). For instance, an antibiotic chemical may also repel and trichomes in *Nicotiana spp.* may exude alkaloids that are toxic to aphids (Thurston *et al.,* 1966). Overall, therefore, not only different mechanisms but also different strengths of resistance may be required to effect equivalent levels of population suppression of pests with different life histories (Abdel-Sabour *et al*., 2010).

In soybean, both antixenosis and antibiosis were found to be resistance mechanisms against common cutworm (Oki *et al.,* 2012), soybean looper larvae *(Chrysodeixis includens)* (Wille *et al.,* 2017), corn earworm (Rector *et al.,* 2000) and aphids (Bruner, 2012). However, there is no report so far documented on soybean with regards to bruchid.

To ascertain the presence of true or genetic resistance in a cultivar and not the relative preference existing only in a choice situation, no choice tests are often used (Smith *et al.,* 1993). The no-choice test ensures an even distribution of test insects on cultivars and excludes any possibility of their escape from infestation. The technique has important implications in screening for resistance against mobile insect pest such as bruchids (Saxena and Khan, 1984).

Host plant resistance to bruchids in legumes apart from having physical, biochemical basis it also has genetic basis. To understand the genetic basis or behavior of host plant resistance as a trait, mating of parental lines has to be done (Acquaah, 2007). The parental lines selection can be performed by particular mating designs such as line X tester, North Carolina designs I, II, and III and diallel. The diallel design gives the best in terms of amount of information and is the most important for determining general combining abilities (GCA) and specific combining abilities (SCA) (Choudhary *et al.,* 2004). Full diallel mating design with parents and reciprocals is important where parental lines are purposely selected. Using full diallel, resistant genes against bruchids have been reported in mungbean, common beans, green grams, cowpeas and chickpea (Keneni *et al.,* 2011). Both additive and non-additive gene effects were reported as important in the inheritance of resistance in legumes to bruchids (Keneni *et al.,* 2011). Maternal effects and cytoplasmic effects have also been reported in other legumes such as cowpea (Adjadi *et al.,* 1985).

Considering the global economic importance of soybean a lot of effort has been geared towards the development of improved varieties with the aim of increasing its production and productivity. In Uganda, systematic research began in the late 1930s (Tukamuhabwa *et al.*, 2012). According to Tukamuhabwa and Oloka (2016) the main objectives of soybean breeding program in Uganda are to develop soybean varieties that are high yielding with medium maturity (<120 days), resistant to diseases and pest with focus on soybean rust disease and groundnut leaf miners, resistant to lodging and pod shattering, promiscuous in the formation of active nodules with local rhizobia, rich in protein and oil contents, having high pod clearance, and having general end-user acceptance in terms of seed appearance and other traits. Indeed, breeding efforts have led to the release of a number of varieties such as Kabanyolo 1, Kabanyolo 2, Congo, Nam 1, Nam 2, Namsoy 3, Namsoy 4, Maksoy 1N, Maksoy 2N, Maksoy 3N, Maksoy 4N, Maksoy 5N and Maksoy 6N. Landmark achievements have also been made on adoption of these improved varieties as Tukamuhabwa *et al.* (2012) reports that almost all soybean varieties grown in Uganda are improved.

Although other constraints to soybean production in Uganda have been given research attention (Tukamuhabwa *et al.,* 2012; Maphosa, 2013,) storage pests have not been attended to significantly (Tukamuhabwa and Oloka, 2016). Furthermore, the mission statement for the breeding program does not include a focus in breeding for resistance to storage pests and as such nothing geared towards breeding resistance for storage pests has been done so far; not only in Uganda (Tukamuhabwa *et al.,* 2012) but also world wide because the crop has had no issues of storage pests until recently (Srinives *et al.,* 2007) and in Uganda, (Tukamuhabwa, 2015 personal communication).

1.2 Problem Statement

The emergence of bruchids as storage pest on soybean causing extensive damage in Uganda is a new challenge (Tukamuhabwa, 2015 personal communication). There is no information whether all varieties released in Uganda have become susceptible. There is no information whether the bruchid species attacking soybean in Uganda is new or there is just a host-plant switch.

Although bruchids are known to attack many legume species, there is lack of information regarding magnitude of damage caused to soybean by bruchids. In addition, there is little information documented for soybean regarding how long can soybean be safely stored without protection.

Whether the bruchid attacking soybean in Uganda is a new species or it is just host-plant switch is a question that has until now not been resolved. Nonetheless, host plant resistance remains key solution because it is the most cost effective and environmental friendly method. Fortunately, Uganda has 566 collection of soybean germplasm being conserved at MUARIK (Tukamuhabwa and Oloka 2016) which can be valuable germplasm if it harbours alleles for bruchid resistance. However, there is no information on bruchid resistance status of this germplasm indicating that there are no sources of resistance to bruchids identified so far in soybean in Uganda.

Furthermore, the basis and mechanisms of resistance to bruchids attack has not yet been established in soybean. Soybean is known to contain biochemicals for defence against pests. However, relationships between physical and biochemical traits in soybean in relation to bruchid resistance have not been established.

Besides, no genetic studies have been carried out on soybean bruchid resistance anywhere in the world. Therefore, information regarding combining abilities and nature of gene actions governing the inheritance of bruchid resistance is not available. This is an impediment in the progress for development of varieties with resistance.

1.3 Justification

Bruchids are the most important insect pests of stored grain legumes (Dent, 2000), because their damage starts in the field and continues along the value chain. Damage by bruchids is irreversible and direct on the grain (Kananji, 2007). Due to the bruchids' high fertility, ability to re-infest and short generation times, even low initial infestation rates can lead to tremendous damage (Yamane, 2013). Consequently, bruchids cause overall seed weight loss, loss of seed viability and altered nutritional quality due to the presence of insect frass and excrement. When left unattended, bruchids can cause up to 100% loss (Tembo *et al*., 2016). The nature of these losses is especially tragic for poor farmers in the tropics since the insects consume dry matter already produced using limited resources.

Proper pest identification is the first basic component in integrated pest management programme (Dent, 2000). Identity of the pest is important in designing a pest control strategy. Proper pest identification helps to know if the pest is a key pest which is an indication of the extent of the problem. Pest control is a costly activity, so if pest is not properly identified it

will lead to wrong measures being applied which is just a loss of resources. Therefore, it was imperative to have the pest identified by the National Agricultural Research Laboratories-Uganda (NARL).

Screening germplasm for bruchid resistance would identify effective and adaptable sources of resistance to bruchids and thus avail progenitors that can be used for a soybean bruchid resistance breeding programme. Screening for resistance would probably identify released varieties with high resistance to bruchids and recommend them to farmers. It would also help in identifying whether the recent reports on bruchids damage on soybeans was due to genetic deterioration or just host-plant switch.

Soybean has been reported to contain and produce biochemicals that have negative toxicological or antinutritional effects on pests (Barbehenn and Constabel, 2011). Since each biochemical factor may be conditioned by a different single gene, identification of biochemicals associated with bruchid resistance in soybean would facilitate selection of desired parental genotypes and cross combinations that enhance resistance to bruchids.

The development of an appropriate breeding strategy to improve resistance to bruchids in soybean requires knowledge of the inheritance of resistance and gene actions controlling it. An inheritance study would provide information on the combining abilities and maternal effects which allows the selection of parental genotypes which are good combiners and crosses which would produce combinations with superior desirable trait (Maphosa *et al.*, 2012). This problem of bruchids if not dealt with in a sustainable manner, it might downplay efforts aimed at increasing production of soybean in Uganda since 1930s.

Therefore this study sought to determine the response of different genotypes to *C. chinensis* infestation, identify metabolites associated with resistance to bruchids and identify parents capable of originating superior individuals resistant to the bruchid in soybean.

Through this research, subsequently, resistant varieties, with traits preferred by farmers will be developed and deployed to farmers. This will contribute significantly to reducing post harvest losses caused by bruchids and thus improving food security and nutrition in Uganda and the entire sub Saharan region.

1.4 Objectives

1.4.1 Main objective

The main objective of this study was to contribute to reduction of soybean post harvest losses caused by bruchids.

1.4.2 Specific Objectives

The specific objectives of the study were to:-

1. Identify sources of resistance to bruchids in the available soybean germplasm in Uganda.
2. Determine the biochemicals associated with bruchid resistance in soybean
3. Determine the mode of inheritance of resistance to bruchids in soybean.

1.5 Hypotheses

To achieve the above objectives it was hypothesized that:-

1. Soybean accessions available in Uganda react differently to bruchid infestation because they are from different geographical origins.
2. Soybean contains more than one type of metabolite which are responsible for and associated with bruchid resistance
3. The mode of inheritance of resistance to bruchids is quantitative and additive with parents expressing different combining abilities

CHAPTER TWO

LITERATURE REVIEW

2.1 Bruchids and their management

There are about 1300 species of seed beetles in the sub family Bruchidae from Chrysomelidae family. Out of these, 20 are recognized as pests of stored legume seeds especially in developing countries (Credland, 2000). Four species are cosmopolitan (Credland, 2000) and these include: *Callosobruchus maculatus, C. chinensis, Acanthoscelides obtectus* and *Zabrotes subfasciatus*. However, Srivastava and Subramanian (2016) reported that in addition to the four, other species of *Callosobruchus* including *C. analis, C, rhodesianus* and *C. subinnotatus* constitute a secondary group of storage pests while *Bruchus pisorium, B. rufimans* and *Bruchidius atrolineatus* are important as pests in the field and early stages of storage.

Out of these, three species have been reported on soybeans; *C. analis* in Indonesia (Naito, 1999), *C. maculatus* in Australia and India (Bailey, 2010a; Sharma and Thakur 2014c) and *C. mackenziei* in Australia (Bailey, 2010b; Rees 2010). Unlike field pests and diseases, damage caused by pests on stored products is irreversible (Dent, 2000; Kananji, 2007). Each emerging female for example, quickly finds a mate and if food is readily available, produces about 100 offsprings (Yamane, 2013). Exit holes formed by these insects cannot be erased. In crops such as cowpea, one generation takes about a month and after three or four generations, losses due to *Callosobruchus spp* are very severe (Yamane, 2013). Bruchids in stored seeds are a major problem because of their ability to re-infest stored seed. In other grain legumes, losses of up to 100% have been reported after 3-6 months of storage (Ofuya and Reichmuth, 1993; Credland, 2000).

Reports on losses due to bruchid damage varies among countries (Ali *et al.*, 2004), due to environmental effects, as such, accurate data on the scale of damage are scarce. For example, losses up to 38% have been reported in common beans in Malawi. Uganda reported losses of up to 90% (Ebinu *et al.*, 2016) while Kenya and Tanzania reported as high as 78% losses within six months of storage (Kananji, 2007). Some of the damages by bruchids include; consumption of seeds (Singh, 2009), loss or conversion of nutrients, reduced germination of seeds and contamination with filthy materials composed of insect fragments, exuviates,

excreta and moulds (Ileke *et al,* 2013). It is for these reasons that bruchids are therefore a major obstacle to achieving food security in developing countries.

In principle, any pest control measure should aim at reducing pest's biological fitness, which consequently leads to control of pest population (Credland, 2000). Optimal management of bruchids is a challenging task (Srinives *et al.,* 2007). Over the years, several methods of bruchid control have been employed by farmers and researchers. These methods range from store hygiene, physical, cultural, biological, chemical control and use of inert materials. Chemical control appeared to be the most effective and efficient control method (Adebowale and Adedire, 2006), but it has adverse effects on both human and environment. Chemicals require a recurring expenditure and for their safe use, appropriate level of education is required (Credland, 2000; Dent, 2000; Singh, 2009). Extensive use of chemical pesticides increases the production costs; reduces the population of natural enemies (parasites and predators) and leads to development of pesticide resistance (Singh, 2009).

Bruchids can also be controlled using biological measures. This involves the application of pathogens and/or a range of invertebrate predators, parasites and parasitoids (Dent, 2000). Amevoin *et al.* (2007) reported that a parasitoid *Dinarmus basalis,* used to control cowpea bruchids in West Africa farmers' stores, reduced damage from 30 to 10%. Soundararajan *et al.* (2012) also demonstrated the efficacy of the same bio control agent (*Dinarmus spp*.) for control of *C. maculatus* in blackgram. A few attempts were made in India for control of bruchids (Soundararajan *et al.,* 2012). However, application of biocontrol technologies presents challenges as it involves continuous monitoring and rearing of the bio agent (Dent, 2000). The time of introduction of bio control agent has to be well studied and understood taking into account the initial rate of seed infestation by the bruchids. The cost of reared natural enemies must be judged in terms of the value of the crop protected by using the agent and in comparison to the cost of competing pest control options such as chemicals. Dent (2000) further states that biological agents may also have some environmental impact because once introduced and established, it is often very difficult and impossible to eradicate.

Furthermore, to avoid the effects of pesticides, physical control of bruchids has also been attempted and reported. Ofuya and Reichmuth (1993) used physical means to manage *C. maculatus* and *A. obtectus* where 100% nitrogen (N_2) at 25-32^{0}C respectively and at 70 ±5% relative humidity was used to kill adults, eggs, larvae and pupae. This type of method is

definitely out of reach for the smallholder farmers and middle scale processors.

Another method for managing bruchids in legumes is the use of host plant resistance. Use of host plant resistance to manage bruchids was reported by Amusa *et al.,* (2013), Kabeh and Lale (2008), in cowpea, Kananji, (2007) in beans and Somta *et al*. (2008) mungbeans. Host plant resistance has the advantages of being sustainable, environmentally friendly and being cost effective. However, little or no work has been done on soybean especially regarding storage pests. Host plant resistance involves morphological barriers and physiochemical properties of the crop. Screening soybean for resistance using morphological parameters can be done and then resistance can be associated with biochemicals. Soybean has long been known to contains an array of biochemicals that are antinutritional (Cabrera-Orozco *et al.,* 2013). Sharma and Thakur (2014c) reported variations in soybean genotypes susceptibility to *C. maculatus.* This was an indication that soybean genotypes contain varying amounts of antinutritional factors and if associated with resistance it would help in selection for parents for breeding for resistance to bruchids. On the other hand, since resistance to bruchids in other legumes has been reported to be a trait with complex inheritance patterns (Keneni *et al.,* 2011), molecular markers can be utilized for identifying chromosomal regions that contain genes controlling complex traits (Dargahi *et al.,* 2014).

2.2 Sources of resistance to bruchids

Breeding progress depends on the magnitude of genetic variability within the germplasm, heritability of the trait under question and the level of selection intensity applied (Keneni *et al.,* 2011). The higher the levels of these components for a given trait, the higher the genetic gain expected from each cycle of selection. Resistance is a relative attribute and is measurable only in relation to susceptibility (Singh, 2009). The degree of insect resistance may be grouped into 5 categories: Immunity, high resistance, low resistance, susceptibility and high susceptibility (Painter, 1951). In many legumes, different sources of resistance to storage insect pests have been identified from cultivated varieties, germplasm collections and species of wild relatives (Singh, 2009; Somta *et al.* 2007; Chen *et al.* 2007). Painter (1951) and Singh (2009) reported that so far no cultivated legume has been reported to be immune to bruchids. In general, genes for complete resistance to insect pests and storage insects in particular are rare in nature for cultivated species (Acosta-Gallegos *et al.,* 2007 and Singh, 2009). However, they have often been reported in species of wild relatives for a number of legume crops (Somta *et al*., 2006; War *et al.,* 2017). Nevertheless, a few cases of complete

resistance have been reported in haricot bean (Ishimoto *et al*., 1995), mung beans (War *et al.,* 2017), field pea, cowpea, black gram and chickpea germplasm collections (Keneni *et al.,* 2011). In mungbean out of 525 accessions screened, 17 were found to be free from bruchid infestation (War *et al.,* 2017). In most cases, bruchid resistance has been found in the unimproved traditional germplasm (Kananji, 2007; Singh, 2009; Keneni *et al*., 2011).

Talekar (1987) reported that host plant resistance to insects attacking soybean and mungbean in the tropics was sought for; no resistance was found for bruchids in soybean but in mungbean. However, results from a comparative study on the varietal preference and developmental behaviour of *C. maculatus* on thirteen soybean varieties by Sharma and Thakur (2014c); revealed that on the basis of developmental behaviour among all the genotypes, one genotype (bragg) was totally resistant; 5 were relatively resistant while other 7 varieties, were susceptible to *C. maculatus*. Similarly in cowpeas, Amusa *et al.* (2013) found resistance to cowpea bruchid in a cultivated variety called IT81D-994 and Lephale *et al.* (2012) reported cowpea genotype Red caloona to be resistant to *C. maculatus.*

Although many sources of bruchid resistance in legumes have been identified, few if any have been identified in soybean. In Uganda, soybean breeding focus has been on the agronomic traits and diseases, consequently high yielding and non pod shattering varieties have been released (Tukamuhabwa and Maphosa, 2010). However, close examination of the breeding work reveals that no attention was given to response of these varieties to storage insect pests and this lack of positive selection for resistance to storage pests might have led to production of varieties with improved other qualities but susceptible to bruchids hence the recent report of bruchids. Soybean germplasm has not been extensively explored for resistance to storage pests. No cultivar of soybean showing resistance against storage pests has been released so far in the world (Bansal *et al.,* 2013). However, with reports from other legumes, therefore there is hope that resistance to bruchids in soybeans could be found in available germplasm either in Uganda or else where. These findings further indicate that there is genetic variability in genotypes with respect to bruchids, but also creates awareness that resistance to bruchids in cultivated genotypes is low highlighting the need for serious studies on genetic and biochemical factors which influence resistance in soybeans.

2.3 Mechanism of resistance to storage insect pests

Association of insects and leguminous plants is a co-evolutionary process and both have

evolved precisely to avoid the defensive systems of each other (War *et al.,* 2017). The defensive system of legumes against insect pests generally comprises three important mechanisms non-preference (antixenosis); antibiosis and tolerance (Painter, 1951; Singh, 2009). For storage pests, antixenosis and antibiosis are the applicable mechanisms (Keneni *et al.,* 2011; Dent, 2000). These resistance mechanisms manifest through morphological, physiological and /or biochemical traits (Dent, 2000).

The term non-preference has subsequently been replaced by antixenosis (All *et al.,* 1999), because non-preference refers to the insect and this is incongruous with the notion of resistance being a property of the plant. Antixenosis is a term derived from the Greek word xeno (guest) that describes the inability of a plant to serve as a host to an arthropod. Therefore, antixenosis is the resistance mechanism employed by the plant to deter colonization by an insect. Insects may orientate towards plants for food, oviposition sites or shelter but certain plant characteristics may be a biochemical or morphological factor, or a combination of both may deter the insect (Dent, 2000). Plants that exhibit antixenotic resistance would be expected to have reduced initial infestation and/or a higher emigration rate of the pest than susceptible plants (Singh, 2009). The impact of antixenosis on the population dynamics is complex, with some of the effects paralleling those of antibiosis (Thomas and Waage, 1996). For instance, reduced oviposition through non-preference is equivalent to reduced fecundity (Kar and Ganguli, 2016), and it can also increase larval movement thereby slowing development time (Gevina and Mohan, 2016) or increasing juvenile mortality (Ofuya and Reichmuth, 1993). Increased emigration from the crop due to antixenosis has the equivalent effect of increased adult mortality (Thomas and Waage, 1996). However, antixenosis is reported to play no role in conferring resistance to bruchids in pulses (Seram *et al.,* 2016; Tomooka *et al.,* 2000; Srinivasan and Durairaj 2007; Sharma and Thakur 2014c).

Antibiosis in contrast to antixenosis is the mechanism by which a colonized plant is resistant because it has an adverse effect on an insect's development, reproduction and survival. These antibiotic effects may result in a decline in insect size or weight (Kananji, 2007) an increased restlessness (Somta *et al.,* 2006), poor accumulation of food reserves affecting the survival of hibernating or aestivating stages (War *et al.,* 2017), or have an indirect effect by increasing the exposure of the insect to its natural enemies (Singh, 2009). Lale and Kolo (1998) observed that resistance to *C. maculatus* in three cultivars of cowpea was conferred mainly

by reduced egg-hatching which may reflect antibiosis rather than antixenosis.

2.4 Basis of resistance to bruchids

2.4.1 Morphological basis of resistance

The first point in the insect pest-plant host relationship at which the plant may show resistance is in deterring oviposition by the insect. The rate of insect population is known to be affected by the resistance of a particular genotype by causing a reduction in the rate of oviposition through physical or mechanical barrier (Semple, 1992). The barrier may either limit access into the grain or make it unsuitable for oviposition. The barrier may make it difficult for eggs to adhere to the seed or prevent the larva's penetration into the seed when they are hatched (Lephale *et al.*, 2012). The physical characteristics of seeds can determine the acceptability for oviposition but may not be related to the antibiotic nature of the seed (Messina and Renwick, 1985). Nwanze *et al.* (1975) showed that rough seeds were less acceptable to *C. maculatus* than smooth ones. On the other hand, Murdock *et al.* (1997) indicated that varieties with smooth and glossy seed coat constantly are more resistant than rough seeded varieties suggesting that other factor besides seed coat appearance affect cowpea's resistance to bruchid infestation. In a soybean study with 13 genotypes all the genotypes were highly preferred by *C. maculatus* for egg laying with variations except 1 (harasoya) which was with intermediate surface texture (Sharma and Thakur, 2014c). Sharma and Thakur (2014c) further explained that the variation in egg laying was attributed to seed coat texture and physical characteristics of the genotypes. However, in general egg counts have not shown to be predictive enough in resistance studies as other variables such as percent adult emergence, total development time, growth (susceptibility) index and percent loss in weight (Redden and McGuire, 1983; Jackai and Asante, 2003; Sharma and Thakur, 2014c). In a study by König *et al.*, (2016) it was concluded that under no choice test conditions, bruchids lay eggs regardless of the quality of the host. Basically this happens because bruchids are desperate to contribute to their species at all cost.

Desroches *et al.* (1995) found that the seed coat in a faba bean (*Vicia faba)* acts like a physical barrier against penetration by *C. chinensis* and C. *maculatus.* They found that only 45–58% of the neonate larvae perforated through the seed coat to the cotyledons. A similar type of resistance against *C. maculatus* was also reported on cowpea (Eddie and Amatobi 2003). Amusa *et al.* (2013) and Lephale *et al.* (2012) reported that different cowpea genotypes possessed different characteristics and therefore response to bruchid infestation

was different and went further to indicate that some characteristics which include testa thickness and hardness in the genotypes may influence cowpea seed response to bruchid attacks. Apart from hindering penetration into the seed, seed coat is also reported to hinder adult bruchid emergence. Ali and Smith (2001) reported that up to 70% adult bruchid emergence of *C. chinensis* was observed from decorticated faba seeds compared to 14.2% from whole grains indicating that seed coat was a barrier to emergence. However, Amusa *et al.* (2014) reported that in the analysis of seed coat resistance, no significant difference was observed in number of eggs laid, mean bruchid development time, percentage bruchid emergence, percentage seed damage and susceptibility index between the smooth and rough seed coats indicating that seed coat nature was not responsible for bruchid resistance in cowpea.

Some studies have attributed grain resistance to differences in grain size (mass) and asserted that the larger grains supply more food and space for insect growth and that the smaller grains or grains with less mass offer more resistance to pests attack than the larger grains (Singh, 1974). Mei *et al*. (2009) reported that there was strong association between small seed size and resistance to *C. chinensis* in mungbean. However, this is true to some extent in some genotypes, because in a study by Amusa *et al.* (2013) two genotypes (IT99K-494-6 and IT81D-994) showed no significant difference in the grain size, yet showed different level of tolerance to the bruchid infestation. This indicated that grain size did not affect the genotype's resistance to the bruchid attack.

2.4.2 Biochemical basis for bruchid resistance

A plant cell produces two types of metabolites, primary and secondary metabolites. Primary metabolites are involved directly in growth and metabolism and these include carbohydrates, lipids and proteins. A number of studies have reported primary metabolites conferring resistance to insect pests, such as western flower thrips (Jager *et al.*, 1996), bruchids (Gatehouse *et al.,* 1987) .

Mphuru (1981) reported that the ability of soybean to resist the attack of bruchid was attributed to the presence of high fat contents in the seeds. Pajni (1986) reported that *C. chinensis* could not successfully develop on *Glycine max* due to low carbohydrates-protein ratio. Sales *et al.* (2000) reported that storage proteins called vicilins, from cowpea and other legumes confer resistance by strongly binding to several chitin-containing structures of the midgut of *C. maculatus* and *Z. subfasciatus*. Vicilins isolated from *C. maculatus*-resistant

cowpea seeds and from several other legumes slow down larval development of this insect (Sales *et al.,* 2000). Lin *et al.* (2005) reported that the presence of a novel protein Vr D1 in mung bean variety (VC 6089 A) was associated with greater resistance for the development of eggs of *C. maculatus* and caused greater adult mortality by 96%. Carbohydrates are also reported to confer resistance to bruchids. Heteropolysaccharides which are carbohydrates in haricot beans were reported to confer resistance to *Acanthoscelides obtectus* and *Zabrotes subfasciatus* (Gatehouse, 1987). However, Sharma and Thakur 2014a reported that carbohydrates, proteins and fats were not responsible for resistance to bruchids in cowpea, chickpea and soybean. Higher amount of proteins were observed in susceptible variety of mungbean than in the resistant isogenic line indicating that the higher the protein content the more the susceptible the genotype (Khan *et al.,* 2003). Such contradictory results suggest need for further studies.

On the other hand enzymes have also been reported to play a role in bruchid resistance. Studies have indicated that the different α-amylase inhibitor classes, lectin-like, knottin-like, cereal-type, Kunitz-like, γ-purothionin-like and thaumatin-like could be used in pest control especially in bruchid control since bruchids are strongly depended on starch availability in the seeds (Franco *et al.,* 2002). Franco *et al.,* (2002) reported that α-amylase inhibitors from *Phaseolus vulgaris* seeds are detrimental to the development of cowpea weevil (*Callosobruchus maculatus)* and adzuki bean weevil (*Callosobruchus chinensis*). Patterns of α-amylase expression vary in *Z. subfasciatus* fed on different diets, apparently in response to the presence of antimetabolic proteins such as α-amylase inhibitors, rather than as a response to structural differences in the starch granules. Bean bruchids, such as the Mexican bean weevil larvae, also have the ability to modulate the concentration of α-glucosidases and α-amylases when reared on different diets (Franco *et al.*, 2002).

Soybean trypsin inhibitor (Kunitz type), benzoyl-arginine-p-nitroanilide (BA*p*NA), N-succinyl-L-alanine-pro-L-leu-*p*-nitroanilide (SAAPL*p*NA), leucine-*p*-nitroanilide (L*p*NA), benzoyl-tyrosine-*p*-nitroanilide (BT*p*NA) and azocasein have been reported to retard growth and development of insects such as *Manduca sexta* (Shukle & Murdock, 1983), *Bactrocera cucurbitae* (Kaur *et al.,* 2009) and *Callosobruchus maculatus* (Macedo *et al.,* 2002). Therefore, there is a possibility of these inhibitors retarding growth for *Callosobruchus chinensis.*

Usually biochemicals involved in resistance to pests are products of secondary metabolism (Minney, 1990). Secondary metabolites are organic compounds which are not involved in primary metabolism of the cell (Manisha, 2017). Secondary compounds accumulate in the seed, which is not able to respond defensively to damage since it is in a quiescent state; and these secondary compounds can be considered to have a role specific to the seed since they often disappear soon after germination (Minney, 1990). Swain (1977) reported that there are probably 400,000 secondary compounds used as defense in plants. To increase the insect resistance of cultivated varieties plant breeders are interested in understanding resistance mechanisms that operate in different varieties or why bruchids attack one genotype but not another. Franco *et al.,* (2002) reported that plants have evolved a certain degree of resistance through the production of aproteic defence compounds, such as antibiotics, alkaloids, terpenes and cyanogenic glucosides. Guo *et al.* (2012) reported that furanocoumarin compound bergapten which is a plant secondary metabolite when incorporated into artificial diet, it retarded cowpea bruchid development, decreased fecundity, and caused mortality at a sufficient dose through altered expression of 543 midgut genes in response to dietary bergapten.

Tannins are secondary metabolites which have been reported to retard bruchid development and kill the larvae, however in reports by Janzen, (1977) and Janzen *et al.,* (1977) it was expressed that since tannins are confined to the region near the testa and to the testa itself, many bruchids by pass this level of defense by passing the testa through the gut undigested or boring through the testa without ingesting. New reports by Barbehenn and Constabel (2011) strongly oppose the reports of Janzen *et al.* (1977). Barbehenn and Constabel (2011) report that tannins have negative effect on insects and the negative effects appear to be more toxicological than antinutritional. This therefore means that apart from increasing larval development period, tannins also have direct killing properties thereby reducing the number of adult bruchid emergence. Lepiniec *et al.,* (2006) reported that it was important to note that tannins are not made in all parts of the plant; some plants make tannins only in the seed coat, where they are incorporated into a complex polymer including other flavonoids that are thought to protect the seed against desiccation and other abiotic stresses.

How tannins affect bruchid development and their population dynamics depends on tannin-protein interactions. Factors that affect tannin–protein interactions include the molecular size, shape of the tannin and ratio of tannin to protein (Barbehenn and Constabel, 2011). Larger

molecular weight tannins and with higher ratio of tannin to protein are better protein precipitants than smaller tannins indicating that plants or genotypes with higher ratio of tannin to protein will display higher resistance to insect infestation. However it is not yet clear whether this display of higher resistance associated with tannins is due to feeding deterrence, decreased protein utilization efficiency or toxicity. Lattanzio *et al.,* (2012) reported that while tannin–protein complexation likely has little impact on insect nutrition, it is possible that oxidized tannins do react with proteins to decrease their nutritional quality. Quinones are capable of covalently binding several essential amino acids in vitro (e.g., methionine, histidine, and lysine). Tannin prooxidant activity and toxicity in herbivores is likely when tannins oxidize to form high levels of semiquinone radicals and quinones. Phenolic autoxidation is well-known to occur in the presence of oxygen and the dismutation of semiquinones readily forms quinones (Barbehenn and Constabel, 2011).

Tannins show toxic effects on insects which do not normally feed on tanniferous plants. Tannic acid (16–22% dry wt.) directly or oxidative stress (indirectly) produce fatal lesions in the midguts of insects (Lattanzio *et al.,* 2012). Lesions are thought to be due to tannins permeating the peritrophic envelopes (Galati *et al.,* 2002) and then binding with membranes of the midgut epithelium (Bernays *et al.,* 1981). The peritrophic envelope is a proteoglycan-rich sheath that is secreted around the food bolus as it passes through the midguts of insects (Barbehenn, 2001). Impermeable peritrophic envelopes were thought to protect polyphagous insects, but subsequent work found that several GGs in tannic acid were able to permeate these peritrophic envelopes (Barbehenn *et al.,* 1996). Oxidative stress in the midgut tissues could result from the absorption of peroxides produced by tannin oxidation, or from the absorption of low molecular weight phenolics that redox-cycle in the midgut tissues (Galati *et al.,* 2002).

Phytic acid reduces vital minerals due to its chelating effect (Hassan, 2013). Phytic acid present in soybean seeds binds to minerals and metals to form phytate (chelated forms of phytic acid with magnesium, calcium, iron, and zinc). Phytate is not digestible and is an impermeable molecule through cell membranes of insects.

Flavonoids are plant secondary metabolites, derivatives of 2-phenyl-benzyl-γ-pyrone, present ubiquitously throughout the plant kingdom (Mierziak *et al.,* 2014). Flavonoids are reported to play defensive role in many insects because of their antioxidative activity. Their presence can alter the palatability of the plants and reduce their nutritive value, decrease digestibility, deter

insects from feeding or even act as toxins (Dakora, 1995). Flavonoids are reported to increase mortality of the tobacco armyworm (*Spodoptera litura*), *Lymantria dispar* and in rice, three flavone glucosides inhibit digestion in *Nilaparvata lugens* (Mierziak et al., 2014). Salunke *et al.* (2005) reported that purified flavonoids were toxic to adults and eggs of *C. chinensis* depending on dose and exposure period in mungbean. However flavonoids are attractants or feeding/growth stimulators for certain insect species, (Mierziak *et al*., 2014). Sales *et al.* (2000) reported the existence of flavonoid detoxification by bruchids in legumes which leads to host specificity. Due to coevolution insects have developed ways of detoxifying flavonoids leading to high seed damage even in the presence of sufficient amounts of flavonoids.

Peroxidases are ubiquitous in nature being found in all living things (Khan *et al.,* 2014). The plant peroxidases, belonging to Class III peroxidase, are implicated in various vital processes of plant growth and development throughout the plant life cycle including cell wall metabolism, lignification, suberization, reactive oxygen species (ROS) metabolism, auxin metabolism, fruit growth and ripening, defense against pest and pathogens (Pandey *et al.,* 2017). Jager *et al.* (1996) reported that roles of peroxidases in plant defense are multifaceted. Peroxidases generate reaction oxygen species, which regulate defense-related signal transduction pathways, initiate hypersensitive reaction, and strengthen cell walls through enhanced lignification and cross-linking (Jager *et al.,* 1996). The potential direct detrimental effect of peroxidases on insect herbivores is also from their role as digestibility reducers (Keneni *et al.,* 2011). Quinones and other oxidative species produced by peroxidases can react with side chains of either free amino acids or proteins, thus reducing the nutritive value of ingested food in the herbivore's gut. The action of enzymes in the gut, such as peroxidase can also promote or inhibit phenolic oxidation (Barbehenn *et al.,* 2011). Peroxidase has been reported to be involved in the bruchid resistance in mungbean (Khan *et al.,* 2003). Pavithravani *et al.* (2013) evaluated 12 rice bean genotypes which showed varying response to peroxidase indicating peroxidase provides protection to rice beans against bruchid infestation. Babu and Hedge (2012) assessed ten accessions of *Dolichos lablab* peroxidase activity and found that tolerant genotypes had higher peroxidase activity than susceptible genotypes. Furthermore, activity of peroxidase in pods of field beans exhibited negative correlation with infestation of bruchid which indicated that increase in enzyme activities resulted in less infestation. Peroxidase is an antinutritive enzyme which decreases the nutritive value of wounded tissues by cross-linking proteins or catalyzing the oxidation of phenolic toxic metabolites to an undesirable component called quinines which acts as feeding

repellants and inhibits the growth of larvae in the seed (Babu and Hedge 2012).

Plant defensive compounds are not universally toxic to pests (Dowd *et al.,* 1983). Dowd *et al.* (1983) reported that insects, to ably feed on plants with defensive chemical factors, they neutralize the effects of the compounds involved. There are three main ways through which detoxification takes place; insects store the chemical in an unaltered form where it would not be harmful (sequestering), insects increase the excretion rate with the chemical remaining unaltered and biochemical alteration of the compound so that it will not harm the consumer.

2.5 Gene action and inheritance of resistance to bruchids

Genes, comprised of DNA (deoxyribonucleic acid), are the basic units of inheritance. Gene action is the functioning of a gene in determining the phenotype of an individual and can be grouped into two categories, additive and non-additive. The non- additive gene expression may exhibit dominance, over- dominance and epistasis (Falconer, 1981; Acquaah, 2007). To develop an efficient and successful resistance breeding programme, understanding the genes controlling resistance of a trait is fundamental. Literature on bruchid resistance inheritance studies in soybean is scanty. The genetic control of resistance to storage insect pests may range from monogenic to polygenic (Dent, 2000; Singh, 2009). Mostly additive and dominant genes may govern storage insect pest resistance in many legumes but a few cases of cytoplasmic gene effect have also been reported (Singh, 2009; Keneni *et al.*, 2011).

Inheritance studies of resistance to storage pests have previously been conducted in legumes and cereal crops. Cardona *et al.* (1990) reported that resistance to storage pests in legumes is conferred by a seed protein known as arcelin. Kornegay and Cardona (1991) reported that resistance conferred by arcelin, a seed protein, was controlled by two recessive complementary genes. While R'omero Andreas *et al.* (1986) found that the inheritance of arcelin, which is believed to confer resistance to *Z. subfasciatus* in wild beans, was controlled by a single dominant gene. However, Goosens *et al.* (2000) reported that there is no evidence for insecticidal activity of arcelin-5 therefore arcelin is not responsible for resistance in legumes. Goosens *et al.* (2000) experiments strongly suggested that the arcelin- 5 protein on its own does not provide satisfactory resistance levels to bruchids. However, Goosens *et al.* (2000) further reports that because arcelins show high biochemical stability, they could add to the effect of other insecticidal products and further impair bruchid development. This suggests that more studies are needed.

The inheritance of resistance to bruchids seems complicated (Garza *et al*., 1996). The inheritance of resistance to the bean pod weevil (Apion *godmani* W.) in dry beans was conditioned by two genes that were segregating independently (Garza *et al.*, 1996). They further reported that one gene pair in each of the accessions was non- allelic. In an F2 segregation analysis of black gram (Vigna *mungo* L.) for resistance to *Callosobruchus maculatus* F, Dongre *et al.* (1996) reported a 15:1 ratio of resistant to susceptible, indicating epistatic gene action for resistance controlled by duplicate genes. In a study done by Adjadi *et al.* (1985) where the parental F_1, F_2, and backcross populations involving three resistant and two susceptible cowpea parents were bioassayed in the laboratory for bruchid reaction on an individual plant basis; the reaction of F_1 seeds was similar to that of seeds from the maternal parent, indicating that the genotype of the maternal plant controls bruchid infestation. The F_2 seeds derived from F_1 plants represented the true hybrid population and the mean adult emergence was similar to that of the susceptible parents, indicating complete dominance of susceptibility. No reciprocal differences were observed. The F_2 plants derived from the six crosses segregated into a ratio of 15 susceptible: l resistant, indicating digenic inheritance. These results were further supported by the backcross data. The F_1 plants from the backcross involving the resistant parent segregated into a 3 susceptible: l resistant ratio, whereas those involving the susceptible parent were uniformly susceptible. Adjadi *et al*. (1985) reported that resistance to bruchids in cowpea is controlled by two recessive genes symbolized rcm_1 and rcm_2. However, in a similar study Dongre *et al.* (1996) found that resistance to cowpea bruchid was controlled by two dominant duplicate genes. These contradictions indicate that genetic studies are crop, pest and geographic specific therefore calls for specific studies in Uganda for *C. chinensis.*

Combining ability studies on maize weevil by Kang *et al.* (1995) and Tipping *et al*. (1989) found that additive gene effects were more important than non-additive gene effects. Derera *et al.* (2001a, b) investigated gene action for weevil resistance in both free-choice and no-choice tests and found significant additive, non- additive and maternal effects. Dhliwayo *et al.* (2005) reported that both general combining ability (GCA), which is defined as average performance of individual lines in crosses and specific combining ability (SCA), which is the deviation of some crosses from the expected value (sum of the GCA values of the two parents involved), were also found to be important for resistance to maize weevil. Dhliwayo and Pixley, (2003) reported the importance of reciprocal effects on maize weevil resistance.

Cockerham (1963) suggested partitioning of reciprocal effects into maternal and non-maternal effects is useful in determining whether maternal or extranuclear factors are involved in the expression of a trait.
Genetic analysis of grain resistance to weevils is reportedly complicated (Widstrom, 1989; Serratos *et al.,* 1994) because the weevil feeds on diploid and triploid tissues, which are both maternal and biparental in origin due to the fact that grain tissues belong to two different generations and have different gene doses from the parents (Serratos *et al.,* 1994).

In a study done at Chitedze Research station in Malawi on bean bruchids it was found that additive and dominance effects were highly significant for adult bruchid emergence and were adequate in explaining the resistance. The analysis further showed that epistasis was not important for resistance to bruchids in beans. The average degree of dominance of 1.4 implied that the action of genes was in the overdominance range. Thus, although both additive and dominance action governed the expression of resistance, the dominance component was relatively more important. The low heritability in the narrow sense further reinforced the usefulness of dominance effects in explaining bruchid resistance, which suggests that breeding for bruchid resistance cannot be easy; because the dominance effects are not fixable in self- pollinating crop like beans (Kananji 2007).

2.6 Summary

Literature review has shown that until 1999 soybean had no problems with bruchids both in nature and laboratory. In Uganda, until 2015 soybean was free of storage pest problems. There is a possibility that while breeding for traits desired by the consumer some traits which are responsible for resistance to pests such as bruchids have been lost. In addition, increased production, climate change and development of bruchid biotypes could also have led to bruchids problem being manifested in Uganda now. Either one and/or all of these factors could have happened but the standing issue currently is that there is a bruchid in Uganda which is devastating soybean. How much damage and economic loss does this new pest cause on soybean in Uganda? This information will help the breeding program in Uganda and Africa as a region to make informed decisions.

Based on damage caused on other legumes, coupled with very suitable proliferation tropical conditions and African storage conditions where different farm products are kept together highlights the intensity of the problem at hand. Furthermore, literature review has indicated

that there is no information on whether there is variability in the germplasm available in Uganda. Breeders usually utilize the available variability in breeding programs before looking for extra sources.

Crops confer different resistance mechanisms for different pests. From literature it is clear that there is no information on mechanisms and factors of resistance deployed by soybean against bruchid attack. Therefore there is a need to study soybean resistance mechanism to bruchids so that the information can be used in the breeding program. Literature so far has documented genetic analyses information on bruchid resistance for crops such as cowpea, field peas, chickpea and mungbean but none for soybean anywhere.

From this review it is clear that genetic studies are applicable to the specific germplasm, specific crop and the set of testing environments; hence results from such studies cannot be generalised (Falconer, 1989). Therefore, the findings from other areas may not have a direct application for bruchid resistance in soybean in Uganda.

CHAPTER THREE

RESISTANCE IN UGANDA SOYBEAN GERMPLASM TO ADZUKI BEAN BRUCHID

3.1 Introduction

Bruchids damage on legume seeds leads to significant losses (Yamane, 2013). Losses of seed quantity (weight losses) and seed quality deprive farmers of the benefits of their labour (Tembo *et al*., 2016). Losses due to bruchids are not just losses of food and seed but loss of all the resources that go into creating food, i.e. labour, land, water, fertiliser, insecticide *etc*. (Hodges and Bernard, 2014). Serious losses due to storage pests sometimes occur and these may result from agricultural developments for which the farmer is not pre-adapted. In the case of legumes, these include the introduction of high yielding varieties that are more susceptible to pest damage, additional cropping seasons that result in the need for harvesting and drying when weather is damp or cloudy, increased climate variability, or significant increase in production (Hodges and Bernard, 2014). In addition, the arrival of new pests can be a problem.

Although bruchids are known to attack many legume species (Onyido *et al*., 2011; Credland, 2000), literature indicates that there is lack of information regarding damage caused to soybean by bruchids. Most previous reports have been done on other legumes such as cowpea, chickpea (Sharma and Thakur, 2014a) and common beans (Kananji, 2007); but little is documented for soybean, suggesting that damage on soybean by bruchids has previously been considered negligible. Nevertheless, Tukamuhabwa (2015 personal communication) indicated that soybean had started being seriously damaged by bruchids in some parts of Uganda.

One of the major bruchid attacking stored legumes is *Callosobruchus chinensis* Linn (Coleoptera), commonly called adzuki bean bruchid or chinese bruchid (Spradbery, 2013). *C. chinensis* is a well-known pest of adzuki bean, cowpea and pigeon pea (Kuroda *et al.*, 1996). *C. chinensis* has been reported on soybean a couple of times (Naito, 1999; Sharma and Thakur 2014b). A record of *C. chinensis* in Uganda was first published in 1978 then in 1995 on pigeon peas (Nahdy, 1995).

Realising how damaging bruchids can be on legumes, different control methods have been undertaken by farmers; of which pesticides have been the principal means (Dent, 2000).

However, pesticides have drawbacks associated with their use such as pest resistance, destruction of beneficial insects, environmental contamination and hazards to the user; in addition to them being expensive for subsistence farmers (Dent, 2000). Resistant varieties, therefore, could provide a sustainable environmental friendly method to reduce soybean pre and post-harvest losses due to *C. chinensis.* This intervention could assist farmers and processors in long term storage of soybeans. In Uganda, soybean germplasm has not been evaluated for resistance to storage pests. No cultivar of soybean showing resistance to storage pests has been released so far in the world (Bansal *et al*., 2013). The existence of genotypic variations in response to bruchid infestation was reported in some legume species, such as cowpea (*Vigna unguiculata*) (Deshpande *et al.,* 2011), pigeon peas (*Cajanus cajan*) (Affognon *et al*., 2016), rice beans (*Vigna umbellata*) (Somta *et al*., 2006), chickpeas (*Cicer arietinum*) (Kar and Ganguli, 2016) and mungbean (*Vigna radiata*) (War *et al.,* 2017). Genotypic variations from the preceding studies were associated with physical and chemical signals by the adult female to detect diets that will provide better larva development and higher nutritive value (War *et al*., 2017). Unfortunately, such studies have not been extensively done on soybean. The objective of the study was to assess damage caused and identify sources of resistance to bruchids (*C. chinensis)* on soybeans germplasm in Uganda.

3.2 Materials and Methods

3.2.1 Study area

The study was carried out in 2015 and 2016 at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) in Central Uganda. MUARIK is located between longitude 32^{o} 37E, Latitude 0^{o} 28 N at an altitude of 1200 m above sea level (Sserumaga *et al.*, 2015). The area receives an average annual rainfall of 1150 mm and has an average temperature of 21.5^{0}C (Fungo *et al*., 2011).

3.2.2 Bruchid rearing

C. chinensis used in this study were obtained from a culture established in a laboratory at MUARIK. The bruchids which initiated the culture were collected from National Crops Resources Research Institute (NaCCRI) soybean stores at Namulonge in Uganda. The laboratory culture was established at MUARIK by allowing the collected samples of insects to oviposit on three commercially grown varieties; Maksoy 2N, Maksoy 3N and Maksoy 4N. The insects were reared on 1 and 5 kg of seed placed in 1 L Kilner glass jars and 10 L plastic buckets, respectively (Plate 3.1a). The jars and buckets were capped with muslin cloth to

allow ventilation, but prevent insects from escaping. The populations were maintained by regularly transferring the bruchids to new grains. A sample from the reared bruchid population was confirmed to be *Callosobruchus chinensis* by the National Agricultural Research Laboratories (NARL), Kawanda in Uganda (Plate 3.1b) using diagnostic protocols by Farrell *et al.,* (2015).

3.1a 3.1b

Plate 3.1a Rearing of *Callossobruchus chinensis* and 3.1b *C. chinensis* used in the study

3.2.3 Soybean germplasm collection

A total of four hundred and ninety eight genotypes were used in the study: Uganda (321), introductions from USA (56), AVRDC-Taiwan (110) and Zimbabwe (11). All this germplasm was available in the germplasm collection of Makerere University Centre for Soybean Improvement and Development. Prior to utilization in the experiment, all seed samples were oven dried at 30 ^{0}C for 24 hours, to ensure that eggs or adult insects from the field were killed (Amusa *et al.*, 2014).

3.2.4 Research design

A sample of 100 soybean seeds was drawn from each of the 498 genotypes and weighed to give baseline information of 100 seed weight. Samples of 50 seeds each were placed in different plastic petri dishes and weighed to determine the initial seed weight. The soybean seeds in each petri dish were artificially infested with 20 randomly selected adult bruchids of 1-3 day old, from the established bruchid colony. The experiment was carried out under no-choice test method as described by Somta *et al.,* (2008). Petri dishes were laid out in a

randomised complete block design, with insect infestation days as blocks (Dent, 2000). The experiment was replicated three times (Plate 3.2). Infestation was done at 3 days interval per replicate (Kananji, 2007). Bruchids were removed from the soybean samples after 10 days (Kananji, 2007). The experiment was carried out under room temperature conditions.

To validate the observed resistance, three genotypes that expressed resistance to *Callosobruchus chinensis*, six popular varieties of Uganda, one most susceptible genotype and 19 moderately resistant genotypes were re-subjected to the bruchid infestation in a no-choice test method, as described in the preceding paragraph.

Plate 3.2: Experiment Lay out RCBD

3.2.5 Data collection

Data was collected on seed weights, number of eggs laid and number of adult bruchid emergence. Eggs laid on each of the 50 seeds were counted on day 11 after removal of infestation bruchids (Kananji, 2007). Emerging adult insects were counted and removed daily until there was no new insects emergence for 5 consecutive days (Lephale *et al.,* 2012). Then final weight of seed samples in each petri dish was taken. All weights were taken using electronic balance (Mettler Toledo, model: ML 104/01). Total number of eggs laid was taken as an indicator for oviposition (Amusa *et al*., 2014); while the number of adult bruchid emergence (ABE) was taken as an indicator for magnitude of infestation (Emeka, 2010). From these data, the following variables were derived:

(i) Seed weight loss %, which is an economic loss indicator (Amusa *et al.,* 2014), was calculated as follows:

$$= \frac{100\ x\ (iwt - fwt)}{iwt} \qquad (3.1)$$

where; iwt = Initial seed weight, fwt = Final Seed weight for the sample.

(ii) Growth Index (GI), which is an indicator of genotype suitability for development of insects (Wijenayake and Karunaratne, 1999) was calculated as:

$$= \frac{\%ABE}{MDP} \qquad (3.2)$$

(iii) The median development period (MDP) was calculated as the number of days from the middle of oviposition (d 5) to the first progeny emergence (Kananji, 2007).

(iv) Dobie susceptibility Index

The data on the number of adult bruchid that emerged and the median development period was used to calculate the Dobie susceptibility index (Dobie, 1974) for each genotype using the formula:

$$= \frac{Log_e\ Y\ x\ 100}{t} \qquad (3.3)$$

where; Y = total number of adult bruchid emerged and t = median development period.

If no insect emerged over the test period, the Dobie susceptibility index value was equal to zero (DSI=0) (Derera *et al.,* 2001a). The modified Dobie (1974) susceptibility index ranging

from 0-9 was used to classify the soybean genotypes; where, 0-1 =resistant; 2 - 3 = moderate resistant; 4 -5 = susceptible and ≥ 6 highly susceptible (Radha and Susheela, 2014). The genotypes with a high susceptibility index (DSI) were considered susceptible and those with a low susceptibility index as resistant. This was based on the assumption that a few insect progenies would emerge out of a resistant genotype and insect progeny development would take a longer time in a resistant than in a susceptible genotype (Kananji, 2007).

(v) Seed size determination

Seed size was categorised based on Tukamuhabwa and Oloka, (2016). Basing on this information, any genotype with 100 seed weight less than genotype Maksoy 1 was considered as small (<12000mg), genotype same as Maksoy 1 (12001-14000mg) was categorized as medium; genotype of the same size as Maksoy 2 (14001-20000mg) was considered as large and genotype with higher 100 seed weight than Maksoy 5 (>20000mg) was considered very large. Four soybean seed size categories were determined.

3.2.6 Data analysis

Data were subjected to one-way ANOVA, with blocking using GenStat Statistical Package 12th edition. Prior to analysis data sets were tested for ANOVA assumptions and data on GI and MDP were transformed using Logarithm (base10) function. Based on ANOVA DSI results, genotypes were then grouped into four categories; resistant, moderate/intermediate resistant, susceptible and highly susceptible. Regression and correlation analyses were done to determine relationships (Amusa *et al*., 2013) between traits. Hierarchical cluster analysis was carried out to determine relationships among all genotypes. Hierarchical cluster analysis grouped genotypes into classes according to their similarities based on the morphological parameters. Similarities were calculated from each parameter by using Euclidean test (Harding and Payne, 2012).

3.3 Results

3.3.1 General observation on studied variables

The analysis of variance results for the parameters used to assess soybean resistance to *Callosobruchus chinensis* are presented in Table 3.1. Significant differences were observed (P<0.05) in 100 seed weight, initial weight, final weight, percent weight loss, adult bruchid emergence, Dobie susceptibility index, percent insect emergence, growth index and median

development period; indicating genetic variability in the studied germplasm. No significant differences ($p>0.05$) on number of eggs laid were observed among the genotypes.

Descriptive statistics of the studied parameters for 498 genotypes are presented in Table 3.2. The studied germplasm was genetically diverse and showed greatest variability on percent seed weight loss (CV=56.99%), followed by growth index (CV=50.52%). Median development period showed least variability (CV=11.67%). The calculated single variable percent coefficients of variation indicated that percent seed weight loss in soybean germplasm was more dispersed than DSI.

Table 3.1: Mean squares for selected traits used to assess soybean resistance to *Callosobruchus chinensis* in Uganda

		Mean square									
Source of Variation	**d.f**	**100 seed wt**	**Initial wt**	**Final wt**	**% wt loss**	**Eggs**	**Adults**	**DSI**	**% IE**	**GI**	**MDP**
Genotypes	499	37590000	4304000	4631000	108.31	17.04	13.08	4.2	5.901	0.1011	0.002737
Blocks	2	1224000	4302	10910	4.47	2.34	2.38	0.999	0.3	0.4603	0.001529
Residual	998	12260000	276200	407400	74.97	17.38	10.39	3.26	3.815	0.3565	0.001662
Probability		<.001	<.001	<.001	<.001	0.595	0.001	<.001	<.001	<.001	<.001
%CV		25.4	7.6	10.3	82	38.3	44.3	35.6	30.2	45.9	2.7

Key: d.f =degrees of freedom, DSI= Dobie susceptibility Index, IE=Insect Emergence, GI= Growth Index, MDP= Median development Period

Table 3.2: Descriptive Statistics of selected traits used to assess resistance to *Callosobruchus chinensis* in Uganda

Parameter	Mean	Minimum	Maximum	CV (%)
100 seed weight (mg)	13865	5225	26481	18.00
Initial seed weight (mg)	6942	2713	13538	17.29
Final seed weight (mg)	6220	2309	12322	20.00
Number of eggs laid	136.10	24	312.30	37.88
Adult emergence	64.53	2.67	172.30	47.91
Percent insect emergence	45.36	6.31	90.67	33.82
Percent seed weight loss	10.47	0.02	27.18	56.99
DSI	5.07	0.70	8.14	23.35
Growth Index	2.09	0.07	5.75	50.52
MDP	31.58	18.67	43.33	11.67

3.3.2 Relative susceptibility

Figure 3.1 presents DSI ranges of the studied 498 genotypes. Less than 1% of the genotypes were resistant, 19.08% of the genotypes showed moderate resistance, 54.82% were susceptible and 25.5% were highly susceptible indicating genetic variability in the studied germplasm. Genotype AVRDC G8527 had the lowest DSI (0.704), followed by PI G89 (1.67), whereas AVRDC G 2043 had the highest DSI (8.14), (Table 3.4).

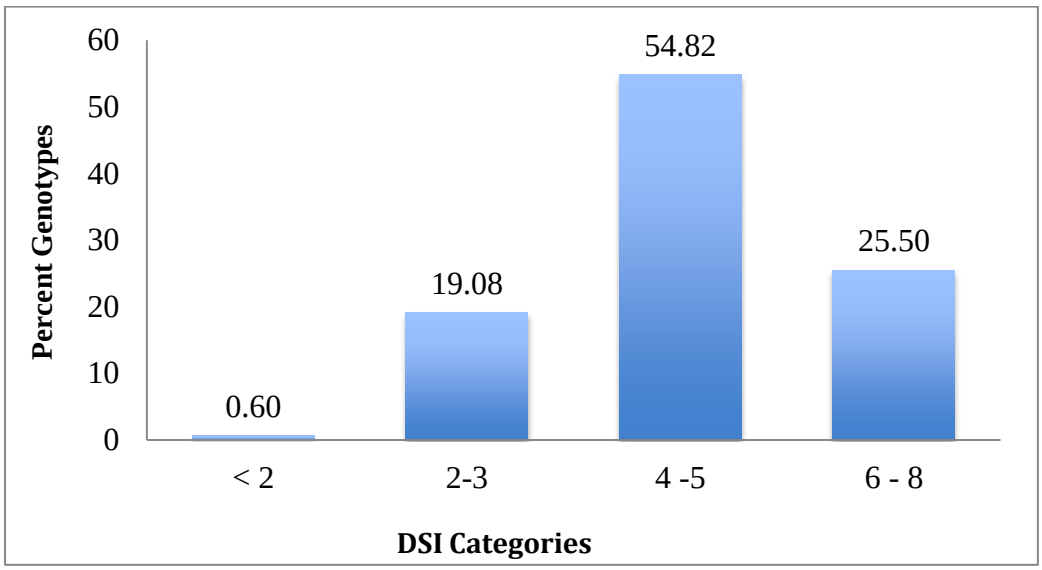

Figure 3.1: Ranking of soybean genotypes based on Dobie Susceptibility Indices (DSI)

3.3.3 Seed size

The results on seed size of the studied genotypes based on 100 seed weights are presented on

Table 3.3. From the studied genotypes, 56.24% had their 100 seed weights above the experimental mean of 13,898 mg. Most of the genotypes (43.17%) were large seeded; followed by medium seeded (39.76%) genotypes. The largest seeded genotype was AGS 329 (26481 mg); followed by AGS 292 (22367 mg); whereas the smallest seed size was observed on USA 33 (5225 mg); (Table 3.4).

Table 3.3: Seed size categories of the studied soybean genotypes in Uganda

Category	Range (mg)	Genotypes (%)
Small	5000 < 12000	15.66
Medium	12001 < 14000	39.76
Large	14001 < 20000	43.17
Very large	20001 < 27000	1.41

3.3.4 Bruchid injury and soybean seed weight loss

Genotype AVRDC G8527 had the least seed weight loss (0.02%), followed by AVRDC SRE-B-15C (0.36%); whereas USA 7 recorded the highest seed weight loss (27.18%), (Table 3.4). Over 45% of the genotypes had percent weight loss above the experimental mean (10.47) within three months of storage. The resistant genotypes had average weight loss of 3.85%, the moderate resistant genotypes had weight loss of 5.44% and the susceptible genotypes had 9.85% loss; while the highly susceptible genotypes had an average weight loss of 15.68%.

3.3.5 Bruchid population dynamics

3.3.5.1 Oviposition

C. chinensis laid eggs on all 498 studied genotypes. Over 50% of the screened genotypes had eggs less than experimental mean (136.1) (Table 3.4). Genotype OBS 116 had the highest number of egg counts (312), followed by NGDT 1.33-2 with 306 eggs. On the other hand AVRDC G8527 had the least number of eggs (24) (Table 3.4). However, there was no significant difference amongst genotypes on the number of eggs laid on them.

3.3.5.2 Magnitude of infestation

Genotype AVRDC G8527 had the least mean of adult bruchid emergence (2.67), while the highest mean was observed on USA 7 (172) (Table 3.4). The mean adult bruchid emergence for the resistant genotypes was 14.22; the moderate resistant 29.10, susceptible genotype 45.47; while the highly susceptible genotypes had a mean of 100.25 adults.

Table 3.4: The 10 most resistant and 10 most susceptible genotypes of the 498 Evaluated in Uganda

Genotype	Source	Seed coat colour	Seed size	100 seed weight	Initial weight (mg)	Final weight (mg)	% weight loss	Number of eggs	Number of adults	% IE	MDP (days)	GI	DSI	Status
AVRDC G 8527	AVRDC (Taiwan)	Green	Small	8335	4198	4197	0.02	24.00	2.67	6.31	27.00	0.07	0.70	R
PI G89	AVRDC (Taiwan)	Cream	Medium	12137	6402	6081	4.46	26.33	4.67	23.86	43.33	0.12	1.67	R
G 7955	AVRDC (Taiwan)	Cream	Medium	13031	6293	5839	7.08	131.00	35.33	44.27	25.67	0.95	1.82	R
Elite Lines 4.11-11	Uganda	Cream	Large	14407	6824	6623	3.10	42.67	20.33	32.66	26.67	0.63	2.05	MR
S-Lines 13.2A	Uganda	Cream/black	Small	8455	3977	3842	3.44	140.00	12.00	7.18	23.67	0.36	2.12	MR
S-Lines 9.2	Uganda	Cream	Small	9566	6411	4926	15.94	52.00	14.00	17.58	24.33	0.38	2.12	MR
AVRDC SRE-B-15C	AVRDC (Taiwan)	Cream/black	Large	16495	8248	8219	0.36	77.00	7.67	9.21	36.67	0.24	2.14	MR
PI G49	AVRDC (Taiwan)	Green	Small	11105	6928	5759	13.86	76.33	11.00	11.03	38.67	0.34	2.15	MR
AVRDC 8586	AVRDC (Taiwan)	Green	Small	11756	5866	5591	4.60	74.67	19.33	15.13	24.67	0.58	2.31	MR
PI G43	AVRDC (Taiwan)	Cream/black	Medium	12563	5881	5833	0.78	34.00	9.00	29.64	36.00	0.25	2.34	MR
BSPS 52 C-1	Uganda	Cream	Large	14324	7356	6041	17.26	187.00	125.33	69.50	29.33	4.43	7.01	VS
BSPS 75B	Uganda	Yellow	Medium	13426	6869	5134	25.05	184.30	112.33	61.02	29.00	3.87	7.12	VS
Bulindi 56	Uganda	Cream	Medium	12422	6129	4702	23.21	229.00	151.67	66.19	30.33	5.05	7.15	VS
Bulindi 31	Uganda	Cream	Medium	12639	6496	4947	23.90	239.30	160.00	66.82	30.67	5.21	7.19	VS
S-Lines 3.17	Uganda	Cream/black	Small	10368	7526	5974	20.66	272.70	149.67	69.45	30.00	5.00	7.24	VS
Obs 116	Uganda	Yellow	Large	14757	7381	5848	20.78	312.30	146.33	47.08	29.67	4.95	7.31	VS
USA 7	USA	Yellow	Medium	13662	6708	4896	27.18	290.70	172.33	60.47	30.33	5.75	7.31	VS
Bulindi 4B	Uganda	Yellow/green	Medium	13162	7549	5712	24.37	282.30	157.67	56.22	31.00	5.37	7.33	VS
Bulindi 77B-1	Uganda	Yellow	Medium	13666	7033	5761	18.06	225.00	119.00	50.34	27.67	4.68	7.51	VS
AVRDC G 2043	AVRDC (Taiwan)	Yellow	Large	16726	8227	7342	10.82	216.70	93.33	40.73	24.00	4.18	8.14	VS
F.pr				<0.001	<0.001	<0.001	<0.001	0.595	0.001	<0.001	<0.001	<0.001	<0.001	
LSD (0.05)				6272	941.4	1143.5	15.51	7.468	5.775	3.4987	0.07305	1.0695	3.2345	

* Data was analysed for 498 genotypes but only most resistant and very susceptible are presented for clarity. The Statistics is for the entire experiment (498 genotypes). R=Resistant, MR= Moderate Resistant, S= Susceptible, VS= Very Susceptible, IE = Insect Emergence, MDP = Median Development Period, GI =Growth Index, DSI =Dobie Susceptibility Index. Results for the entire experiment are presented in the Appendix 1.

Of the 498 studied genotypes, 52.41% had the number of adult bruchid emergence below the experimental mean value of 64.53.

3.3.5.3 Insect median development period

Results on median development periods (MDP) of the 498 studied genotypes are presented in Figure 3.2. Eighty-six percent of the genotypes had MDP range between 30-39 days, 13% had MDP range of 16-29 days, and 1% had MDP range of 40-45 days. No genotype had MDP below 15 days. However, 57.23% of the genotypes had MDP above the mean experimental mean (31.58 days). The predominant MDP was 31 days. Genotype PI G89 had the longest MDP of 43.33 days; followed by AVRDC G84051-31-2 (2) with 41 days; while Bulindi 12 had the least MDP of 18.67 days (Table 3.4).

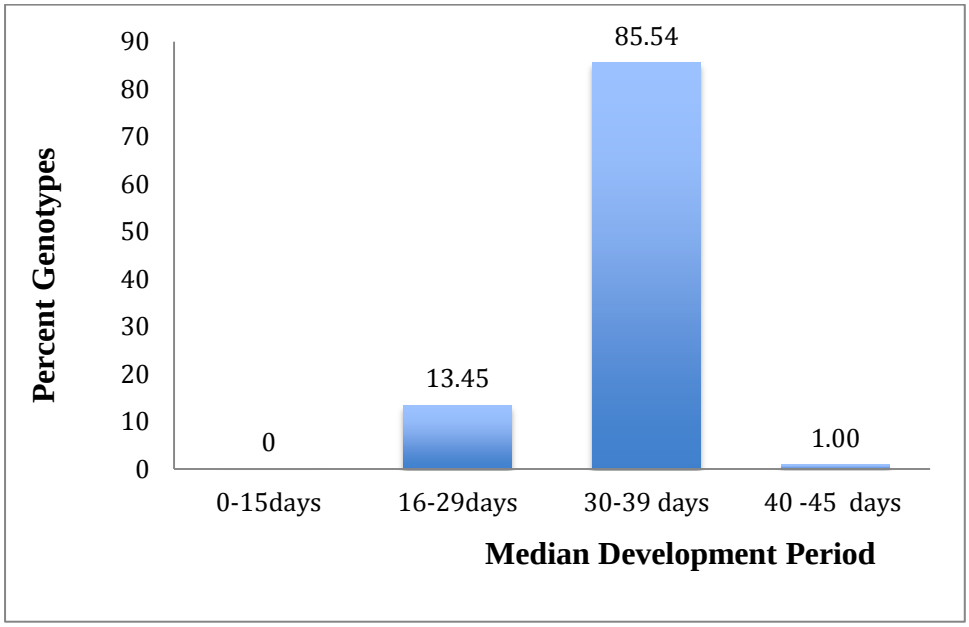

Figure 3.2. Median Development Periods of *C. chinensis* on screened soybean genotypes

3.3.5.4 Insect growth index

Out of the 498 genotypes, AVRDC G8527 had the least growth index (GI=0.066), followed by G89 with GI=0.124; whereas the highest GI was observed from USA 7 (GI=5.75) (Table 3.4). Fifty-three percent of the genotypes had GI below the experimental mean of 2.09.

3.3.6 Relationships between variables

Results of correlation analysis are presented in Table 3.5. There was significant strong positive correlation between adult bruchid emergence and DSI (0.87), eggs (0.88), % weight

loss (0.73) and growth index (0.996). The same trait (adult bruchid emergence) also showed a significant positive correlation with adult bruchid emergence (ABE) (0.59) but a weak significant correlation (0.17) with median development period. DSI had a significant strong correlation with eggs (0.77) and growth Index (0.86). Seed size (100 seed weight) had no correlation with DSI and MDP, but had very weak correlation with eggs, ABE and GI. The results of a simple linear regression analysis of percent seed weight loss against adult bruchid emergence are presented in Figure 3.3. The results revealed that adult bruchid emergence would predict 62% (R^2=0.624) of percent weight loss of seed.

Table 3.5: Correlation coefficients (r) for experimental parameters, under *Callosobruchus chinensis* no-choice artificial-infestation on the 498 genotype samples

	100 seed weight	%weight loss	Number of eggs	ABE	% IE	DSI	MDP
% weight loss	0.0738*	-					
Eggs	0.0527*	0.6458**	-				
Adults	0.0559*	0.7316**	0.875**	-			
% IE	0.019ns	0.3573**	0.2614**	0.589**	-		
DSI	0.0419ns	0.5474**	0.7711**	0.8659**	0.669**	-	
MDP	-0.014ns	0.0551*	0.2024**	0.1741**	0.3865**	0.41**	-
GI	0.0603*	0.7293**	0.8669**	0.9962**	0.5828**	0.86**	0.14**

** Significant at p<0.01; * Significant at p<0.05; ns =Not significant. %IE= Percent Insect Emergence; DSI= Dobie susceptibility Index; MDP= Median Development Period, ABE=Adult bruchid emergence

Cluster analysis of the 27 most resistant and 14 most susceptible genotypes is presented by the dendrogram in Figure 3.4. Two major groups designated as cluster I and cluster II, were observed. Five sub-clusters are highlighted with brown dots. Genotypes from same susceptibility category (DSI basis) are presented with the same font colour. Genotypes in red font are resistant to bruchids, black are moderate resistant and the genotypes in blue are the very susceptible. Cluster analysis revealed that the resistant genotype AVRDC G8527 was closely related to a moderate resistant genotype S-line 13.2A (Figure 3.4). In cluster I, genotype AVRDC G4890-21-13-13 was very unique from the entire group. Analyzing its characteristics, AVRDC G4890-21-13-13 had a very high growth index (GI) as compared to other genotypes in the same group. Group II comprised of susceptible genotypes, with Bulindi 14A and AVRDC G2043 being very similar.

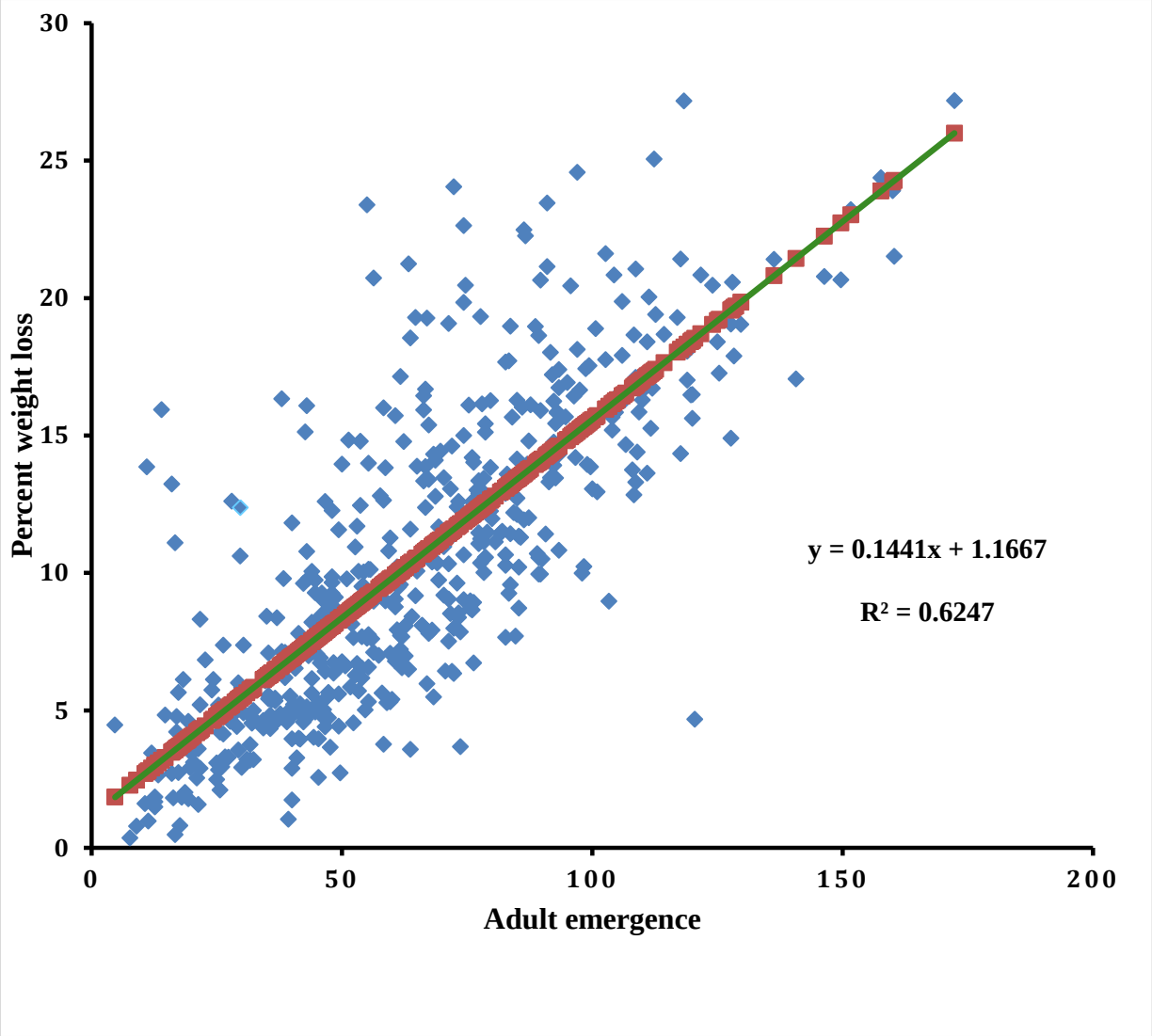

Figure 3.3: Regression of percent seed weight loss of soybean against adult bruchid emergence for the 498 genotypes

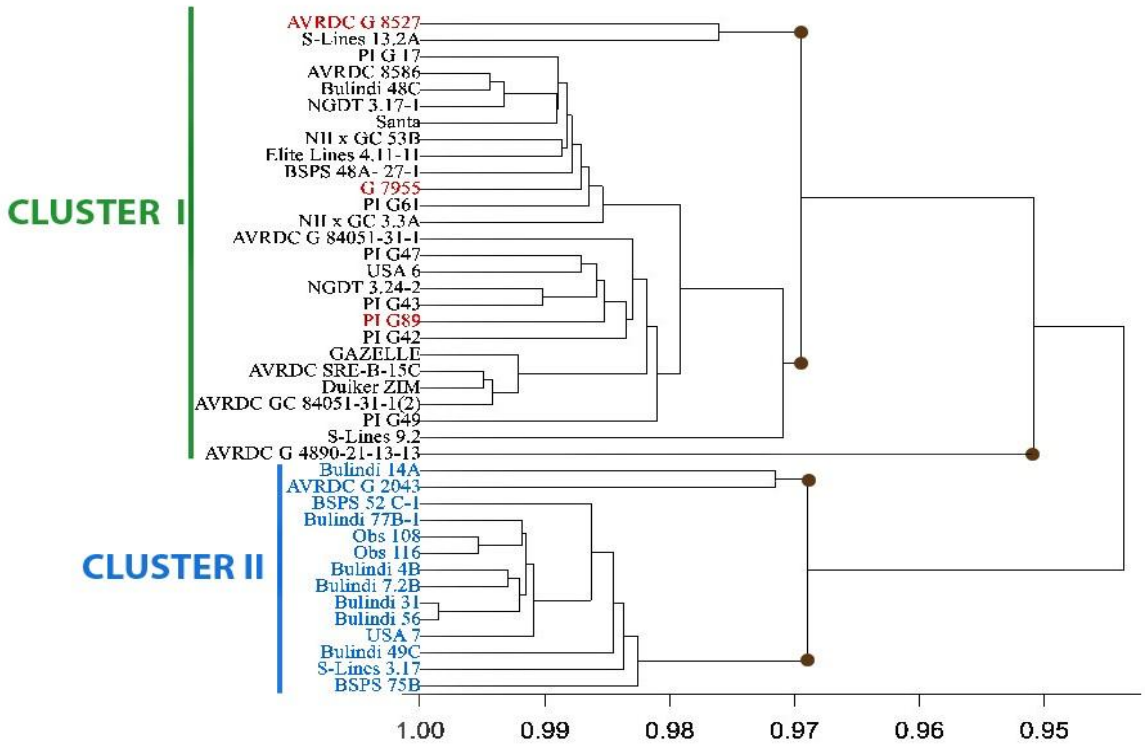

Figure 3.4: Relationships amongst 27 most resistant and 15 most susceptible genotypes.

3.3.7 Confirmation of resistance

Confirmation of resistance results for those genotypes re-subjected to a no-choice test are presented in Table 3.6. Two genotypes AVRDC G8527 and G89 were confirmed resistant to *Callosobruchus chinensis*. Ugandan commercial varieties Maksoy 2N, Maksoy 3N, Maksoy 5N and UG 5 were confirmed susceptible while Maksoy 1N and Maksoy 4N were moderately resistant. It was also confirmed AVRDC G8527 was more similar to S-Line 13.2A as earlier alluded to in section 3.4.6 (figure 3.4).

Table 3.6: Confirmation of resistance to *Callosobruchus chinensis* on selected genotypes

Genotype	Iwt	Fwt	swl	%swl	Eggs	ABE	%IE	GI	MDP	DSI
PI G89	3.39	3.16	0.23	6.78	82.49	12.80	15.52	0.36	43.44	2.55
AVRDC G8527	5.12	4.79	0.33	6.42	97.82	12.60	12.98	0.30	42.67	2.58
G43	2.63	2.57	0.06	2.35	69.49	4.70	6.86	0.17	75.00	2.80
S-Line 13.2A	5.40	4.75	0.65	11.99	195.82	26.00	29.93	0.85	37.00	3.25
SRE-B-15C	7.66	7.41	0.25	3.22	194.82	24.70	14.03	0.35	40.33	3.42
AVRDC 8586	4.73	4.46	0.27	5.84	169.49	31.20	21.10	0.52	40.67	3.42
USA 6	4.96	4.74	0.22	4.58	127.16	28.30	30.35	0.76	40.33	3.59
Maksoy 4N	9.23	8.52	0.71	7.67	85.16	43.30	60.25	1.57	30.67	3.66
NAM III	4.44	3.88	0.56	12.51	133.49	51.00	35.08	0.97	38.33	3.67
Maksoy 1N	3.21	2.91	0.31	9.49	70.82	27.30	42.54	1.12	38.00	3.77
SANTA	8.47	7.83	0.64	7.69	100.16	54.00	58.20	1.64	36.67	3.81
NII X GC53B	7.26	6.73	0.53	7.17	168.16	48.30	28.60	0.72	39.67	3.90
NIIX GC 3.3A	7.68	7.07	0.60	7.93	116.49	44.00	37.50	1.02	37.67	4.25
G42	4.04	3.62	0.42	10.21	65.16	42.30	65.18	1.75	37.33	4.28
AVRDC 8451-31-1	7.03	6.37	0.66	9.36	106.16	61.00	50.65	1.41	36.67	4.28
NGDT 3.24-2	6.67	6.11	0.55	8.29	79.16	49.00	64.30	1.74	37.00	4.39
SIESTA	8.99	8.34	0.65	7.23	98.49	62.70	64.24	1.65	39.00	4.55
Gazelle	6.35	5.87	0.49	7.73	76.49	46.00	64.29	1.83	35.33	4.62
G61	5.14	4.33	0.81	15.70	198.82	70.30	39.80	1.07	37.67	4.65
G49	4.35	3.62	0.73	16.75	179.49	67.70	37.36	1.05	36.00	4.90
Bulindi 48C	6.90	5.98	0.92	13.11	82.16	84.00	72.29	1.95	37.00	4.97
G7955	6.26	5.25	1.01	16.11	216.82	100.00	45.73	1.21	38.00	5.25
S-Line 9.2	4.64	3.85	0.79	16.82	161.16	82.00	52.60	1.47	36.00	5.29
Duiker	8.00	6.89	1.11	13.85	140.49	97.00	67.75	1.94	35.00	5.62
Maksoy 2N	4.11	3.49	0.62	15.36	126.49	61.70	48.84	1.27	20.67	8.66
Maksoy 3N	7.23	6.26	0.97	13.45	132.49	77.30	57.97	1.66	21.47	8.79
AVRDC 2043	4.75	3.76	0.99	21.00	106.38	63.20	62.63	1.75	19.67	9.15
UG 5	8.00	6.54	1.46	18.12	202.16	131.00	67.09	1.92	21.22	9.88

Maksoy 5N	4.78	3.72	1.06	22.06	185.82	111.70	59.91	1.65	20.33	10.07
Fpr	<0.001	<0.001	0.004	0.004	0.291	0.055	0.5	0.509	<0.001	0.04
LSD (0.05)	0.47	0.68	0.569	8.98	124.7	62.59	370.8	10.61	9.74	1.03

Iwt=Initial seed weight, Fwt= Final seed weight, swl=Seed weight loss, IE=Insect Emergence, MDP = Median Development Period, GI =Growth Index, DSI =Dobie Susceptibility Index.

3.4 Discussion

This study was set out to quantify the damage caused by *C. chinensis* and identify the sources of resistance in the germplasm in Uganda. *Callosobruchus chinensis* was used to challenge 498 soybean genotypes under no choice condition, in a laboratory. The results in this study demonstrated that soybean genotypes responded differently to *C. chinensis* infestation (Table 3.1), indicating that there is variability in genotypes susceptibility levels. The variation in genotype susceptibility was basically due to variations in adult bruchid emergence, percent weight loss, median development period and growth index. The study identified two genotypes AVRDC G8527 and PI G89 as resistant to *C. chinensis.* These could be progenitors for bruchid resistance in soybean breeding. Since less than 1% of the genotypes were resistant, it implied that search for resistance gene sources might have to go further. This is in agreement with Dong *et al*. (2001), who reported that sources of resistance to bruchids from cultivated legumes are low.

The variability showed by DSI indicated existence of genetic diversity among tested germplasm collection and could provide parent materials for genetic studies and breeding for resistance. Mechanisms of resistance were beyond the scope of this study; nonetheless, it can be speculated that genotypes possess different intrinsic and extrinsic factors of different levels, which conferred different resistance levels most probably through antibiosis. Osman and Ibrahim (1991) reported that bruchids resistance in soybean was due to presence of saponins, anti-nutritional factors, high fat and protein content, which inhibit larval development. Osman and Ibrahim (1991) further reported that both antixenosis and antibiosis mechanisms were important in soybean resistance to bruchids. Similar findings were reported by Lephale *et al*. (2012) in beans and Amusa *et al.* (2013) in cowpea.

The hypothesis of variability to *C. chinensis* infestation was further highlighted by variations among test genotypes in percent weight loss (Table 3.4). The resistant genotypes were not immune to *C. chinensis*, but suffered considerably less weight loss compared to the susceptible genotypes. Weight loss is an economic loss indicator and an economic loss of 10.47% within three months of storage justifies intervention measures to avoid enormous yield losses. The percent seed weight loss observed in this study was higher than that reported by Sharma and Thakur (2014a), which was 4.93%. Reports on losses due to bruchid

damage vary from crop to crop (Sharma and Thakur, 2014b); (Ebinu *et al.,* 2016) and genotype to genotype (Sharma and Thakur, 2014c) and (Gevina *et al.,* 2016).

Lack of significant difference among genotypes on number of eggs laid, implied that the genotypes did not influence oviposition by female bruchids. This finding therefore implied that no genotype was totally rejected for oviposition. Results of this study demonstrated that oviposition trait alone failed to distinguish genotype suitability for *C. chinensis* development. This finding is in agreement with the theory that where the insects have no choice, females oviposit on hosts in which the chances for larval survival are low or absent (König *et al.*, 2016). This survival behaviour is basically associated with unpredictability of the environmental resources. Somta *et al.,* (2006) and Swella and Mushobozy (2009) reported that *Callosobruchus spp* could oviposit on any seed, even though the seed might not be suitable for development of insects. Amusa *et al.* (2014) reported that the number of eggs laid by insects was less important than the rate of oviposition in its influence of the rate of multiplication. Nonetheless, the number of eggs is important as a resistance assessment trait because it helps to know whether there was a likelihood of getting adults since no adults would emerge from zero laid eggs (Azeez and Pitan, 2014).

Number of adult bruchid emergence indicates the magnitude of infestation and the loss of market value of the crop (Emeka, 2010). The resistant genotypes like AVRDC G8527 were characterised by delayed and low adult bruchid emergence suggesting antibiosis mechanism; on the contrary, susceptible genotypes were associated with early adult bruchid emergences in high numbers. Each adult bruchid emerging leaves a hole on the seed, which implied loss of appeal in the market (Kananji, 2007), and could lead to loss of seed viability (Kumar and Kalita, 2017). It can be speculated that genotype variations in magnitude of infestation by bruchids observed in this study could be due to differences in levels of antinutritional factors in genotypes (Amusa *et al.,* 2014).

Median development periods varied markedly among genotypes, with genotypes PI G89 and AVRDC G84051-31-2 (2) having the longest development periods (Table 3.4); indicating that such genotypes probably were either hard-textured (Soumia, 2015) and difficult to ingest or digest by the larvae; partially toxic (Gevina *et al.,* 2016); and/or nutritionally inadequate to support optimal development rates of the pest (Hiiesaar *et al.,* 2009). Nutritionally inadequate diets have been reported to extend development period (Kananji, 2007) due to antibiosis

(Hiiesaar *et al*., 2009). The results also indicated shorter development periods compared to results of Sharma and Thakur (2014a), who reported it to be 40-50 days, implying that soybean can no longer be stored safely for more than a month without some form of protection in Uganda. This finding has a negative impact on the farmers who would be forced to sell their produce as soon as they harvest a situation characterized by low prices and thus low profits.

Insect Growth Index, which is an indicator of genotype suitability to *C. chinensis* development, showed that the insect had capacity to infest and develop on all soybean genotypes tested, but with varying difficulty. Larval development within the seed depends on chemical composition of the grain (Sharma and Thakur, 2014c). The inability of *C. chinensis* to develop at the same rate in the genotypes was probably an indication that genotypes had varying contents of saponin (Swella and Mushobozy, 2009), fat content (Tripathi *et al.*, 2013), protein-carbohydrates ratio (Srinivasan and Durairaj, 2007) and/or other biochemicals. Maximum growth of *C. chinensis* was on genotype USA 7, implying that this genotype had the least anti-nutritional factors and, therefore, was the most suitable genotype for development of the bruchid; while AVRDC G8527 had the most anti-nutritional factors which made it the least suitable.

Plant breeding is facilitated when desirable genes are strongly associated on the chromosome (Acquaah, 2007). Absence of significant correlation between 100 seed weight and DSI in this study (Table 3.5) suggests that the association between these two variables was curvilinear or non-linear (Acquaah, 2007). This explanation was true for all other non-significant associations, with correlation coefficients closer to zero. Furthermore, these results suggested that resistance in soybean did not really depend on the nutritional factors nor space but presence of anti-nutritional factors which may not depend on the seed size or seed density (Sharma and Thakur, 2014c). The weak correlation between 100 seed weight with eggs and adults suggested that oviposition and adult bruchid emergence did not depend on 100 seed weight which was similar to what Dent (2000) reported that seed weight was a very complex variable in legumes and as such it does not have linear relationships with other variables (Acquaah, 2007).

The correlation coefficients (Table 3.5) suggested that the number of adults' emergence could be used for predicting resistance in soybean because it had an almost perfect positive

correlation with growth index and strong correlation with DSI. Similar findings were reported by Kananji (2007), who worked on beans and Hiruy and Getu (2018) on maize. The strong positive correlation between GI with percent weight loss indicated that rapid insect growth and development could lead to high percent seed weight losses. On the other hand, resistant genotypes reduced the number of adult bruchid emergence thereby minimizing the post-harvest losses. If the resistance in the genotypes with low GI could be enhanced it would be an environmental friendly way of reducing losses from *C. chinensis.* The breeding implication is that genotypes with high adult bruchid emergence, means high DSI, GI and % seed weight loss; which means increased susceptibility, increased suitability for *C. chinensis* development and high economic loss for the farmer. Consequently, it means when breeding for resistance to *C. chinensis*; there is need to select against adult bruchid emergence.

Regression analysis of percent seed weight loss against adult bruchid emergence (Figure 3.3) implied that post-harvest losses due *C. chinensis* increased with increase in adult bruchid emergence which eventually would lead to increased economic losses. Adult bruchid emergence explained 62% of the variability in percent seed weight loss. The information generated is important for determining economic injury levels for *C. chinensis* in soybean in Uganda. Tefera *et al.,* (2011) and Musa and Adeboye (2017) reported that increased adult bruchid emergence produces a corresponding increase in percent weight loss in grains until there is no more food for larva development in the grains.

Cluster analysis was performed to see if observations naturally group themselves in accord with the already measured variable DSI (Berkerey, 2007). Results from cluster analysis of 27 most resistant and 15 most susceptible genotypes (Fig 3.4) implied that geographical distances between sources of accessions were not associated with genetic distances among genotypes. Since genotypes did not cluster according to sources of origin it confirmed that these genotypes were never selected for resistance before (Bansal *et al.,* 2013). However, the genetic gap between resistant and susceptible genotypes was evident suggesting that the variability was an important trait for classification of germplasm. The results reflected the resistance categories which further confirmed the variability presented and discussed in Table 3.1 (Berkerey, 2007). The similarity of genotype AVRDC G8527 to S-line 13.2A suggest that these genotypes would be equally used as sources of resistance genes to *C. chinensis*.

Results from the confirmation test (Table 3.6) indicate that AVRDC G8527 and PI G89 can

confidently be used as sources of resistance to *C. chinensis*. The results further indicated that the officially released varieties (Maksoy 2N, 3N, 4N and 5N) in Uganda were susceptible, and will thus need to be introgressed with bruchid resistant genes.

3.5 Conclusions

Based on the datasets generated in this study, it is apparent that; *Callosobruchus chinensis* is a major storage pest for soybean in Uganda, causing damage of up to 10.5 % through seed weight loss within 3 months of storage. Soybean genotypes have varying degrees of resistance to *C. chinensis* with AVRDC G8527 being the most resistant followed by PI G89. The resistant genotypes identified in this study could be used as sources of resistance genes to introgress in the soybean cultivars of choice which are susceptible to *C. chinensis.*

However, identification of sources of resistance to bruchids through infestation takes long time. Since the findings much more pointed to antibiosis which means presence of chemical factors as such there is need to find out if the resistance in identified sources is associated with biochemical factors. Identification of sources of resistance through biochemical analysis would be faster and make determination of resistance reliable. This is considered in detail in the next chapter.

Identification of resistance sources is but the beginning of a long venture to deploy insect resistance genotypes into agricultural production systems. There is therefore need to carry out a genetic analysis to understand the nature of gene actions for effective use in breeding programme. This is considered in detail in Chapter 5.

CHAPTER FOUR

BIOCHEMICALS ASSOCIATED WITH ADZUKI BEAN BRUCHID RESISTANCE IN SOYBEAN

4.1 Introduction

Soybean (*Glycine max*) contributes 25% to the global edible oil and about two-thirds of the world's protein concentrate for livestock feeding (Agarwal *et al.,* 2013). Soybean has all of the essential amino acids in adequate quantities except for methionine and tryptophan (Zarkadas *et al*., 1993) which makes quality of protein from soy products almost equivalent to animal sources but with far less saturated fat and zero cholesterol (Young, 1991; Zarkadas *et al*., 2007). Soybean nutrition value plays a great role in reducing diabetes, heart diseases, rickets (Agarwal *et al.,* 2013), osteoporosis, memory loss, fibroids and cancer (Hassan, 2013). Soybean is a key forex earner for countries such India, Brazil, Argentina, USA (Agarwal *et al.,* 2013), Malawi, Zambia and South Africa (Tinsley, 2009).

Soybean production is however threatened by post harvest losses due to bruchids which are the major storage pests in legumes worldwide (Onyido *et al.,* 2011). Adults are non feeders, it is the larvae of the bruchids that burrow into seedpods and seeds and the insects usually continue to multiply during seed storage (Franco *et al.,* 2002). The damage causes extensive losses, especially if the seeds are stored for long periods (Spradbery, 2013). Bruchids infest soybean to satisfy their food and shelter requirements resulting in qualitative as well as quantitative losses. *Callosobruchus chinensis* is an internal feeder as such is only discernible after considerable damage is caused (Srivastava and Subramanian, 2016). *C. chinensis* causes losses of 32-64% varying between crops as well as genotypes within the crop (Swella and Mushobozy, 2009).

Use of pesticides to manage bruchids is effective; however it is expensive, dangerous to humans, the environment and pests easily develop resistance. The most environmentally friendly and cost effective method especially in the developing world to control bruchids would be the use of resistant varieties. Host plant resistance to pests is ubiquitous, but there exists great variation in levels expressed by plants (Dent, 2000). The level of resistance will obviously depend on the specific morphological and biochemical defenses utilized by the plant (Dent, 2000).

From chapter three it was evident that substantial genetic variations exist in the soybean germplasm. Investigation indicated that resistant soybean seeds adversely affected bruchids by reducing adult bruchid emergence and elongating the median development period (Plate 4.1). These variations demonstrated that genotypes exhibited different capacities in affecting biology of *C. chinensis* which we speculated that it might be through antibiosis (Guo *et al.,* 2012). Effect of chemical composition of grains and host plants on the host plant resistance has been reported by a number of workers (Keneni *et al.,* 2011; Pavithravani *et al.,* 2013; War *et al.,* 2013 and War *et al.,* 2017). Plant secondary metabolites such as lignins, tannins, alkaloids, quinines and lectins play an important role in the seed defense against insects (Pavithravani *et al.,* 2013). Studies have documented that soybean contains a number of compounds that are responsible for resistance to herbivorous attack such kunitz trypsin inhibitor, nicotine, mitogen activated protein kinase, peroxidase and terpenoids (War *et al.,* 2015). These compounds have been reported to be associated with resistance to field pests such as looper larvae (Wille *et al*., 2017), *Helicorvepa armigera*, and aphids (Wang *et al.,* 2015). However, there is limited information on compounds associated with *C. chinensis* resistance in soybean. Studies to determine metabolites associated with resistance to *C. chinensis* would provide suitable information in parents selection for breeding and help to confirm resistance identified through observation of morphological traits.

It was therefore deemed necessary to carry out an investigation with the aim of identifying biochemicals that are associated with *C. chinensis* resistance in soybean genotypes. In this study it was hypothesized that (i) soybean contains more than one type of metabolite which are responsible for and associated with bruchid resistance; (ii) soybean genotypes contain varying amounts of resistance metabolites. Identification of biochemicals associated with resistance would quicken the selection of sources of resistance for a bruchid resistance breeding programme in soybean.

Plate 4.1: Bruchids damage on soybean genotypes. Note variability on the number of emergence holes between the two genotypes

4.2 Materials and methods

Eight soybean genotypes with differing response to bruchid infestation were used in this study, (Table 4.1). Genotypes were selected based on their performance in the screening test (Chapter 3) and seed availability. Seed materials used in the study were from the same field at MUARIK. For each genotype, three biological replicates were taken to the laboratory. All reagents used in the study were sourced from BDH Laboratory suppliers–Uganda. This work was done at National Crops Resources Research Institute (NacRRI) Laboratory in Uganda.

For each genotype, concentration of secondary metabolites and nutritional factors were determined. The secondary metabolites which were studied included peroxidase activity, flavonoids, phenolics, alkaloids, tannins, phytic acid, lipid peroxidation and total antioxidants while the nutritional factor were reducing sugars, starch and protein. These metabolites were selected because they have been associated with resistance to field pests in soybean and storage pests in other legumes. Seeds were ground into fine powder using a mortar and pestle.

4.2.1 Nutritional factors

Proteins were determined using a method as described by Nuwamanya *et al.* (2014) with modifications. A sample of 0.3 g was weighed into clean tubes and 2 ml of distilled water added. The samples were heated in a water bath at 80 ^{0}C for 30 minutes, then left to settle. Samples were filtered and 0.1ml transferred to clean tubes in triplicate. Then 3 ml of Bradford reagent were added and mixed with the samples, then left to stand for colour development. Absorbance was read at 595nm and bovine serum albumin was used to prepare

the standard curve to estimate the protein concentration in samples (Sales *et al.,* 2000).

Starch content was estimated by following the method reported by Parthiban *et al.* (2012) with some modifications. One hundred mg of the sample was homogenized in hot 80 percent ethanol to remove sugars. The residue was retained after centrifugation. The residue was washed with hot 20% ethanol till the washings did not give colour with anthrone reagent. The residue was dried well in a water bath and 5 ml of water and 6.5 ml of 52% perchloric acid were added. Starch was extracted at 0°C for 20 minutes. The extract was retained after centrifugation and extraction repeated with fresh perchloric acid. The extracts were pooled after centrifugation and the volume was made up to 100 ml with 52% perchloric acid. To 0.2 ml of the extract, 0.8 ml of distilled water and 4 ml of anthrone reagent were added and the resultant mixture was heated for 8 minutes in a boiling water bath and cooled rapidly. The colour intensity was read at 630 nm using a spectrophotometer (Parthiban *et al.*, 2012).

A modification of the method by Nuwamanya *et al*. (2014) was used to estimate the reducing sugars. A sample of 0.5 ml from the soaked soya beans was placed into a test tube, followed by addition of 0.5 ml of 5% phenol solution. The contents were shaken well and 1 ml of distilled water was added, followed by addition of 1 ml of concentrated sulphuric acid. The samples were gently shaken and then left to cool and develop a blue colour in the dark for 10 minutes. Absorbances were read in a spectrophotometer at 490nm (Parthiban *et al.,* 2012). A glucose standard curve was used to estimate the reducing sugars in the samples.

4.2.2 Secondary metabolites

Based on their biosynthetic origins, plant secondary metabolites can be divided into three major groups: (i) flavonoids, allied phenolic and polyphenolic compounds; (ii) terpenoids and (iii) nitrogen-containing alkaloids and sulphur-containing compounds. Phenolics range from simple, low molecular-weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols. Phenolics can be classified into two groups: the flavonoids and the non-flavonoids (Crozier *et al.*, 2016).

Flavonoids are polyphenolic compounds comprising fifteen carbons, with two aromatic rings connected by a three-carbon bridge. The main subclasses of flavonoids are the flavones, flavonols, flavan-3-ols, isoflavones, flavanones and anthocyanidins (Ho, 1992).

The main non flavonoids of dietary significance are the C_6–C_1 phenolic acids, most notably

gallic acid, which is the precursor of hydrolysable tannins, the C_6 –C_3 hydroxycinammates and their conjugated derivatives, and the polyphenolic C_6–C_2–C_6 stilbenes (Crozier *et al.*, 2016).

To determine the phenolic acids in the soybean seed, the Folin-Ciocalteu method adopted from Wong *et al.* (2009) was used. Total phenolic acids were extracted using methanol (50 ml), from 1 g of the sample with continuous swirling for 1 hour at room temperature using an orbital shaker. Extracts were filtered under suction and stored at -20 °C for further use (Wong *et al.,* 2009). Soybean extract of 300 µl in triplicate were introduced into test tubes to which 1.5 ml of Folin-Ciocalteu's reagent (diluted 10 times with distilled water) and 1.2 ml of sodium carbonate (7.5% w/v) were added respectively. The reaction mixture was shaken, and then allowed to stand for 30 minutes at room temperature and the absorbance of the resulting blue coloured mixture was measured at 765 nm against a blank prepared by dispensing 300 µl of distilled water instead of sample extract. Total phenolic acid content was expressed as gallic acid equivalent (GAE) in mg/g.

Table 4.1: Soybean materials used in the study and their susceptibility variables

			Weights			Insect Susceptibility Variables					
Genotype	**Source**	**Resistance status**	**Initial**	**Final**	**% loss**	**Eggs**	**ABE**	**% IE**	**MDP**	**DSI**	**GI**
AGS 292	Taiwan	Very susceptible	10678	9616	9.95	171.7	89.67	51.72	29.67	6.459	3
AVRDC G8527	Taiwan	Resistant	4198	4197	0.02	24	2.67	6.31	27	0.704	0.07
PI G89	Taiwan	Resistant	6402	6081	4.46	26.3	4.67	23.86	43.33	1.667	0.12
Maksoy 1N	Uganda	Susceptible	6312	5833	7.6	112.7	56	50.83	32.67	5.221	1.71
Maksoy 2N	Uganda	Very susceptible	6698	5995	10.5	186.3	90	48.22	31	6.359	3.02
Maksoy 3N	Uganda	Susceptible	8660	8270	4.5	104.3	40.33	36.45	19.67	4.048	1.36
S-Lines 13.2A	Uganda	Moderate resistant	3977	3842	3.44	140	12	7.18	23.67	2.12	0.36
S-Lines 9.2	Uganda	Moderate resistant	6411	4926	15.94	52	14	17.58	24.33	2.123	0.38

%IE=percent Insect emergence, MDP= Median Development Period, GI= Growth Index, DSI= Dobie susceptibility index.

Source: Msiska *et al.* (2018), from chapter 3 (objective1).

Total flavonoid content was determined using aluminum chloride method as reported by Kale *et al.* (2010). A methanolic extract of 0.5 ml was dispensed into test tubes, followed by 1.5 ml of methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was shaken, and then allowed to stand at room temperature for 30 minutes before absorbance was read at 514 nm. Total flavonoid content was expressed as quercetin equivalent (QE) in mg/g material.

Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes: hydrolyzable and condensed tannins (King and Young, 1999). Tannins are defined as antinutrients of plant origin because they can precipitate proteins, inhibit digestive enzymes and decrease the utilization of vitamins and minerals (Amarowicz, 2007). In this study, condensed tannins were determined. The quantitative tannin content in samples was estimated by the method of Price and Butler (1977) as reported by Mrudula and Prabhu (2014) with some modifications. Known concentration of methanolic extract were taken and made up to 0.5ml using distilled water. To this reaction mixture, 1 ml 1% potassium ferricyanide ($K_3Fe(CN)_6$) and 1ml 1% $FeCl_3$ were added and the volume was made up to 10 ml with distilled water. The reading of the resultant solution was measured spectrophotometrically at 720nm after 5 min using tannic acid as a standard. The tannin content was expressed as mg of tannic acid equivalent/100g of sample.

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species (Pisoschi *et al.*, 2011). Antioxidants are involved in the defense mechanism of the organism against the attack of free radicals. Antioxidants can be enzymes, non enzymatic and nutritional compounds. Examples of antioxidants are peroxidases (Pisoschi *et al.*, 2011), flavonoids (Heima *et al.*, 2002), tannins (Amarowicz, 2007), albumins (Pisoschi *et al.*, 2011), and polyphenols (Heima *et al.*, 2002). The total potential antioxidant activity of the investigated soybean genotypes was determined using a method described by Ahmed *et al.*, (2015) with some modifications. Plant material was extracted using 50% aqueous methanol and then centrifuged to separate residues from supernatant. In a test tube, 0.1 ml of the plant extract was added, followed by 2.5 ml of phosphate buffered saline (PBS). Thereafter, 2.5 ml of 1% potassium ferricyanide was added and the solution incubated for 20 minutes at 50^0 C. After, 2.5 ml of 80% phosphoric acid was added. The sample was centrifuged at 10000 rpm for 10 minutes and 5ml of supernatant was transferred into a fresh tube. Then 5ml of distilled water was added,

followed by 1 ml of 0.1% ferric chloride. The sample was mixed well and absorbance read at 700nm.

Alkaloids were determined using the method of Babu and Hedge (2012) modified by Vijay and Rajendra (2014) where 1 g of sample was ground in 5 ml of 90% ethanol and then refluxed at 85^{o} C for 40 minutes. The sample was filtered and the filtrate evaporated to dryness at 45^{o} C in a vacuum concentrator. The resulting residue was dissolved into 3 ml of phosphate buffer (pH = 4.5) and transferred into a separating funnel. The resulting solution was mixed with 3 ml of bromocresol green solution and let to stand for 30 minutes. Then 5 ml of chloroform was added and vortexed for 2 minutes, then let to settle for 10 minutes. The lower layer in the funnel was then separated off. The extraction was continued for 3 more times and the extracts were mixed in a volumetric flask. Using 90% ethanol as blank, extracts were analyzed by using a UV–Vis spectrophotometer at a wavelength of 418 nm (Vijay and Rajendra, 2014).

Lipid peroxidation was determined in terms of content of malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, which is considered a useful index of general lipid peroxidation. The MDA was calculated using the extinction coefficient, ε= 155 nMol^{-1} cm^{-1}), following the method of Heath and Packer (1968) as reported by (Hodges *et al.*, 1999):

MDA equivalents (nmol.ml^{-1}) $= \left[\frac{(A_{532}-A_{600})}{155000}\right] 10^{6}$

where A_{532} nm represented the maximum absorbance of the TBA-MDA complex, A_{600} nm the correction for nonspecific turbidity, and 155000 the molar extinction coefficient for MDA.

Ground soybean sample (0.1 g) was homogenized in 5 ml 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged for 5 min (15000 x g, 4.0 ^{0}C) and 1ml aliquot of the supernatant collected was mixed with 4 ml of 0.5% (w/v) thiobarbituric acid (TBA) diluted in 20 % (w/v) TCA. The mixture was incubated in water bath at 95 ^{0}C for 30 minutes and then the reaction was stopped by quickly cooling the sample in an ice bath. The sample was further centrifuged for 10 minutes (10000 x g, 4,0 ^{0}C) (Taulavuori *et al.*, 2001). Absorbance was measured at 532 and 600 nm. The value of non specific absorption at 600nm was then subtracted. MDA content was then expressed as nmol MDA per g.

Peroxidase activity was determined using a method by Shannon *et al*. (1966). A 100mg sample of each genotype was extracted with 2 ml of 0.1 M potassium phosphate buffer (pH=7.5) containing 1% mM EDTA, 1% polyvinylpyrolidone (PVP) and 10mM β-mercaptoethanol using prechilled pestle and mortar. The contents were then transferred into eppendorf tubes and centrifuged (PrismaR, Edison, New Jersey, USA) at 1,000 rpm for 20 minutes. Thereafter, 0.02 ml of the supernatant were placed into a test tube containing 3 ml of 0.1M potassium phosphate buffer (pH=6.5). The reaction mixture, without the H_2O_2, was measured as a blank. The reaction was initiated by adding 0.8M H_2O_2 and the breakdown of the H_2O_2 was monitored for 2.5 minutes at a 30 second interval, at 24 ^{0}C, by recording absorbances at 470 nm in a spectrophotometer (Biowaveii+, Cambridge, England). The activity of the enzyme was calculated and expressed as absorbance units/min/mg (Mizobutsi *et al.,* 2010).

Phytic acid was determined using a method reported by Yahaya *et al*. (2013). Four grams of ground sample was soaked in 100 ml of 2% hydrochloric acid (HCl) for 3 hours and then filtered through two layers of filter paper, 25 ml of the filtrate was placed in a 250 ml conical flask and 5 ml of 0.3% ammonium thiocyanate (NH_4SCN) solution was added as an indicator, 53.5 ml of distilled water was then added to reach the proper acidity. This mixture was titrated against ferric chloride ($FeCl_{3)}$ solution, which contains about 0.00195 g of iron per ml of $FeCl_3$ solution until a brownish yellow colour that persisted for 5 minutes was obtained (Ileke, 2014). The result was multiplied by factor 1.95 to obtain phytate P. Phytate P result was multiplied by factor 3.55 to convent to phytate.

4.3 Data Analysis

All absorbance readings and titration measurements were done in triplicates. One way Analysis of variance (ANOVA) with genotype as a treatment structure of seed metabolites concentrations was done using Genstat 12th edition statistical software package. Prior to analysis data sets were tested for ANOVA assumptions. Correlation analysis was carried out between the metabolites and the morphological susceptible parameters in Genstat 12th edition.

4.4 Results

The results of the quantitative estimation of secondary metabolites associated with resistance to *Callosobruchus chinensis* in soybean are presented in Table 4.2. Significant differences ($P<0.05$) were observed amongst genotypes for tannins, flavonoids, total anti-oxidants and phenolic acids. Genotypes did not significantly differ in the concentration of alkaloids and phytic acid. Table 4.3 presents results on primary metabolites estimation in different soybean genotypes. Genotypes did not show significant differences for lipid peroxidation, proteins and starch but for reducing sugars. Comparison of the concentrations of phenolic acids, flavonoids, tannins and alkaloids is presented in Figure 4.1. Tannins were the most abundant secondary metabolites.

4.4.1 Secondary metabolites

Analysis of variance showed significant differences in tannins among soybean genotypes ($P<0.021$) (Table 4.2). Tannin concentration for the studied genotypes ranged from 0.296 to 1.845 mg of tannic acid equivalents per 100g sample. Genotype AGS 292 had the lowest tannin concentration while AVRDC G8527 had the highest. Tannin concentration was highest in resistant genotypes and lowest in susceptible genotypes.

Total antioxidants (TAOX) concentration was highest in resistant genotypes and lowest in susceptible genotypes ($P<0.023$). AVRDC G8527 had the highest total antioxidants concentration (1.98 AU $min^{-1}mg^{-1}$) followed by Maksoy 3N (1.78 AU $min^{-1}mg^{-1}$) while susceptible AGS 292 had the least concentration (0.39 AU $min^{-1}mg^{-1}$).

Flavonoids concentration was highest in susceptible genotypes than in the resistant genotypes ($P<0.001$). The most susceptible genotype, AGS 292 had the highest concentration of flavonoids (31.22 mg QE per 100g) followed by Maksoy 2N (22.14 mg QE/100g) while AVRDC G 8527 had the least content of flavonoids (5.13mg QE/100g) (Table 4.2).

Alkaloid concentration in soybean seeds ranged from 0.14 - 0.27 mg of AE per g of extract. The highest content was measured in AGS 292 and the lowest in Maksoy 2N (Table 4.2). Nevertheless, there were no significant differences in alkaloid concentration between the resistant genotypes and susceptible genotypes.

The results of Phytic acid content in different soybean genotypes showed that there were no

significant differences among the soybean genotypes. The highest phytic acid was in S-Line 13.2A (11.19mg/100g) and lowest was in AGS 292 with 8.88mg per 100g (Table 4.2).

The highest phenolic acids concentration was recorded in a resistant genotype PI G89 (1051 mg of GAE per 100g) followed by AGS 292 (554.4 mg GAE per 100g) while the least concentration was recorded in Maksoy 1N (139.1 mg GAE per 100g) (Table 4.2).

Table 4.2: Mean concentrations of the secondary metabolites in 8 soybean genotypes

Genotype	Tannins	TAOX	Flavonoids	Alkaloids	Phenolics	Phytic Acid
	mgTAE /100g	$AUmin^{-1}mg^{-1}$	mgQE/100g	$AUmin^{-1}mg^{-1}$	mg GAE/100g	mg/100g
AGS 292	0.296	0.391	31.22	0.27	554.4	8.88
AVRDC G8527	1.845	1.978	5.13	0.17	148.5	10.15
PI G89	1.21	1.324	5.88	0.19	1051.1	9.23
Maksoy 1N	1.013	1.176	6.11	0.16	139.1	10.04
Maksoy 2N	0.394	0.509	22.14	0.14	572.1	9.58
Maksoy 3N	1.651	1.778	8.37	0.16	368.6	10.61
S-Line 13.2A	0.441	0.565	8.56	0.26	367.6	11.19
S-Line 9.2	0.968	1.112	10.91	0.25	249.7	10.96
P-Value	0.021	0.023	<.001	0.369	0.043	0.175
LSD	*0.937*	*0.957*	*9.209*	*0.143*	*532.985*	*1.878*

TAOX= Total antioxidants, TAE= tannic acid equivalent, QE =Quercetin equivalent, GAE= Gallic Acid equivalents, AU= Absorbance Units

However though genotypes showed significant difference in phenolic acids, there were no defined differences in phenol concentrations between the susceptible and resistant genotypes suggesting that it may not be a good trait for determining resistance or susceptibility to bruchids in soybeans. There were no significant differences in lipid peroxidation amongst soybean genotypes indicating no difference in extent to which genotypes caused *C. chinensis* organ damage (Table 4.3). The highest lipid peroxidation activity was observed for PI G89 (1.16 nmol $MDA^{-1}g$) followed by Maksoy 3N (0.51 nmol $MDA^{-1}g$) and lowest in AVRDC G8527 and Maksoy 1N (0.01 nmol $MDA^{-1}g$).

4.4.2 Primary metabolites

There were no significant differences in starch content among the different genotypes indicating that starch had no effect on seed resistance to *C. chinensis*. Highest concentration was observed in S-Line 13.2A (64.13 $mg^{-1}100g$) and lowest was in AGS 292 (18.69 $mg^{-1}100g$).

Protein concentration did not differ significantly amongst the studied soybean genotypes. Highest protein concentration was observed in Maksoy 3N (48367.49 mg^{-1} 100g) and the lowest in S-Line 13.2A (28900.27 mg^{-1}100g). The results indicate that proteins were not responsible for resistance to *C. chinensis* in soybean.

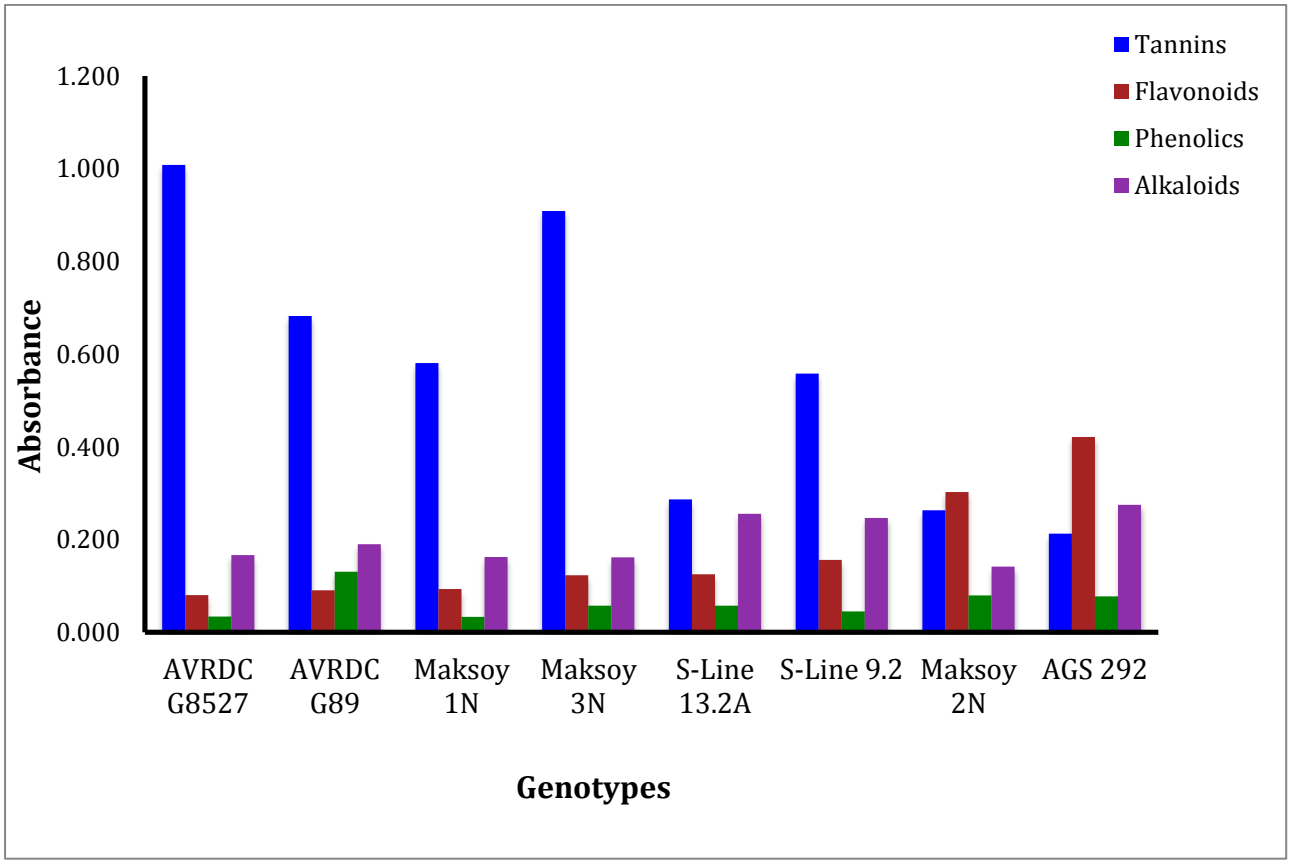

Figure 4.1: Comparisons of secondary metabolites Absorbance concentrations

Genotypes showed significant differences in concentration of reducing sugars (P<0.035) Table 4.3. The highest reducing sugar concentration was on AGS 292 and Maksoy 3N (0.007mg^{-1}100g) while the lowest reducing sugar concentration was recorded in Maksoy 2N (0.005 mg^{-1}100g). However, there was no trend observed between the resistant and susceptible genotypes signifying that reducing sugars may not be the basis for resistance to *C. chinensis* in soybean.

Table 4.3: Mean concentrations of the primary metabolites in 8 soybean genotypes

Genotype	Lipid Peroxidation	Protein	Reducing Sugar	Starch
	nmol MDA/g	mg/100g	mg/100g	mg/100g
AGS 292	0.02	35913.02	0.007	18.69
AVRDC G8527	0.01	33180.78	0.006	48.89
PI G89	1.16	47069.67	0.006	50.13
Maksoy 1N	0.01	39943.08	0.006	38.84
Maksoy 2N	0.02	43221.77	0.005	38.56
Maksoy 3N	0.51	48367.49	0.007	27.58
S-Line 13.2A	0.06	28900.27	0.006	64.13
S-Line 9.2	0.15	40785.52	0.006	49.01
P-Value	0.462	0.952	0.035	0.065
LSD	*0.583*	*38590.56*	*0.00117*	*27.165*

LP= Lipid peroxidation, PA= Phytic acid, RS= Reducing sugars, MDA= Malondialdehyde

4.4.3 Peroxidase Activity

Analysis of variance indicated that there were significant variations amongst genotypes for peroxidase activity (P<0.001), Table 4.4. However, peroxidase activity was not statistically affected by time (P=0.998) and the interaction between time and genotypes (P=1.00).

Table 4.4: Peroxidase Activity for 8 genotypes over 150 Seconds

SOV	d.f.	Mean Squares	F pr.
Blocks	2	0.060317	
Genotype	7	0.079175	<0.001
Time	5	0.000492	0.998
Genotype X Time	35	0.000016	1.00
Residual	94	0.008848	
Total	143	1.509642	

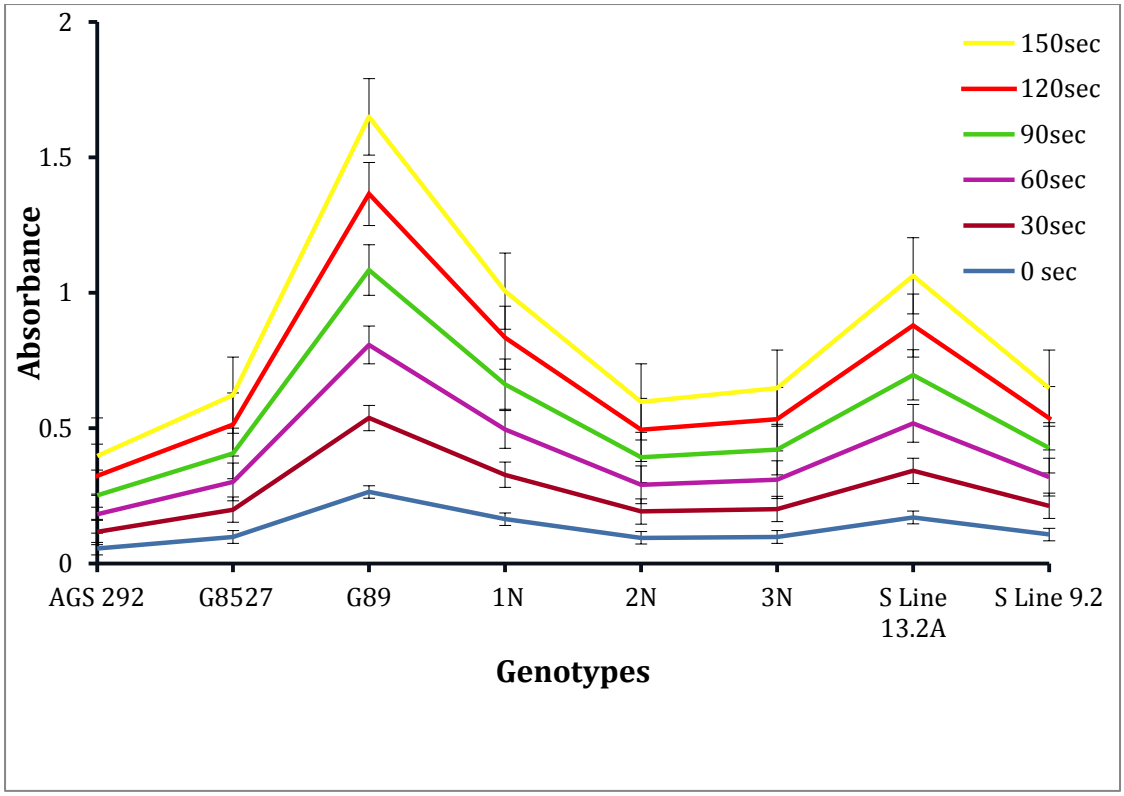

Figure 4.2: Peroxidase absorbances of soybean genotypes over 150 seconds

The results on peroxidase activity for the studied genotypes over a period of 150 seconds are presented in Figure 4.2. 0 sec was the immediate absorbance reading when the H_2O_2 was added and the sequential readings taken after every 30 seconds. Resistant genotype PI G89 had the highest peroxidase activity (0.28 AU min^{-1} mg^{-1}) followed by genotype S-Line 13.2A (0.18 AU min^{-1} mg^{-1}) while the least activity was observed on a susceptible genotype AGS 292 (0.07 AU min^{-1} mg^{-1}). Results in Figure 4.2 further shows genotypes consistency in their response with time; for example genotype PI G89 had highest activity at all observational times.

Correlations coefficients for biochemical and susceptibility variables are presented in Table 4.5. Peroxidase had a significant positive correlation with median development period ($r=0.69^{*}$). However, peroxidase activity had negative relationships with percent seed weight loss (r=-0.32), adult bruchid emergence (r= -0.50), DSI (r= -0.42), eggs (r= -0.41), insect growth index (r=-0.51) although not significant. These results indicate that peroxidase activity had a significant association with *C. chinensis* resistance through median development period.

Total antioxidants correlated negatively with resistance parameters such as percent seed weight loss (r=-0.15), number of eggs (r=-0.38), number of adult bruchid emergence (r=-0.17), dobie susceptibility index (r=-0.27), insect growth index (r=-0.18) and positively with median development period (r=0.38) even though not significantly. Tannins had a significant relationship with total antioxidants (r=0.99**) and flavonoids (r=-0.71*). Furthermore, tannins negatively correlated with percent seed weight loss, adult bruchid emergence, DSI, and insect growth index.

Flavonoids had a significant strong relationship with initial seed weight (r=0.70**). Results further showed a strong negative relationship between flavonoids and TAOX (r=-0.73**), a moderate positive relationship with adult bruchid emergence (r= 0.54) and DSI (r=0.53). Flavonoids had a negative association with MDP (r=-0.34). Phenolics showed a negative relationship with adult bruchid emergence, weight loss and DSI. Phenolics positively correlated with MDP (r=0.54) and peroxidase (r=0.55).

Table 4.5: Correlations for biochemical and susceptibility parameters of the different soybean genotypes

Variables	Protein	%wt L	Adults	Alk	DSI	Eggs	Flav	GI	I wt	MDP	Prx	Phe	RS	TAOX
Prot	-													
%wt L	0.36	-												
Adults	0.22	0.43	-											
Alk	-0.5	0.37	0	-										
DSI	0.26	0.45	0.98***	-0.02	-									
Eggs	-0.07	0.3	0.85**	0.21	0.85	-								
Flav	-0.1	0.26	0.54	0.37	0.53	0.46	-							
GI	0.23	0.42	0.99***	0	0.98**	0.85**	0.55	-						
I wt	0.39	0.58	0.85**	0.2	0.83**	0.59	0.70**	0.86**	-					
MDP	0.44	-0.06	0.01	-0.08	0.02	-0.21	-0.34	0.01	0.2	-				
Prx	0.24	-0.32	-0.5	-0.11	-0.42	-0.41	-0.58	-0.51	-0.4	0.69*	-			
Phe	0.47	-0.13	-0.08	0.05	-0.05	-0.13	0.22	-0.06	0.27	0.54	0.55	-		
RS	0.27	0.4	0.62	0.26	0.51	0.39	0.22	0.63	0.75*	0.47	-0.22	0.16		
TAOX	0.29	-0.15	-0.17	-0.51	-0.27	-0.38	-0.73**	-0.18	-0.26	0.37	0.13	-0.25	0.27	-
Tan	0.29	-0.16	-0.17	-0.51	-0.27	-0.38	-0.71**	-0.17	-0.24	0.37	0.12	-0.23	0.29	0.99**

Key for Table 4.5: Prot= Protein, %wtL= Percent seed weight loss, Alk= Alkaloids, DSI=Dobie susceptibility Index, Flav=Flavonoids, GI=Growth Index, Iwt=Initial seed weight, MDP=Median development period, Prx= Peroxidase, Phe=Phenolics, RS=Reducing sugars, TAOX=Total antioxidants, Tan=Tannins.

4.5 Discussion

Callosobruchus chinensis has been considered to be one of the most important storage pest on soybean in Uganda (Tukamuhabwa, 2015 personal communication). Host plant resistance is an important component of an integrated pest management strategy for the control of *C. chinensis* (Nahdy, 1995). Currently, some genotypes were identified as sources of resistance; however, empirical data on levels and basis of resistance within the soybean germplasm in Uganda is limited. This was the motivation for the study.

In this study, biochemical analysis was done on resistant, moderately resistant and susceptible genotypes to determine the basis for resistance to *C. chinensis.* Results from the present study indicated that resistant genotypes had higher tannin and total antioxidants concentration, peroxidase activity and lower flavonoids concentration compared to susceptible genotypes. Resistant and susceptible genotypes did not differ in concentration of nutritional factors such as starch and protein. Correlation analysis revealed that tannins, total antioxidants and peroxidase were correlated negatively to DSI, adult bruchid emergence, seed weight loss, insect growth index and percent insect emergence but positively to MDP.

Variations observed in tannins, total antioxidants, flavonoids, and peroxidases amongst soybean genotypes explained the differences observed in resistance levels of the genotypes and therefore be crowned as the basis for soybean resistance to *C. chinensis.* The results indicate that there is an array of compounds found in seeds that act either additively or synergistically against bruchids. They act either directly on bruchid through antibiosis or develop the non-preference for insects feeding on the seeds (War *et al.*, 2017). The secondary metabolites serve to reduce or destroy the palatability of the plant in which they are produced (Sharma and Thakur, 2014a).

Results of comparison of soybean secondary metabolites (Figure 4.1) indicate that phenolic acids, tannins, alkaloids and flavonoids were all present in all soybean genotypes studied. This finding is in agreement with the findings of Pereira *et al.* (2009) which state that phenolic acids, tannins, alkaloids and flavonoids are ubiquitous in seeds. The findings further highlight that there is a bigger potential in utilizing soybean antioxidants as a basis for resistance in addition to physical factors. Chung (2009) reported that tannins, flavonoids and isoflavones are the large constituents of the soybean antioxidants. Chung (2009) further

reported that cultivars of soybean have varying concentration of antioxidants. Presence of antioxidants indicate the capacity of a genotype to cause vital damage to organs in insects which is a firm defense mechanism related to bruchids (Kolawole and Kolawole, 2014).

Findings in this study showed that flavonoids were higher in susceptible genotypes than resistant genotypes (Table 4.2). This finding is agreement with the report by Lattanzio *et al.* (2006) who reported that most plants contain an array of flavonoids which phytophagous insects usually differentiate. Lattanzio *et al.* (2006) went further to explain that some flavonoids are feeding and oviposition stimulants to insects implying that genotypes with high concentrations of such flavonoids will be susceptible to insect pest attack. Alkaline midgut pH, surfactants, and the peritrophic membrane all may help these species tolerate flavonoid concentrations in the diet (Sales *et al.*, 2000). Furthermore, in comparison to many other secondary metabolites, flavonoids are apparently not very toxic to and have a low physiological activity in most insects (Harbone, 1980).

Significant differences observed in phenolic acids among genotypes (Table 4.2), did not show any defined pattern between the susceptible and resistant genotypes suggesting that it may not be responsible for susceptibility of genotypes to bruchids in soybean. Similar observations were reported by Mahatma *et al*. (2011) who reported increased content of phenolics, but without conferring any resistance. This could be attributed to the fact that plant phenolic acids were not toxic to insects unless prophenoloxidase genes are lost or the levels of phenolic acids exceed the catalytic activity of the gut prophenoloxidases (Wu *et al*., 2015). Prophenoloxidases which are produced in the foreguts detoxify phenols in the midgut of insects.

Tannins are the most abundant secondary metabolites made by plants. The significant differences in tannin concentration amongst resistant and susceptible genotypes implied that tannin played a role in soybean resistance to *C. chinensis*. Tannins are generally considered to be deleterious to phytophagous insects. Tannins may affect the growth of insects in three main ways: they have an astringent taste, which affects palatability, and decreases feed consumption; they form complexes of reduced digestibility with proteins and they act as enzyme inactivators (Winkel, 1998). Barbehenn and Constabel (2011) reported tannin toxicity in insects is thought to result from the production of high levels of reactive oxygen species (ROS), which react with high pH guts, forming semiquinone radicals and quinones.

When developing, larvae fed on the tanniferous soybean, tannins permeated the peritrophic envelopes thereby producing fatal lesions in insects midgut which subsequently led to reduced growth index of the insects in the resistant soybean genotypes (Barbehenn and Constabel, 2011). Since tannins produce astringent taste in food and reduce the bioavailability of proteins and minerals, they are generally not selected for in breeding programmes. This might have led to release of soybean varieties with reduced tannin content and perhaps the reason for increased bruchid infestation.

Genotype Maksoy 3N a susceptible genotype and a popular commercial variety in Uganda showed higher concentration of tannins which was difficult to explain, therefore more studies on the factors creating that situation could provide more explanation about it. This could probably suggest that *C. chinensis* detoxified the tannins through either sequestering, increased level of excretion with the chemical compound remaining unaltered, and/or altered biochemical composition of the compound so that it did not harm the consumer (Dowd *et al.,* 1983).

All genotypes contained phytic acids (PA), but with no significant differences suggesting that the resistance to *C. chinensis* in soybean was not due to PA. This finding was in contradiction with earlier studies in mungbean (Somta *et al.,* 2007) and cowpea (Fawki *et al.,* 2012) which indicated that PA was associated with bruchid resistance. However, Dhole and Reddy (2016) working on mungbean argued that higher concentrations of PA were required for the resistant reaction. Dhole and Reddy (2016) further indicated that even though the resistant gene may be present in a plant, reduced concentration of PA decreases tolerance to biotic stress. Under this reasoning therefore it was speculated that PA concentration in soybean was not high enough to affect bruchid's metabolic activities, growth and development.

The study established that there were no significant differences in proteins, lipid peroxidation and starch amongst genotypes (Table 4.3). This signified that nutritional factors were not associated with resistance to *C. chinensis* in soybean. Even with reducing sugars where genotypes showed significant differences there was no trend observed between the resistant and susceptible genotypes. This finding is of significant importance in soybean breeding programs for resistance to *C. chinensis* because it implies that nutritional factors of soybean will not be affected with breeding towards more bruchid resistance. These findings are in agreement with Sharma and Thakur (2014a) who reported that nutritional factors were not

responsible for resistance to bruchids in chickpea, cowpea and soybean. Sales *et al.* (2000) reported that nutritional factors such as proteins are ineffective against host specific bruchids such as *C. chinensis, C. maculatus* and *Zabrotes*.

Significant differences were observed in peroxidase activity between resistant and susceptible soybean genotypes (Table 4.4). Resistant genotypes showed higher levels of peroxidase activity than susceptible ones indicating that peroxidase negatively impacts *C. chinensis'* growth and development in soybean. These results imply that seeds possess enzyme-based biochemical defenses, which represent a fundamental mechanism of seed survival and longevity (Duan *et al.*, 2014). Similar results were reported in field beans by Babu and Hedge (2012) suggesting that peroxidase enzymes play a defensive role against bruchids. Peroxidases deter the feeding of insects and produce toxins that reduce plant digestibility, which in turn leads to nutrient deficiency in insects with drastic effects on their growth and development (War *et al.*, 2012). Furthermore peroxidases impair nutrition through forming electrophiles which oxidize mono-or dihydroxyphenols thereby directly causing toxicity in the guts of the insects (Zhu-Salzman *et al*., 2008).

Peroxidase in the presence of hydrogen peroxide catalyzes autoxidation of tannin compounds. Oxidized tannins react with proteins and decrease their nutritional quality (Barbehenn and Constabel, 2011). Hong *et al.*, (2015) reported that during plant defence mechanisms peroxidases have a cell wall cross-linking activity (formation of lignin, extensin cross-links, dityrosine bonds) and create a highly toxic environment (Li *et al.*, 2010) by producing vast amounts of ROS (oxidative burst, hypersensitive response; which results in adverse growth conditions for organisms. Results of peroxidase activity over time (Figure 4.2) showed consistency of genotypes position. There were no overlaps between genotypes indicating no interaction between time and genotype. This finding is of interest because it implies stability of the enzyme activity (Jun *et al.*, 2011) in the seed therefore a trait worthy selecting for in breeding for resistance to bruchids.

The correlation analysis results between the susceptibility and biochemical parameters (Table 4.5) indicated that anti-nutritional factors such as tannins, total antioxidants and peroxidase were associated with seed resistance in soybean implying that soybean genotypes with high tannin and total antioxidants would have lower number of adult bruchid emergence, longer median development periods, smaller DSI values, lower seed weight percent loss, delayed

growth index and consequently would be resistant to the *C. chinensis* attacks. In contrast, correlation analysis revealed that high flavonoid concentration would favour high numbers of adult bruchid emergence, seed weight loss, shorter development periods and consequently higher DSI which means more susceptibility to *C. chinensis* suggesting that when breeding for bruchid resistance flavonoids should be selected against. This further poses a challenge in breeding for *C. chinensis* resistance because flavonoids are generally selected for in soybean because some flavonoids such as isoflavones are beneficial in human diets for reduction of cancer and other ailments (Hassan, 2013). Breeding for resistance to *C. chinensis* in soybean would have to strike a balance or trade off with human consumption needs.

The negative relationship between peroxidase and DSI, adult bruchid emergence, weight loss and insect growth index indicated that peroxidase contributed positively to seed resistance to bruchids. This finding agrees with Khan *et al.* (2003) who reported that resistance-related enzymes such as chitinase, β-1,3-glucanase, and peroxidase are also involved in the bruchid resistance. Lattanzio *et al.* (2006) reported that the effectiveness of phenolics as resistance factors to insect feeding is enhanced by oxidation to polymers, which reduce digestibility, palatability and nutritional value. Thus, high levels of polyphenol oxidases and peroxidases, the major phenolic oxidising enzymes of plants, can be correlated with plant resistance mechanisms against insects. The non-significant relationship between peroxidase and seed weight parameters indicate that resistance to *C. chinensis* in soybean is independent of seed size. This finding is in agreement with Sharma and Thakur (2014b) who reported that seed size had no influence in susceptibility parameters of chickpea, soybean and cowpea.

Significant positive correlation between peroxidase activity and median development period implied that peroxidase activity contributed to slow development of *C. chinensis*. Slow development means less number of generations per year. As such, the result consequently means genotypes with higher peroxidase activity would be more resistant to *C. chinensis.* Therefore, peroxidase is amongst the biochemicals associated with *C. chinensis* resistance in soybeans. This finding is in agreement with Sharma and Thakur, (2014a), that peroxidase affected insect metabolic activities and inhibited growth of larvae in soybean hence the longer development periods. From the study it can be suggested that peroxidases confer resistance to *C. chinensis* through prolonged insect development periods. Peroxidase activity may therefore be used as a biochemical marker for bruchid resistance in soybean. This finding has practical applications in that soybean varieties with higher peroxidase activity can

be bred through genetic engineering (Dzhavakhiya and Shcherbakola, 2007). Moderate high negative correlation between peroxidase and flavonoids was an indication that they have an antagonistic relationship. Selecting for peroxidase activity in soybean will lead to negative selection for flavonoids (Yamasaki *et al.*, 1997).

An almost perfect correlation between tannins and total antioxidant signifies that tannins are the greatest component of the antioxidants; therefore during breeding, selecting for antioxidants will mean selecting for tannins as well. Soybean contains other antioxidants such as genstein but in lower quantities (Hassan, 2013).
There is further need to isolate these biochemicals and feed them to bruchids to determine if bruchids are directly affected by the biochemicals.

4.6 Conclusions

In the present research work, evaluated soybean genotypes contained varying concentration of biochemical factors. High tannins, total antioxidants, peroxidases and low flavonoids were the biochemical parameters associated with resistance in soybean. Breeding for resistance against *C. chinensis* in soybean should therefore consider these biochemical besides physical parameters. This study needs to be proceeded with an inheritance study of the associated factors to guide breeders variety development. In future, there is a need to carry out a diet study where bruchids would be fed directly by the biochemicals and then establish whether the metabolites either cause death or increase development periods.

CHAPTER FIVE

GENETICS OF RESISTANCE TO ADZUKI BEAN BRUCHID IN SOYBEAN POPULATIONS

5.1 Introduction

Adzuki bean bruchid (*Callosobruchus chinensis*) is a major storage threat to soybean in Uganda. Although this pest was reported on pigeon peas in the 1990s (Nahdy, 1995), it was not a known pest of soybean until recently as reported by Tukamuhabwa (2015, personal communication). Consequently, there has been limited genetic research work done on soybeans related to *C. chinensis.* To develop an efficient and successful soybean resistance breeding programme, understanding the gene action controlling resistance to adzuki bean bruchid is fundamental. Inheritance studies of resistance to storage pests have previously been conducted in other legumes (Somta *et al*., 2007) and cereal crops (Zunjare *et al.,* 2015). However, information on bruchid resistance inheritance in soybean is scanty. Genetic control of resistance to storage insect pests may range from monogenic to polygenic (Dent, 2000; Singh, 2009), with mostly additive and dominant genes governing storage insect pest resistance in many legumes. However, a few cases of cytoplasmic gene effects have been reported (Singh, 2009; Keneni *et al*., 2011).

Adjadi *et al*. (1985) reported high maternal effects, digenic inheritance and complete dominance of susceptibility indicating that cowpea resistance was controlled by two recessive genes. However, in a cross between mungbean and blackgram, Dongre *et al.* (1996) it was established that resistance to *Callosobruchus maculatus* was controlled by two dominant duplicate genes. Kananji (2007) while working on beans reported that although both additive and dominance gene actions governed expression of resistance, the dominance component was relatively more important for the bean bruchids. Kananji (2007) also reported low narrow-sense heritability in beans, suggesting that breeding for bruchid resistance cannot be easy.

This present study (chapter three), identified two genotypes AVRDC G8527 and PI G89 as possible sources of resistance from the 498 genotypes screened in Uganda (Msiska *et al.,* 2018). In chapter four, the basis for resistance was found to be associated with higher concentrations of some secondary metabolites. However, based on phenotypic performance

of resistance to bruchids and biochemical concentrations, some parents would look superior, though this may not provide adequate information to guide selection of elite parents and desirable cross combination required for systematic breeding. Performance of identified sources has to be assessed through combining ability studies. Information on combining abilities can be obtained by different mating design including a full diallel. GCA and SCA estimates of beans (Kananji, 2007), cowpeas and mung beans (War *et al.,* 2017) for different traits such as seed weight loss, adult bruchid emergence and DSI were reported to be important (Keneni *et al.,* 2011).

The present study was carried out to determine inheritance and the mode of gene action of resistance to *C. chinensis.* The results from this study will help in identification of most promising crosses that will ensure effective breeding for bruchid resistance in soybean.

5.2 Materials and Methods

Nine soybean genotypes Maksoy 1N, Maksoy 3N, Maksoy 4N, AVRDC G8527, G7955, S-Line 13.2A, S-Line 9.2, SREB-15C and UG 5 (Table 5.1) all previously characterized (Objective 1), were selected based on their resistance levels. These were crossed in a screen house at Makerere University Agricultural Research Institute- Kabanyolo (MUARIK) in the year 2016 using full diallel mating design to produce F1s (Plate 5.1) (Choudhary *et al.,* 2004). Soybean seeds used in the study were provided by the Makerere University Centre for Soybean Improvement and Development.

F1 seeds were grown in pots together with their corresponding female parent in the screen house to generate adequate F2 seeds while at the same time assessing if they were true crosses. The parents and F2 seeds were evaluated for resistance to *Callosobruchus chinensis* in a laboratory in a randomized complete block design (RCBD) with three replicates. A total of 10 seeds were weighed and placed in a petridish after 24 hours oven drying. The seeds were then infested with 10 unsexed 1-3day old bruchids. Data on initial seed weights, number of eggs, adult bruchid emergence (ABE), final weights were collected.

Plate 5.1: Soybean crossing in the screen-house at MUARIK 2016

Table 5.1: Parental Materials used in the study during 2016/2017

ID	Name	Pedigree	Source	Susceptibility status
1	Maksoy 1N	TGX 1835-10E	Uganda	Moderate Resistant
2	Maksoy 3N	GC 00138-29 x Duiker	Uganda	Susceptible
3	Maksoy 4N	Duiker x GC 00138-29	Uganda	Susceptible
4	G7955	Ankur	Taiwan	Resistant
5	AVRDC G8527	-	Taiwan	Resistant
6	S-Line 13.2A	-	Uganda	Moderate Resistant
7	S-Line 9.2	-	Uganda	Moderate Resistant
8	SREB-15C	-	Taiwan	Moderate Resistant
9	UG 5	-	Uganda	Very Susceptible

Source: Msiska *et al.* (2018)

Subsequently, seed weight loss (Amusa *et al.,* 2014), percentage seed weight loss, percentage adult bruchid emergence, growth index (Wijenayake and Karunaratne, 1999), median development period (Kananji, 2007), and susceptibility index (Dobie, 1974) were calculated following the formula already explained in Chapter 3 section 3.3.5.

5.3 Data Analysis

The assumptions of ANOVA were tested before data analysis using GenStat 12th Edition procedures. Data on GI and MDP were transformed using Log (base10) function. Data were then subjected to one-way analysis of variance with genotypes as treatment factor while replication as blocks using GenStat 12th Edition statistical package (Harding and Payne, 2012) following linear statistical model:

$$\gamma_{ij} = \mu + \tau_i + \beta_{j+}\varepsilon_{ij} \quad (5.1)$$

Where, Υij = observed value for the i^{th} genotype in j^{th} block, μ = Overall mean effect, τ_i = Genotype effect (fixed), $\beta_j = j^{th}$ block effect (random), ε_{ij} = Error term.

Means of parameters which were significantly different from the ANOVA were analysed using Analysis of Genetic Designs (AGD-R) statistical package (Rodriguez *et al*., 2015) to generate variance components, GCA, SCA and maternal effects. The statistical model for analysis was based on Griffing (1956) method 1 model 1 as described by Hallauer *et al.* (1988):

$$X_{ijk} = \mu + r_k + g_i + g_j + s_{ij} + m_{ij} + p_{ijk} \quad (5.2)$$

where:

Xijk is the value of the progeny derived from the crossing of the *ith* female parent with *jth* male parent, μ is the mean effect for all progenies, r_k is the replication effect, g_i is the GCA effects of the ith female parent, g_j are the GCA effects of the j^{th} male parent, s_{ij} is the SCA effect specific to the hybrid of the i^{th} female and the j^{th} male genotype, *mij* is reciprocal effects, p_{ijk} is the experimental error for the X_{ijk} observation (k = 1, 2, ..., 81 ; $i = j$ = 1, 2, ..., 9). Grrifing's method 1 model 1 is a numerical approach where genotypes are fixed, includes parents, progenies and reciprocals. Fixed model was used because parental genotypes were selected purposely, based on their levels of resistance to *C. chinensis* (Choudhary *et al.*, 2004).

GCA and SCA effects were estimated as described by Hallauer *et al.* (1988) respectively, as:

$$\hat{\sigma} i = \{1/[n(n-2)]\}(nX_{i.} - 2X_{..}) \quad (5.3)$$

$$\hat{s} ij = X_{ij} - [1/(n-2)](X_i + X_j) + \{2/[(n-1)(n-2)]\}X_{..} \quad (5.4)$$

Significance of GCA, SCA, maternal and reciprocal effects was determined by a *t* tests as described by Griffing, (1956) and Dabholkar (1999). GCA was used to estimate genetic variations in parents. Assuming no additive-by-additive interactions and other higher terms, GCA may be used to estimate heritability for traits. For traits with significant GCA effects, estimates for phenotypic variance, narrow (NSCGD) and broad (BSCGD) sense coefficients of genetic determination were determined. NSCGD and BSCGD are fixed effects equivalents of narrow and broad sense heritabilities. The heritability of a character has a major impact on the methods chosen for population improvement, in breeding, and other aspects of selection (Fehr, 1991). NSCGD, BSCGD and phenotypic variance were calculated by the formulae that follow respectively (Ruming, 2004):

$$NSCGD \sim h^2 = \frac{2\delta^2_{GCA}}{(2\delta^2_{GCA} + \delta^2_{SCA} + \delta^2_e)} \quad (5.5)$$

$$BSCGD \sim H^2 = \frac{(2\delta^2_{GCA} + \delta^2_{SCA})}{[2\delta^2_{GCA} + 2\delta^2_{SCA} + \delta^2_e]} \quad (5.6)$$

$$\delta^2_p = 2\delta^2_{GCA} + \delta^2_e \quad (5.7)$$

Baker's Ratio which estimates the relative significance of additive to non-additive gene effects (Mwije *et al.*, 2014) and allows inferences about optimum allocation of resources in hybrid breeding (Fasahat *et al.*, 2016) was calculated using formula:

$$= \frac{2\delta_{GCA}}{2\delta_{GCA} + \delta_{SCA}} \quad (5.8)$$

where: δ_{GCA}=General combining ability variance, δ_{SCA}= specific combining ability variance

5.4 Results

Mean squares for bruchid susceptibility parameters of soybean seed are shown in Table 5.2. Significant differences were observed amongst F_2 genotypes and parents for seed weight loss, adult bruchid emergence and dobie susceptibility index. No differences were observed amongst genotypes for percentage insect emergence, median development period and growth index in the F_2 genotypes.

Table 5.3 presents means on response of parents and F_2 genotypes to *C. chinensis.* The most susceptible genotypes were UG 5 (DSI=4.99), Maksoy 3N (DSI=5.96) and Maksoy 4N (DSI=6.08). Most resistant genotypes were S-Line 9.2 X Maksoy 3N (DSI=0.002), SREB-15C X Maksoy 3N (DSI=0.002) and SREB-15C X S-Line 9.2 (DSI=0.002). Genotypes with the highest ABE were Maksoy 3N (adults =68), UG 5 (adults=65) and Maksoy 4N (adults=53). The lowest number of ABE was observed in genotype S-Line 9.2 X Maksoy 3N, SREB-15C X Maksoy 3N and SREB-15C X S-Line 9.2 (adult=0.32). All crosses exhibited lower DSI values than their female parent except Maksoy IN and S-Line 9.2 crosses. Crosses with Maksoy 1N and S-Line 9.2 as female parent had higher DSI values than their female parent.

Table 5.2. Analysis of variance for *Callosobruchus chinensis* susceptibility parameters in F_2 generation

Source	df.	swl	swl%	Eggs	ABE	%IE	MDP	GI	DSI
Rep	2	0.03^{ns}	95.6^{ns}	3194.5^{*}	184.96^{ns}	101092^{ns}	239^{ns}	126.3^{ns}	9.52^{*}
Genotype	78	0.05^{**}	266.8^{**}	676.3^{ns}	426.08^{***}	123575^{ns}	218.9^{ns}	158.4^{ns}	4.61^{**}
Residual	154	0.03	163.10	701.50	90.60	91683	172.20	130.80	2.77
Total	234	0.04	197	714.4	203.23	102394	188.3	140	3.44

df.= degrees of freedom, swl= Seed weight loss, swl%=Percentage seed weight loss, Eggs= Number of eggs, ABE= Adult bruchid emergence, %IE=Percentage adult bruchid emergence, MDP=Median Development Period, GI= Growth index, DSI=Dobie susceptibility index, ns=not significant, ***=P<0.001, **=P<0.01, *=P<0.05

Table 5.3: Mean performance of Genotypes to *Callosobruchus chinensis*

Genotype	Wt	Fwt	Wt L	%wt L	Eggs	ABE	%IE	GI	MDP	DSI
1N	0.9768	0.9339	0.043	4.38	57.26	9.98	16.06	0.389	41.31	2.127
1N X 3N	0.801	0.741	0.0601	7.6	28.26	12.32	46.75	1.27	36.64	2.973
1N X 4N	0.6123	0.5673	0.045	7.21	54.93	15.32	32.32	1.016	31.98	3.795
1N X G7955	0.9396	0.8709	0.0688	7.36	20.59	8.98	42.46	0.52	10.31	1.482
1N X G8527	0.5246	0.4952	0.0294	5.7	31.26	5.65	19.75	0.515	40.31	1.363
1N X S-Line 13.2A	0.9618	0.8948	0.067	7.22	31.59	10.32	29.49	0.762	40.64	2.257
1N X S-Line 9.2	1.145	1.0691	0.0759	6.65	28.26	8.65	48.12	1.355	35.31	2.622
1N X SREB-15C	1.2222	1.1437	0.0786	6.5	33.93	12.32	36.62	0.946	24.98	2.154
1N X UG 5	1.0756	0.9535	0.1221	11.47	52.93	21.65	35.71	1.006	36.98	2.758
3N	0.9216	0.7357	0.1859	19.9	70.59	68.98	98.01	3.183	30.98	5.962
3N X 1N	1.0824	0.8152	0.2672	24.61	58.26	17.98	52.4	1.596	33.98	3.431
3N X 4N	1.1363	0.9314	0.2049	17.78	65.59	16.98	24.15	0.694	37.64	3.206
3N X G7955	1.2245	1.0063	0.2182	17.73	32.93	9.65	17.28	0.492	24.64	1.728
3N X G8527	1.251	1.017	0.234	18.38	52.93	15.98	35.96	1.068	34.64	3.115

Genotype	Wt	Fwt	Wt L	%wt L	Eggs	Adults	%IE	GI	MDP	DSI
3N X S-Line 13.2A	0.7835	0.6512	0.1323	16.56	53.59	21.65	41.14	1.125	35.98	3.547
3N X S-Line 9.2	1.1991	1.0032	0.1959	16.1	38.93	18.32	64.94	1.956	32.98	3.765
3N X SREB-15C	0.9016	0.7377	0.1639	18.39	23.93	8.32	27.63	0.847	21.64	2.24
3N X UG 5	1.2044	0.8268	0.3777	34.49	21.59	13.32	66.81	0.965	23.31	2.018
4N	1.163	0.9611	0.2019	18.09	69.93	53.32	79.44	2.368	16.64	6.084
4N X 1N	1.1406	0.7246	0.416	32.49	12.26	5.98	19.74	0.619	10.64	1.305
4N X 3N	1.1472	0.9434	0.2038	17.4	42.26	18.32	45.32	1.372	33.31	3.742
4N X G7955	1.1058	0.9324	0.1734	15.72	27.59	10.65	50.56	1.407	23.98	2.167
4N X G8527	1.1323	0.9024	0.2299	20.87	27.59	12.65	33.57	1.254	19.31	3.379
4N X S-Line 13.2A	0.9546	0.7698	0.1849	19.37	50.26	21.98	50.52	1.594	32.31	4.066
4N X S-Line 9.2	1.4007	1.068	0.3327	24.47	52.26	24.32	42.22	1.295	34.64	3.615
4N X SREB-15C	1.2384	1.0502	0.1882	15.49	24.59	8.65	18.44	0.596	10.31	1.519
4N X UG 5	1.3274	1.0611	0.2663	20.24	47.59	21.65	66.05	1.949	33.64	3.665
G7955	0.9629	0.8544	0.1085	11.38	36.26	11.32	42.42	1.17	35.98	2.499
G7955 x 1N	0.8996	0.8009	0.0987	11.14	39.93	5.65	15.27	0.427	23.98	1.587
G7955 X 3N	0.9432	0.8627	0.0805	9.04	38.93	11.98	33.43	0.895	37.31	2.874
G7955 X 4N	0.9715	0.7164	0.2551	24.21	59.59	28.32	52.39	1.502	33.98	4.156
G7955 X G8527	0.7888	0.6267	0.1622	20.36	53.26	22.65	44.36	1.263	35.64	3.742
G7955 X S-Line 13.2A	0.8378	0.7151	0.1228	14.54	36.93	14.98	46.38	1.306	35.64	3.131
G7955 X S-Line 9.2	0.8193	0.7922	0.0271	2.36	38.34	5.57	-3.15	-0.37	46.81	1.198
G7955 X SREB-15C	0.8805	0.8421	0.0384	4.25	46.26	21.65	48.21	1.36	35.98	3.708
G7955 X UG 5	0.8608	0.7588	0.1019	11.83	17.28	5.02	42.02	0.952	40.89	1.8
G8527	0.7043	0.675	0.0293	4.12	36.93	6.65	54.69	1.452	38.64	1.964
G8527 X 1N	0.8234	0.8088	0.0146	1.94	21.59	4.32	14.27	0.388	25.31	0.97
G8527 X 3N	0.8453	0.5702	0.275	36.21	39.59	10.32	34.23	0.486	34.98	2.138
G8527 X 4N	0.743	0.7057	0.0373	5.09	32.93	14.65	47.86	1.319	36.31	3.269
G8527 X G7955	1.0657	1.0284	0.0373	3.53	61.93	10.65	26.14	0.72	36.64	2.717

Genotype	Wt	Fwt	Wt L	%wt L	Eggs	Adults	%IE	GI	MDP	DSI
G8527 X S- Line 13.2A	1.4175	1.3881	0.0294	2.27	47.26	5.32	11.49	0.301	37.31	1.551
G8527 X S-Line 9.2	1.3298	1.2991	0.0307	2.04	18.93	8.32	26.72	0.841	28.98	1.909
G8527 X SREB-15C	1.03	0.718	0.312	27.32	34.93	4.65	9.12	0.264	24.64	1.566
G8527 X UG5	0.8963	0.8639	0.0324	3.65	15.26	6.98	30.82	0.876	25.98	1.237
S- Line 13.2A X SREB-15C	0.8264	0.7401	0.0863	9.25	37.93	10.32	21.7	0.58	41.31	2.086
S-Line 13.2A	0.8021	0.684	0.1181	15.19	42.59	10.98	32.18	0.902	35.31	2.885
S-Line 13.2A X 1N	0.7478	0.7124	0.0354	4.74	17.26	1.32	21.27	0.491	51.98	0.242
S-Line 13.2A X 3N	0.4729	0.3603	0.1126	26.43	57.26	5.32	10.06	0.26	39.64	1.812
S-Line 13.2A X 4N	0.3236	0.3953	-0.072	-11.66	29.06	3.66	-0.47	-0.15	45.14	0.897
S-Line 13.2A X G7955	0.6021	0.508	0.0941	15.41	47.26	9.65	19.98	0.603	36.98	2.474
S-Line 13.2A X G8527	0.5964	0.5507	0.0457	7.83	16.26	4.32	34.14	0.965	42.98	1.349
S-Line 13.2A X UG 5	0.7291	0.687	0.0421	5.74	23.93	1.98	9.79	0.232	29.64	0.689
S-Line 9.2	0.5936	0.5583	0.0354	6.09	17.59	1.98	9.78	0.288	23.31	0.844
S-Line 9.2 X 1N	0.4741	0.4415	0.0326	6.92	35.93	3.32	9.77	0.233	42.64	0.907
S-Line 9.2 X 3N	0.8529	0.5565	0.2964	25.88	12.59	0.32	1.67	0.042	15.31	-0.002
S-Line 9.2 X 4N	0.5488	0.5255	0.0233	4.93	26.59	3.65	13.44	0.326	42.64	1.247
S-Line 9.2 X G7955	0.513	0.4866	0.0264	5.03	48.93	4.32	9.41	0.245	40.31	1.384
S-Line 9.2 X G8527	0.6163	0.542	0.0742	11.97	34.26	9.32	32.79	0.846	37.98	2.52
S-Line 9.2 X S-Line 13.2A	0.4406	0.4261	0.0144	3.49	26.93	1.32	4.62	0.115	35.64	0.416
S-Line 9.2 X SREB-15C	0.5837	0.4733	0.1105	18.91	47.26	8.32	17.75	0.487	36.64	2.447
S-Line 9.2 X UG 5	0.4706	0.4236	0.047	9.59	18.26	3.65	17.62	0.442	26.64	1.058
SREB-15C	0.6204	0.5503	0.0701	10.3	27.59	10.32	58.33	1.473	37.98	2.677
SREB-15C X 1N	0.5381	0.4777	0.0604	10.47	22.26	7.32	30.23	0.878	22.98	1.956
SREB-15C X 3N	0.714	0.6757	0.0383	5.31	16.93	0.32	1.34	0.035	14.98	-0.002
SREB-15C X 4N	0.5985	0.555	0.0435	8.27	42.59	3.98	9.92	0.252	27.31	1.209
SREB-15C X G7955	0.8048	0.6727	0.1321	16.37	34.93	7.98	20.37	0.573	23.64	1.676
SREB-15C x G8527	0.7062	0.6487	0.0575	8.21	21.59	8.65	38.21	1.302	29.98	2.639

Genotype	Wt	Fwt	Wt L	%wt L	Eggs	Adults	%IE	GI	MDP	DSI
SREB-15C X S-Line 9.2	0.7321	0.6863	0.0457	6.09	24.26	0.32	3.72	0.08	16.64	-0.002
SREB-15C X UG 5	0.8878	0.7975	0.0902	10.3	41.59	11.32	31.97	0.89	40.64	2.473
SREB-I5C X S-Line 13.2A	0.9359	0.758	0.1779	18.83	54.93	13.32	20.94	0.586	36.31	2.729
UG 5	1.2346	0.8795	0.3551	28.07	28.93	64.98	1827	65.23	37.64	4.989
UG 5 X 1N	1.4656	1.2275	0.2381	15.59	51.59	6.65	32.88	0.925	34.64	2.137
UG 5 X 3N	1.292	0.9183	0.3737	31.91	30.26	10.32	27.24	0.886	32.64	2.479
UG 5 X 4N	1.2171	0.9493	0.2678	21.98	23.59	12.32	66.5	1.97	33.64	2.452
UG 5 X G7955	1.4088	1.0626	0.3462	22.78	9.59	1.65	16.63	0.437	32.98	0.667
UG 5 X G8527	1.2726	0.6287	0.6439	48.66	60.59	16.65	45.78	1.266	35.31	3.374
UG 5 X S-Line 13.2A	1.4321	1.089	0.3431	25.25	11.78	0.52	-6.26	-0.25	23.39	0.127
UG 5 X S-Line 9.2	1.3748	0.8906	0.4842	35.43	44.59	13.98	38.23	0.672	30.64	2.771
LSD	*0.2698*	*0.3377*	*0.287*	*25.7*	*42.98*	*15.45*	*491.4*	*21.3*	*18.56*	*2.7*

Iwt= Initial seed weight, fwt=Final seed weight, wt L= Seed weight loss, %wtL=Percentage seed weight loss, ABE= Adult bruchid emergence, %IE=Percentage adult insect emergence, MDP=Median Development Period, GI= Growth index, DSI=Dobie susceptibility index

Results of the mean squares from a full diallel analysis are presented in Table 5.4. Crosses showed significant differences for trait seed weight loss ($p<0.01$), adult bruchid emergence ($p<0.001$) and DSI ($p<0.001$). General combining abilities (GCA) were significantly different for traits swl ($p<0.01$), ABE ($P<0.001$) and DSI ($P<0.001$). Specific combining abilities (SCA) were significantly different for trait ABE ($p<0.001$) and DSI ($p<0.05$) but were not significantly different for swl. Significant differences were observed in reciprocals for parameter swl ($P<0.01$) and ABE ($P<0.05$) but were not significantly different for DSI. The results showed that there were maternal effect differences for swl ($P<0.01$), ABE ($P<0.01$) and DSI ($P<0.05$). Narrow sense heritability for swl was 0.12, ABE (0.17) and for DSI it was 0.11. Broad sense heritability for swl was 0.12, for ABE was 0.55 and 0.19 for DSI. Phenotypic variance for swl was 0.04; ABE was 215.58 and 3.54 for DSI. Seed weight loss had the highest baker's ratio of unity (1) followed by DSI (BR=0.59) and the least was reported in ABE (BR=0.32).

GCA effects for *Callosobruchus chinensis* susceptibility parameters are presented in Table 5.5. Significant positive GCA effects were observed in genotype UG 5 (0.11^{***}) and Maksoy 3N (0.06^{*}) for swl. For ABE, three parents Maksoy 4N (6.59^{***}), Maksoy 3N (6.57^{***}) and UG5 (3.85^{*}) had highest significant positive effects. The highest significant GCA effect for DSI were expressed by Maksoy 4N (0.88^{**}) followed by Maksoy 3N (0.61^{*}). Lowest GCA effects was expressed by genotype S-Line 13.2A (-0.05) for swl ($P<0.05$). For ABE genotypes S-Line 9.2 (-5.19), S-Line 13.2A (-3.51) and SREB-15C (-3.50) expressed lowest significant GCA effects ($P<0.01$). Lowest GCA effects for DSI were observed on genotype S-Line 9.2 (-0.69^{**}).

Negative non significant GCA effects for swl were observed in genotypes SREB-15C, Maksoy 1N, S-Line 9.2, G7955 and G8527. On the other hand non significant positive GCA effects was observed in Maksoy 4N. For ABE negative non significant GCA effects were observed on Maksoy 1N, G8527 and G7955. Negative non significant GCA effects for DSI were observed in Maksoy 1N, SREBC-15C and S-Line 13.2A while positive non significant GCA were expressed by G7955, G8527 and UG 5.

Table 5.4 Mean squares for *Callosobruchus chinensis* susceptibility parameters in soybean

Source of Variation	df	Swl	ABE	DSI
Rep	2	0.03^{ns}	179.00^{ns}	9.23^{*}
Cross	80	0.05^{**}	426.20^{***}	4.88^{***}
GCA	8	0.16^{**}	1100.39^{***}	13.17^{***}
SCA	36	0.02^{ns}	574.49^{***}	4.33^{*}
Reciprocal	36	0.05^{**}	133.50^{*}	3.78^{ns}
Maternal	8	0.13^{**}	316.60^{**}	6.90^{*}
Residuals	158	0.03	88.35	2.70
GCA component		0.002	18.74	0.19
SCA component		0.00	81.02	0.27
Maternal component		0.002	4.36	0.08
Phenotypic Variance		0.04	215.58	3.54
NSCGD		0.12	0.17	0.11
BSCGD		0.12	0.55	0.19
Baker's ratio		1.00	0.32	0.59

df.= degrees of freedom, swl= Seed weight loss, ABE= Adult bruchid emergrnce, DSI=Dobie Susceptibility Index, ns=not significant, ***=P<0.001, **=P<0.01, *=P<0.05, Narrow sense coefficient of Genetic Determination

The estimates of parents' maternal effects are presented in Table 5.5. The genotypes Maksoy 4N and UG 5 had significant positive effects (P<0.01) for seed weight loss while only genotype AVRDC G7955 had significant positive effect (P<0.05) for DSI. Genotypes Maksoy 3N and AVRDC G7955 had significant positive effect (P<0.05) while S-Line 13.2A had significant negative effect for adult bruchid emergence.

The estimates of specific combining ability (SCA) for the different crosses are presented in Table 5.6. The SCA effects results showed that cross UG X SREB-15C had significant (P<0.01) negative SCA effect for seed weight loss. Crosses SREB-15C x Maksoy 3N, SREB-15C x Maksoy 4N, and UG x S-Line 13.2A had significant (P<0.05) negative SCA effects for DSI. The crosses AVRDC G8527 x AVRDC G7955, SREB-15C x AVRDC G7955 and SREB-15C x S-Line 13.2A had significant (P<0.05) positive effect for adult bruchid emergence (ABE) while crosses G7955 x 3N, Maksoy 4N x 3N, SREB-15C x Maksoy 3N, SREB-15C x Maksoy 4N, UG x AVRDC G7955, UG x Maksoy 3N and UG 5 x S-Line 13.2A had significant (P<0.01) negative SCA effect for adult bruchid emergence.

Table 5.5 GCA and maternal effects for *Callosobruchus chinensis* susceptibility parameters

	GCA effects					
Parent	**swl**	**Rank**	**DSI**	**Rank**	**ABE**	**Rank**
Maksoy 1N	-0.04^{ns}	7	-0.21^{ns}	6	-2.41^{ns}	6
Maksoy 3N	0.06*	2	0.61*	2	6.57***	2
Maksoy 4N	0.04^{ns}	3	0.88**	1	6.59***	1
G7955	-0.02^{ns}	5	0.11^{ns}	3	-0.36^{ns}	4
G8527	-0.01^{ns}	4	0.04^{ns}	4	-2.04^{ns}	5
S-Line 13.2A	-0.05*	9	-0.38^{ns}	8	-3.51**	8
S-Line 9.2	-0.04^{ns}	6	-0.69**	9	-5.19**	9
SREB-15C	-0.04^{ns}	8	-0.35^{ns}	7	-3.50**	7
UG 5	0.11***	1	0.01^{ns}	5	3.85*	3
	Maternal effect					
Parent	**swl**	**Rank**	**DSI**	**Rank**	**ABE**	**Rank**
Maksoy 1N	-0.03^{ns}	7	0.38^{ns}	3	2.37^{ns}	3
Maksoy 3N	0.01^{ns}	3	0.39^{ns}	2	2.94*	2
Maksoy 4N	0.07**	2	0.18^{ns}	4	1.41^{ns}	4
G7955	-0.01^{ns}	4	0.46*	1	3.05*	1
G8527	-0.04^{ns}	8	-0.34^{ns}	8	-1.70^{ns}	7
S-Line 13.2A	-0.04^{ns}	9	-0.46^{ns}	9	-2.94*	9
S-Line 9.2	-0.03^{ns}	6	-0.33^{ns}	7	-2.53^{ns}	8
SREB-15C	-0.02^{ns}	5	-0.17^{ns}	6	-1.17^{ns}	5
UG 5	0.09**	1	-0.12^{ns}	5	-1.45^{ns}	6

swl= Seed weight loss, DSI=Dobie Susceptibility Index, ABE=Adult Bruchid Emergence, ns=not significant, ***=P<0.001, **=P<0.01, *=P<0.05

Table 5.6. SCA effects for *Callosobruchus chinensis* susceptibility parameters on soybean F_2 generations

Cross	**DSI**	**swl**	**ABE**
G7955 X Maksoy 1N	-0.58^{ns}	0.01^{ns}	-1.64^{ns}
G7955 X Maksoy 3N	-0.64^{ns}	-0.03^{ns}	-7.12*
G7955 X Maksoy 4N	-0.05^{ns}	0.06^{ns}	1.53^{ns}
G8527 X G7955	0.86^{ns}	-0.01^{ns}	7.32*
G8527 X Maksoy 1N	-0.89^{ns}	-0.07^{ns}	-2.30^{ns}
G8527 X Maksoy 3N	-0.25^{ns}	0.07^{ns}	-3.11^{ns}
G8527 X Maksoy 4N	0.18^{ns}	-0.03^{ns}	-2.63^{ns}
Maksoy 3N X Maksoy 1N	0.58^{ns}	0.01^{ns}	-0.74^{ns}
Maksoy 4N X Maksoy 1N	-0.34^{ns}	0.09^{ns}	-5.26^{ns}

Maksoy 4N X Maksoy 3N	-0.24^{ns}	-0.03^{ns}	-7.24*
S-Line 13.2A X G7955	0.86^{ns}	0.04^{ns}	4.46^{ns}
S-Line 13.2A X G8527	-0.43^{ns}	-0.04^{ns}	-1.36^{ns}
S-Line 13.2A X Maksoy 1N	-0.38^{ns}	0.00^{ns}	0.01^{ns}
S-Line 13.2A X Maksoy 3N	0.23^{ns}	-0.03^{ns}	-1.31^{ns}
S-Line 13.2A x Maksoy 4N	-0.24^{ns}	-0.07^{ns}	-2.00^{ns}
S-Line 9.2 X G7955	-0.35^{ns}	-0.06^{ns}	-1.24^{ns}
S-Line 9.2 X G8527	0.64^{ns}	-0.04^{ns}	4.32^{ns}
S-Line 9.2 X Maksoy 1N	0.45^{ns}	-0.01^{ns}	1.86^{ns}
S-Line 9.2 X Maksoy 3N	-0.26^{ns}	0.08^{ns}	-3.79^{ns}
S-Line 9.2 X Maksoy 4N	0.02^{ns}	0.04^{ns}	0.86^{ns}
S-Line 9.2 X S-Line 13.2A	-0.94^{ns}	-0.05^{ns}	-2.38^{ns}
SREB-15C X G7955	0.71^{ns}	0.01^{ns}	6.95*
SREB-15C X G8527	0.19^{ns}	0.10^{ns}	0.46^{ns}
SREB-15C X Maksoy 1N	0.39^{ns}	0.01^{ns}	4.00^{ns}
SREB-15C X Maksoy 3N	-1.36*	-0.06^{ns}	-10.48**
SREB-15C X Maksoy 4N	-1.39*	-0.02^{ns}	-8.50**
SREB-15C X S-Line 13.2A	0.91^{ns}	0.08^{ns}	7.10*
SREB-15C X S-Line 9.2	0.04^{ns}	0.02^{ns}	1.29^{ns}
UG 5 X G7955	-0.88^{ns}	-0.02^{ns}	-10.56**
UG 5 X G8527	0.04^{ns}	0.104^{ns}	-1.72^{ns}
UG 5 X Maksoy 1N	0.43^{ns}	-0.03^{ns}	0.99^{ns}
UG 5 X Maksoy 3N	-0.59^{ns}	0.07^{ns}	-10.33**
UG 5 X Maksoy 4N	-0.05^{ns}	-0.02^{ns}	-5.18^{ns}
UG 5 X S-Line 13.2A	-1.44*	-0.01^{ns}	-10.79**
UG 5 X S-Line 9.2	0.38^{ns}	0.05^{ns}	-1.56^{ns}
UG 5 X SREB-15C	-0.64^{ns}	-0.16**	-6.42^{ns}

swl= Seed weight loss, DSI=Dobie Susceptibility Index, ABE=Adult Bruchid Emergence, ns=not significant, ***=P<0.001, **=P<0.01, *=P<0.05

5.5 Discussion

The highly significant GCA and SCA mean squares for most of the traits (Table 5.4), indicate the presence of both additive and non-additive gene actions. However, the high Baker's ratio associated with seed weight loss and DSI indicated the preponderance of additive gene action. Maternal effects were found to govern the inheritance of *C. chinensis* resistance in soybean meaning that contribution of maternal genotype to the phenotype of its offspring was beyond the equal chromosomal contribution from each parent.

Genotypes showed variability in response to bruchid infestation as reflected by seed weight loss, adult bruchid emergence and DSI (Table 5.2) implying that crosses and parents exhibited varying levels of resistance thereby providing an opportunity for developing bruchid resistant genotypes in soybean (Wayne *et al.* 2004). Further, these results basically implied that the seed weight loss, adult bruchid emergence (ABE), and DSI could further be used for discriminating genotypes in the genetic analyses. Parameters eggs, MDP and growth index (GI), did not show variability amongst genotypes and thus could not be used in the genetic analyses. These findings are in agreement with several other studies including Kananji (2007) who reported variability in susceptibility parameter in dry bean studies.

The results indicated a decrease in DSI with hybridization except for crosses on Maksoy 1N and S-Line 9.2 (Table 5.3), thus implying that hybridization can increase resistance probably by reducing the number of adult bruchid emergence (ABE) and increasing the MDP. Derera *et al.* (2014) also reported similar findings. Similar results were observed in the reciprocal crossing which showed no significant influence on DSI.

According to Hallauer *et al.* (1988) the average performance of a parent in a hybrid combination is termed GCA, whilst the deviation of the performance of a hybrid from the expectation based on the average GCA effects of the lines that produced the hybrid is termed the SCA. GCA is equivalent to the breeding value and estimated additive genetic variation (Wayne *et al.,* 2004). Significant GCAs were observed among parents indicating that there were differences in performance of genotypes as parents in hybrid combinations (Mwije *et al.,* 2014). Significant differences in the three parameters (seed weight loss, ABE and DSI) were an indication that the genes controlling soybean resistance to *C. chinensis* lack dominance and their action is largely additive, Table 5.4. This suggests that selection of parents to generate resistant crosses and developing resistant pure line cultivars is possible (Mulbah *et al.*, 2015). These results further indicated that selection would be effective and it could be used to fix resistance in cultivars (Fasahat *et al.,* 2016). However, significant SCA mean squares for ABE and DSI were also reported indicating that resistance to *C. chinensis* was also influenced by non additive gene effects signifying the presence of a locus or loci with dominance variation (Fasahat *et al.,* 2016). Presence of significant SCA indicates that a complex type of inheritance to resistance of *C. chinensis* may be involved in some parents (Hakizimana *et al.,* 2004). Similar conclusions were drawn by Kananji (2007) working on beans and Somta *et al*. (2007) on mungbeans. The presence of both additive and non additive

gene actions might indicate transgressive segregation in soybean (Machado *et al.,* 2009). Wayne *et al*. (2004) reported that if parental alleles were purely additive, then an F_2 genotype would deviate from the population mean by the sum of the GCAs of its parents and due to environmental or error effects. Any additional deviation from the population mean would be attributable either to dominance, *i.e.*, intralocus interactions, of alleles or to epistasis, *i.e.*, interlocus interactions (Cai *et al.,* 2012). However, the GCA variances were higher than SCA variances indicating preponderance of additive gene action and progeny selection will be effective for the genetic improvement of bruchid resistance traits.

This study showed that maternal effects were significant (Table 5.4), indicating that resistance to *C. chinensis* depended on the genotype of maternal parent used in hybridization (Vaiserman *et al*., 2013). In fact, all the significant effects of reciprocal crosses were attributed to maternal effects since non-maternal effects were not significant for all the traits implying that seed resistance was controlled by chemicals synthesized by the female parent and transported to the cotyledon and embryo of the seed (Somta *et al.,* 2007). This finding is in agreement with Fernandez and Talekar (1990) and Somta *et al*. (2007), who concluded that resistance to bruchids in mungbean exhibited maternal effect. Maternal effects indicate that the traits had cytoplasmic mode of inheritance pattern implying that when selecting parents for hybridization in soybean for bruchid resistance breeders should give female parent priority (Cai *et al.,* 2012). The significant difference between reciprocal crosses indicated that non- chromosomal maternal effects could have contributed to a heterotic response (Mendes *et al*., 2015), and presence of the interaction of the genes with nuclear factors or the expression of extranuclear genes (Cai *et al.,* 2012).

Seed weight loss and DSI recorded high Baker's ratio of 1 and 0.59, respectively (Table 5.4) and this implied preponderance of additive gene action. The unity baker's ratio for seed weight loss indicated total influence of additive gene effects (Baker, 1978). The Baker's ratio of unity or closer to unity indicated greater predictability of progeny performance based on the GCA alone (Cai *et al.,* 2012) and better transmission of trait to the progenies (Murtadha *et al.,* 2018). In soybean, a self pollinating crop this implied that when non additive genes are lost after some generations, it would practically be possible to fix genes controlling resistance to these traits. The breeding procedures to be adopted for these characters include pure line selection, mass selection, progeny selection, hybridization and selection and heterosis breeding (Choudhary *et al.*, 2004). The Baker's ratio for ABE was 0.32, indicating that non-

additive gene effects were more important than additive gene effects in controlling the inheritance of this trait in the germplasm evaluated. Cai *et al.* (2012) reported that performance of hybrids for traits with low Baker's ratio couldn't be predicted from GCA but from exploiting of the SCA. Therefore, only crossing the two parents with the lowest ABE-GCA effects cannot simply produce the best *C. chinensis* resistant progeny (Hakizimana *et al.,* 2004) as such breeding procedure for this character should be performed using heterosis breeding method (Choudhary *et al.*, 2004).

Estimation of the components of variance for a quantitative trait allows one to evaluate both the degree to which genetics influences the trait and the trait's underlying genetic architecture (Abney *et al*., 2001). The narrow sense heritability results in Table 5.4 indicated that additive gene action was present, but not high as such early generation selection would not be very effective. High narrow sense heritability (NSCGD) of close to 1 indicates that early generation selection would be effective (Hansen, 1989). Adult bruchid emergence had a high broad sense heritability which was an indication of high dominance genetic contribution towards phenotypic variance (Abney *et al*., 2001).

The GCA effects results for the parents (Table 5.5) clearly showed that genotype UG 5 and Maksoy 3N contributed highest to swl, while SREBC-15C and S-Line 13.2A contributed the least to seed weight loss (Table 5.5) suggesting the best donors for developing reduced swl varieties would be SREB-15C and S-Line 13.2A. Genotype UG 5 would generate the populations with highest mean swl while the population with least swl would be generated by S-Line 13.2A (Dias *et al.*, 2017).

The GCA effects for DSI of parental genotypes Maksoy 4N and Maksoy 3N were high indicating that they would increase susceptibility of genotypes in hybridization. On the other hand S-Line 9.2 showed least GCA effects therefore was the best combiner for bruchid resistance amongst the parental genotypes used. Parent line S-Line 9.2 contributed the least to adult bruchid emergence while parent Maksoy 4N and Maksoy 3N contributed the highest to ABE indicating that S-Line 9.2 was best combiner for reduction of ABE. The results from this study indicated that it was not easy to get one donor genotype for all traits of interest (Murtadha *et.al.,* 2018).

Out of the 9 genotypes used in the study, 6 had negative GCA effects and only three (Maksoy 3N, Maksoy 4N and UG 5) had positive GCA effects. Negative general combining abilities

are preferred for pest and disease resistance because they are based on the scale where highest values are associated with more pest infestation (Fasahat *et al.*, 2016).

Significant SCA effects (Table 5.6) were observed in 11 out of 36 of crosses, indicating the presence of non- additive gene effects. Crosses with significant negative SCA effects such as SREB-15C x S-Line 13.2A, SREB-15C x Maksoy 3N, AVRDC G7955 x Maksoy 3N would be beneficial in the development of *C. chinensis* resistant varieties. The significant and positive SCA effect presented by cross AVRDC G8527 (resistant) x AVRDC G7955 (resistant) could possibly be explained by what Kananji (2007) reported that this happens due to quantitative inheritance of genes. However, Symphorien *et al.*, (2018) reported that accidental resistance break down could happen when two or more resistant genotypes are combined. A number of studies (Abakemal *et al.*, 2011; Nagarajan *et al.*, 2017) observed the same result but gave no explanation for it.

Significant and negative SCA effects were observed for the combination UG 5 (susceptible) x Maksoy 3N (susceptible) suggesting that resistance of these progenies was higher or lower than would be expected from the average resistance of their respective parents thereby implying that resistant genotypes could be produced from susceptible parents due to transgressive segregation or inter and intra-locus gene interactions (Hakizimana *et al.*, 2004).

5.6 Conclusions

The study identified SREB-15C, S-Line 9.2 and S-Line 13.2A as useful parents in breeding for resistance to *C. chinensis* based on general combining abilities. The study established that additive and non-additive gene effects governed the soybean resistance to *C. chinensis.* The presence of maternal effects signified the importance of ensuring that parental genotypes with most desired traits are always made females during hybridization. The best progenies from crosses with negative GCAs should further be screened and advanced for the release of resistant varieties.

CHAPTER SIX

GENERAL DISCUSSIONS AND RECOMMENDATIONS

Bruchids cause serious losses to all legume crops in both quality and quantity, particularly in the tropics and sub-tropics where temperatures and relative humidity are high (Keneni *et al.,* 2011). In soybean, *Callosobruchus chinens*is has been reported as a major storage threat in Uganda (Tukamuhabwa 2015, personal communication). However, the quantitative magnitude of damage and susceptibility status of Ugandan soybean germplasm, was not clear. Several management options have been employed for bruchids with varying levels of success, but host plant resistance (HPR) seems to offer better options because it is environmentally friendly, convenient for the farmer and compatible with other integrated pest management technologies (Maphosa, 2013). HPR on legumes has been reported in cowpeas (Sharma and Thakur 2014b), beans (Kananji, 2007) and mungbean (War *et al.,* 2017). For HPR to be used effectively there is need for information on sources, basis and genetics of resistance which was not available. Identifying germplasm with resistance to *C. chinensis* is critical to the success of soybean resistance research program. However, once germplasm with resistance has been identified, other questions quickly arise. Why is the germplasm resistant? What mechanisms of resistance are operating? How is the resistance inherited? How effective is the resistance in reducing storage losses?

This study on genetic resistance of soybean to *Callososbruchus chinensis* provides acumens on most of the above questions. This study therefore aimed at identifying sources of resistance to *C. chinensis* from the available germplasm in Uganda. The study also aimed at finding out if there exists a biochemical basis of resistance, which could enhance the selection of the breeding materials. Further, the modes of gene action and inheritance patterns were examined.

Genotypes showed great variation in response to *Callosobruchus chinensis* infestation. One key finding was that magnitude of infestation by *C. chinensis* in most soybean genotypes was high with mean economic loss of 10.5% within 3months based on seed weight loss. Cluster analysis revealed that resistance to *C. chinensis* was not associated with geographical origin of the soybean genotypes. Based on median development period, most of the soybean

genotypes could no longer be stored for more than 30 days without some form of protection once exposed to the *C. chinensis*. The major contribution of this study was the characterization of germplasm in Uganda into the classes of susceptibility. Screening the soybean germplasm for resistance to *C. chinensis* in a no choice test yielded two sources; genotype AVRDC G8527 and PI G89 based on DSI. A number of genotypes of Ugandan origin showed moderate resistance, including the released variety Maksoy 1N. Based on correlation analysis DSI had no linear relationship with 100 seed weight indicating that seed size of genotypes did not influence resistance to *C. chinensis*. Information generated by this study on percentage weight loss due to *C. chinensis* is very vital for the progression of entomology work to determine the economic injury levels. More replication in time have to be built on this base line information then the economic injury levels for each genotype will be established. Regression analysis demonstrated that there was a positive relationship between adult bruchid emergence and seed weight loss.

The study also demonstrated that significant variations existed in secondary metabolite concentration among soybean genotypes. Biochemical analysis exposed that soybean genotypes' secondary metabolites were associated with resistance but primary metabolites were not associated with resistance. Biochemicals associated with increased resistance were tannins, total antioxidants and peroxidase. Meanwhile metabolites; peroxidase, tannin and total antioxidants were negatively related with flavonoids. It was evident that tannin and flavonoids had antagonistic effects on biology of *C. chinensis*.

Further more in this study both additive and non additive gene actions were evident but traits seed weight loss and DSI were predominantly governed by additive gene action. It was evident that genotype S-Line 9.2 was the best combiner for DSI and ABE while S-Line 13.2A was the best combiner for seed weight loss based on the general combining ability effects. The study demonstrated that genotypes AVRDC G8527 (R), S-Line 9.2 (MR) and Maksoy 1N (MR) had no maternal effects while other six parents were governed by maternal effects.

Economic losses reported in this study were from one generation. In the farmers stores losses higher than this would be expected due to reinfestation. This information is very vital to the farmer to assist in management decisions concerning selection and timing of control measures while governments require yield loss data for food and crop production planning and to assist in the process of resource allocation for research, extension and control

operation (Dent, 2000). The relationship between weight loss and ABE showed a susceptive response because it causes direct damage on the grain. With this direct damage, seed weight declines in direct proportion to the number of *C. chinensis* present (Amusa *et al.,* 2014). Therefore the amount of injury caused by *C. chinensis* was linearly related to the pest intensity. Information generated in this study on damage and seed weight loss is very vital for assessing efficacy of a control measure (Dent, 2000). After breeding resistant varieties it would be important to know how much savings have been made or how much expenditure and losses have been avoided.

Ugandan germplasm was characterized into classes of susceptibility. There were four classes, designated; resistant, moderate resistant, susceptible and very susceptible based on DSI. This indicated that levels of resistance and response to *Callosobruchus chinensis* infestation among genotypes were diverse. Genotypes which were classified as resistant had low susceptibility index, percentage seed weight loss, number of adult bruchid emergence, and extended median development periods. Like any other crop soybean demonstrated that it has some form of defence mechanisms against *C. chinensis.* It could therefore be deduced that soybean genotypes contained various intrinsic or extrinsic defence factors. These factors could be morphological or biochemical. However, these defence factors as much as they are ubiquitous, they are not the same in crops. For example, in mung bean small seed size and hard seed coat were factors responsible for resistance to *C. analis* (Mahato *et al.,* 2015) while in beans, α-amylase inhibitor was responsible for resistance to bruchids (Goosens *et al.,* 2000). In this study, since the results on seed size, a morphological trait, didn't have a significant relationship with susceptibility parameters it was therefore speculated that the differences were influenced by a number of inherent biochemical factors. The effect of these biochemical factors on biology of *C. chinensis* is usually depicted in the number of ABE, MDP, GI and seed weight loss (Kananji, 2007). This study did not explore the possibility of other morphological parameters such seed hardness, seed texture if they have impact on resistance to *C. chinensis*. It is therefore important that studies be carried out with regard to these other traits.

The proposition of resistance to *C. chinensis* in soybean being due to biochemical factors was strengthened by low number of adult bruchid emergence (ABE) and extended median development periods in genotypes classified as resistant. Low ABE and extended MDP pointed out to the possibility of antibiosis being mechanism of resistance to *C. chinensis* in

soybean. An extension to this explanation is that since seed size did not influence resistance which is based on antibiosis it therefore means concentration of antibiotic factors was not depended on seed size of soybean. Furthermore, on breeding perspective it means breeding for resistance to *C. chinensis* will not affect seed size of soybean. It should be noted that large seed size is one of the major attributes preferred by farmers (Tukamuhabwa *et al.*, 2016). In this study heritability of seed size was not evaluated, but Hu *et al.*, 2013 reported that seed size traits are controlled by multiple genes in soybean and are associated with five quantitative trait loci indicating that it is a complex trait. Thus being the case, seed size cannot be used as an indirect way for *C. chinensis* resistance selection.

In most plant-insect interactions, food quality affects mean relative growth rate (Li, 1995) which would thus explain, lower rates of population increase and eventual increase in median development period in genotypes with high tannin, peroxidase and total antioxidants. Median developmental periods increase hyperbolically on low food quality because many insects grow and develop slowly, survive poorly, achieve smaller sizes and produce few offspring (Price, 1985). It is worth noting that median development periods from the study were shorter than what was reported by Sharma and Thakur (2014a). It is a known fact that where insects have a short development period the insects population develop resistance to insecticides. Genotypes that had the shortest median development periods would give higher rates of population increase and develop resistance to insecticides more quickly than comparatively where there is extended development period (Li, 1995). How short development periods lead to development of insecticide resistance was beyond this study, however suffice to say that it has to do with hormones that regulate juvenile stages and chitin synthesis (Doucet and Retnakaran, 2012). Shorter development periods observed in this study, highlight the need for breeding for extended development periods which translates into genotypes which are resistant to *C. chinensis*. However, since breeding for resistance takes a long time, it is therefore imperative that Uganda should put in place other storage practices for management of *C. chinensis* in the interim period such as use of hermetic bags, metallic silos just to mention a few.

The central tenet of Ehrlich and Raven's theory is that evolution of plant chemical defenses is followed closely by biochemical adaptation in insect herbivores (Wheat *et al.*, 2007). This theory explains therefore why soybean defences were incomplete; bruchids were able to infest and develop on seeds despite the presence of plant defence compounds indicating that

there was no immune genotype in the germplasm (Dent, 2000). Plant breeding is human-mediated evolution aiming at hastening evolution process (Acquaah, 2007); it is therefore important that selection for resistance traits associated with secondary metabolites be pursued. However, since *C. chinensis* will follow suit in upgrading its defence mechanisms; stacking/pyramiding two or more resistance genes with different modes of action into a single genotype would reduce the possibility of resistance breakdown.

This work didn't profile the changes that have happened in soybean genotypes since they were introduced to Uganda but it was evident from the results that most released varieties were susceptible, for example; Maksoy 2N, Maksoy 5N and AGS 292. In fact, according to Moreira *et al.* (2018) plant domestication conducted over several millennia has resulted in the modification of specific plant traits to enhance vegetative or reproductive growth (depending on the type of tissue or organ that is being selected), to increase nutrient content, or to improve taste for human consumption. Moreira *et al.* (2018) states that at the same time, however, selective breeding has frequently led to a reduction in levels of plant physical or chemical defences in many cases by direct selection because these traits are harmful or distasteful to humans and livestock. Alternatively, selection for larger organs or increased productivity and better nutritional value has simply diluted defence levels in crop plants or reduced defences in cases where growth and defences trade off (Keneni *et al.,* 2011). As a result of lowered defence levels, domesticated plants are generally more susceptible to pathogen infection and damage by phytophagous insects compared to their wild relatives. This, therefore, indicates need to breed for increased metabolites to a level where they would be effective while at the same time not affecting the nutritional levels of soybean. Suffice to say, most tannins are made only in the seed coat, where they are incorporated into a complex polymer including other flavonoids that are thought to protect the seed against dessication and other abiotic stresses (Barbehenn *et al.,* 2011). This implies that selecting for high tannin content in soybean will not face challenges since soybean are processed whereby the seed coat is removed before consumption by livestock and human beings. Tannins have been reported to reduce ovulation in animals but once soybean is processed there is no need for this fear. Unfortunately, it was not determined whether the tannins decreased insect growth because of feeding deterrence, decreased protein utilization efficiency, or toxicity.

Meanwhile, this study did not explore the role of individual metabolites, as such it is possible that the presence of one influences the capabilities of another since metabolites are produced

in pathways. For example tannins, flavonoids and phenolic acids are all produced in the shikimic-phenylpropanoid pathway, they could have been acting in synergy or additively to produce specific effects (Guo *et al.,* 2012). Efficient production of gallic acid negatively affects the synthesis of shikimic acid and its products such as flavonoids, and condensed tannins. For example, phenolic acid (gallic acid) is a precursor of hydrolysable tannins in the shikimic pathway (Kumar and Pandey 2013). This therefore clearly explains why phenolic acids did not reach the toxic concentration to affect bruchids. The antagonistic effects of these tannins with flavonoids might not be actually strange looking at the shikimic pathway. The pathway either produces condensed tannins or flavonoids. It is documented that in a cell it is not possible to have both tannnis and flavonoids being produced at the same time (Lattanzio *et al.,* 2012). Production of one hinders the other, that is likely why genotypes that contain high tannins had low flavonoids and the vice versa. In breeding perspective it means breeding for high flavonoids which are required nutritionally more than tannins means increased susceptibility to *C. chinensis*. This might have been the reason why most of the released varieties were found susceptible to *C. chinensis*. This type of inderdependence makes breeding for such traits complicated and difficult using conventional breeding techniques. Given the complex associations between numerous plant variables and insect performance, linking cause and effect between secondary metabolite levels and bruchid performance needs more studies. It is therefore worthwhile considering isolation and transgenic transfer of tannins, peroxidase and total antioxidants gene to popular high yielding soybean varieties to confer *C. chinensis* resistance. Another major challenge is to show what combinations of defenses produce synergistic or additive effects that would increase bruchid resistance. For example if tannins are an important component of plant defenses, are there other specific chemical or physical factors that make tannins more effective? What is envisaged currently is to carry out artificial diet bioassays and should secondary metabolites be lethal to *C. chinensis* then in vitro plant regeneration system coupled with advanced genetic transformation techniques would make it possible to transfer bruchid resistant gene from diverse sources. As such, further work has to be done to assess metabolite action. However, based on this study's findings, it was reasonable to claim that metabolites; tannins, total antioxidants and peroxidase act together to reduce *C. chinensis* infestation.

The findings on Maksoy 3N were of great interest in this study; during the screening study Maksoy 3N showed susceptibility to *C. chinensis* however the results on biochemical analysis showed that it had high tannin contents just as good as resistant genotypes

suggesting that *C. chinensis* was capable of detoxifying the tannins or Maksoy 3N has a gene that is different from other varieties. This finding is intriguing, and requires further studies to be done on Maksoy 3N to understand this phenomenon.

This study identified that resistance was governed by both additive and non additive genes signifying that resistance to *C. chinensis* is a complex trait. When a trait is complex national programs of the developing countries often find it beyond their capability to effectively manage the trait, although suffice to say that there are some programs that have quite successful HPR programs (Tefera, 2015). The trait is also not one that fits private seed companies very well, as they are often required to apply their resources on more short-term research projects. Smaller local seed companies usually find such complicated traits well beyond their very limited resources (IITA, 2015). Since resistance to *C. chinensis* is a complex trait, it lends itself to the application of more advanced scientific techniques such as marker assisted selection but also bigger programs such as IITA may need to take up further research on this.

The absence of maternal effect on potential resistance sources genotypes AVRDC G8527 (R), S-Line 9.2 and Maksoy 1N (MR) is very encouraging to the breeder because it implies they respond well to selection while the association of the potential lines (S-Line 13.2A) with cytoplasmic inheritance poses more challenges for the breeder as the presence of maternal effects reduces the response to selection. However, it is was not clear from the study that maternal effects would persist up to F_6 or F_7 when release of soybean lines is done. This needs to be given further attention.

Nevertheless, as varietal breakthroughs against *C. chinensis* are still far from fruition, whatever level of genetic resistance achieved so far must be integrated with other cultural, chemical and biological control methods available to obtain immediate synergetic effects.

In conclusion, this study provide valuable insights into the understanding of the basis and mechanisms that underlie soybean response to *C. chinensis* infestation that could be useful to identify the most resistant soybean genotypes to bruchids. Two genotypes were identified as resistant to *C. chinensis*. Identified resistant genotypes had high tannins, peroxidase and total antioxidant concentrations but were not adaptable to Ugandan conditions hence breeding is inevitable for the introgression of the resistant genes into adapted high yielding varieties.

Conventional breeding methods proved to have challenges in tapping the resistant genes from the identified sources due to poor combining abilities; therefore, we call for the use of biotechnological tools.

Recommendations

C. chinensis is a major storage pest in Uganda which needs interventions to be put in place to safeguard the produce. From the study it was found that Maksoy 1N a released variety in Uganda had moderate resistance to *C. chinensis* therefore it is recommended for use by farmers. Genotype SREB-15C, S-Line 9.2 and S-Line 13.2A are recommended potential parents for the bruchid resistance breeding programme. The crosses between SREB-15C x Maksoy 3N, and SREB-15C x S-Line 13.2A would be recommended as start up material for the bruchids breeding programme.

Challenges

Generation of starting material both soybean seed and bruchids population was a challenge in this study. Screening large numbers of germplasm for bruchid resistance requires a lot of seed, as such a lot of time was consumed in trying to generate enough seed coupled with some introduced materials could not germinate. Raising thousands of insects to use in the study as per when required was a big nightmare. There is need for proper planning in such type of studies in future and there is further need to establish sustained cultures for the *C. chinensis* in the laboratory.

This study could not be done in multi location for ethical reasons, in future multi location evaluation might be necessary so as to study genotype by environment interactions.

Future Perspective

The number of resistance sources in the available germplasm was low and furthermore, there was no complete resistance as such there is need to continue with the search for more sources even in the wild relatives of soybean. Profiling soybean germplasm for the changes that have occurred through the soybean breeding program would also be important to find out if there is something that has been lost in the way.

Biochemical analysis revealed some metabolites that were associated with resistance, to establish if the metabolites cause resistance there is need to carry out a diet study where bruchids would be fed directly by the biochemicals and then establish whether the

metabolites either cause death or increase development periods.

Marker assisted breeding would be a good strategy for the development of resistant cultivars to *Callosobruchus chinensis*. Therefore, there is need to identify quantitative trait loci associated with resistance to bruchids.

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Appendix 1: Effect of Soybean Genotype on resistance parameters

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
AGS 292	22367	10678	9616	9.95	171.7	89.67	51.72	29.67	6.459	2.999
AGS 329	26481	13538	12322	8.99	105	46.33	43.64	36.33	4.34	1.287
AGS 338	21302	10529	9965	5.31	135	59.67	39.04	33.67	5.027	1.965
AVRDC 8586	11756	5866	5591	4.6	74.7	19.33	15.13	24.67	2.31	0.576
AVRDC B-11-13	16521	9107	7690	11.24	176.7	77.67	46.26	32	5.93	2.438
AVRDC G 10427	17697	8997	8440	6.17	125.7	54	39.32	30.33	5.511	1.851
AVRDC G 1882	18262	8834	7934	10.23	184.3	98.33	52.48	30.67	6.381	3.276
AVRDC G 2043	16726	8227	7342	10.82	216.7	93.33	40.73	24	8.144	4.175
AVRDC G 2843	16332	8109	7592	6.49	199	63.33	29.88	33.67	5.167	1.874
AVRDC G 487021-26-3-1	16063	7655	7297	4.67	128.7	42.67	27.6	35.67	4.16	1.317
AVRDC G 4890-21-13-13	18491	9053	8599	5.12	82.7	43	22.69	24.33	2.501	1.381
AVRDC G 50	18197	8251	7884	4.42	82.7	49.33	57.11	31.67	5.2	1.586
AVRDC G 548360	18276	9292	8801	5.31	133	55.33	44.14	31	5.76	1.851
AVRDC G 57	16764	7722	7284	5.48	185.7	68.33	41.54	32.33	5.651	2.157
AVRDC G 7955	12363	6475	5737	11.27	96.3	59.67	57.22	34.33	5.038	1.896
AVRDC G 7959	9796	4836	4387	9.26	155	46	28.76	33	4.954	1.394
AVRDC G 84051-31-1	14443	7255	7025	3.18	159	31.67	19.47	35.33	4.082	0.914
AVRDC G 85037-2-3	12485	6490	6199	4.52	97.7	34.33	26.1	36.67	3.549	1.072
AVRDC G 8527	8335	4198	4197	0.02	24	2.67	6.31	27	0.704	0.066
AVRDC GC 00138-28	16242	7225	6932	4.77	49.3	17	29.91	38	3.011	0.507
AVRDC GC 2043	16922	8664	8223	4.93	104.3	44.67	37.58	30.33	4.247	1.389
AVRDC GC 48702-26-3	20870	10170	9556	5.97	125	67	57.48	29.33	6.124	2.287
AVRDC GC 84051-31-1	16099	7838	7460	4.84	143	42.67	27.7	33	4.673	1.297
AVRDC GC 84051-31-1(2)	16751	7965	7617	4.21	84.7	21	20.66	41	2.879	0.637
AVRDC GC 85037-2-3-54	11917	5999	5568	7.21	162.3	61.67	44.53	32.33	5.489	1.903
AVRDC PI 606405	12537	6610	5665	14.39	232	109	45.51	31.67	6.311	3.533
AVRDC PI 606505	12731	5895	5558	5.38	85.3	36	38.35	29.67	4.359	1.259
AVRDC PI 615434	57091	7168	6057	15.62	254	120	46.03	31.67	6.559	3.934
AVRDC PI 615437B	11596	5986	5601	6.42	118.7	46.67	38.71	34	4.781	1.341
AVRDC PI 628908	14350	6893	6839	0.8	67.7	17.67	31.18	35	3.47	0.507
AVRDC PI 628909	13058	6415	5558	13.46	174	70.33	45.88	33.33	5.532	2.154
AVRDC PI 628919	14406	8129	6910	14.32	119.7	68.33	57.81	31.33	5.847	2.181
AVRDC SRE-B-15C	16495	8248	8219	0.36	77	7.67	9.21	36.67	2.143	0.24
AVRDC SRE-D-11-13	13498	6948	5831	16.47	292	119.67	32.56	34.33	5.763	3.99
AVRDC SREC-14A	14500	6432	6094	4.9	89	42	42.48	34.33	4.524	1.311
AVRDC SS 86045-23-2	14189	6995	6665	4.87	108	43.33	41.38	34	4.579	1.327
BLP 50	13780	5772	5615	2.73	40.7	17.33	41.88	32	3.628	0.554
BSPS 24.2A-3	14310	7789	6475	16.75	253.3	110.67	43.66	30	6.788	3.732
BSPS 42	17120	8001	7424	6.55	95	62	68.29	29	6.154	2.169
BSPS 43	12532	5808	5698	1.81	48.7	16.33	34	33.67	3.592	0.484
BSPS 48A- 25	*	7073	5906	16.1	128.7	75.33	57.51	32	5.762	2.454
BSPS 48A- 27-1	*	7286	7071	2.91	36	19.67	43.78	22	2.83	0.58
BSPS 48A- 28	*	7000	6317	9.79	164.7	59	35.77	33	5.324	1.927
BSPS 48A- 4.8	*	7380	7015	4.99	78.7	32.33	43.09	32	4.537	1.024

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
BSPS 48A- 8	*	7684	7178	6.35	150.3	48.33	32.36	31	5.16	1.741
BSPS 48A-01	14914	7542	6703	11	112.7	69.33	40.84	34	4.441	2.314
BSPS 48A-06	17766	8955	8002	10.66	219.7	74.33	33.51	30.33	6.14	2.504
BSPS 48A-09	17393	7070	6606	6.73	87.7	48.33	42.81	35	3.786	1.491
BSPS 48A-1	12499	6549	6071	7.17	131.7	40.67	29.08	31.33	4.739	1.27
BSPS 48A-12A	15471	7631	7429	2.56	134	45.33	32.58	34.33	4.755	1.344
BSPS 48A-13	17085	8164	7429	9.25	110	48	36.93	31.67	4.833	1.546
BSPS 48A-14	15585	7990	7207	9.74	176	69.33	40.64	29.67	6.272	2.426
BSPS 48A-16	15428	7223	6333	11.45	206.7	77.33	33.85	30	6.187	2.765
BSPS 48A-16B	17387	8753	7845	10.36	151.3	77.67	46.82	33.67	4.817	2.339
BSPS 48A-17	13912	7056	6688	5.2	153.3	21.67	19.18	21	3.2	0.689
BSPS 48A-18	13269	7015	6540	6.72	122	45.67	32.22	34.33	4.401	1.413
BSPS 48A-19	16169	7688	7304	4.54	113	52.33	51.62	30.67	5.567	1.726
BSPS 48A-20	15089	7652	7403	3.26	70	26.67	30.08	36.67	3.405	0.824
BSPS 48A-21B	12345	6412	5951	7.1	120.3	44	35.11	32.33	4.785	1.493
BSPS 48A-24	14310	6341	3986	4.67	204	120.5	63.87	30.5	6.774	3.97
BSPS 48A-27-10	14735	7257	6702	7.51	174.3	71.33	40.56	33.67	5.48	2.169
BSPS 48A-27-9 SPS	16582	7800	6918	11.34	147	85.33	59.2	32.67	5.949	2.634
BSPS 48A-29	15573	7729	6934	10.45	121.3	68.33	55.54	31.67	5.567	2.119
BSPS 48A-3	13595	6868	5305	22.63	219.7	74.33	30.29	33.33	5.271	2.247
BSPS 48A-30	14531	7133	6896	3.29	60.7	27.33	39.31	35	3.653	0.828
BSPS 48A-31	*	7451	6871	7.8	142.7	67.33	47.32	31.67	5.708	2.161
BSPS 48A-3B	*	6996	6391	8.54	138.7	73.33	56.42	32.67	5.584	2.345
BSPS 48A-5	14478	8189	7722	5.65	65	47.33	37.22	33	5.043	1.465
BSPS 48A-7	14015	7000	6509	6.91	94.3	45.67	49.68	34	4.84	1.352
BSPS 48A-9	13533	6621	5572	15.67	156.3	94.67	56.24	31.33	6.099	3.006
BSPS 48A-9-6	16323	7673	6862	10.56	155	78.67	50.52	30.67	6.178	2.633
BSPS 48A-9.6 SPS	14880	7446	6471	13.31	145	91.33	57.94	29.33	6.423	3.169
BSPS 48B	12697	6086	5407	11.21	123.7	71.33	53.26	31	5.649	2.362
BSPS 52 C-1	14324	7356	6041	17.26	187	125.33	69.5	29.33	7.007	4.427
BSPS 7.6	12942	6592	6136	6.83	85	22.67	33.79	34.67	3.597	0.705
BSPS 70	13246	5936	5341	10.13	106	55.33	50.17	32.33	4.919	1.798
BSPS 75B	13426	6869	5134	25.05	184.3	112.33	61.02	29	7.116	3.868
BSPS SRB 48A-27-3	10103	7293	6428	12	228.7	87.33	37.83	33	5.654	2.794
BSPS SRB 48A-27-4	14061	7161	6118	14.67	158	106.67	65.34	30.67	6.49	3.438
BSPS SRB 48A-27-5	15586	7594	6368	15.82	246	93	33.73	33.67	5.605	2.901
BSPS SRB 48A-27-7	14477	7576	6759	10.8	225.3	59.33	30.95	34	5.068	1.85
BSPS SRB 48A-27-8	13778	7281	6696	8.07	181.7	62.67	39.8	32.33	5.39	1.941
BSPS SRB 48A-9-1	15337	7879	7165	9.04	152	71.33	47.04	31.67	5.854	2.279
BSPS SRB 48A-9-4	16095	7701	7414	3.67	138.3	73.67	51.02	30.33	5.814	2.43
BSPS SRB 48A-9-5	15039	7405	7165	3.27	95.7	41	29.79	19	4.189	1.418
BSPS SRB 48A-9-6	15671	7680	7357	4.33	122	35.67	26.9	34.33	4.162	1.006
BSPS SRB 48A-9-7	16485	8251	7142	13.3	171.7	108.67	65.17	30.33	6.642	3.658
BSPS SRB 48A-9-8	16044	7955	7244	8.92	181.7	76.33	50.47	30.67	6.041	2.464
BSPS SRB 48A-9-9	16080	7910	7458	5.65	69.7	17.33	28.96	34.67	3.498	0.499

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
Bulindi 1	9772	5481	4974	9.27	102.3	44.67	47.19	31.33	4.942	1.447
Bulindi 11	11244	6275	4697	24.57	166.7	97	61.25	30	6.562	3.355
Bulindi 119-1A	13164	7012	5757	18.02	161	91.67	56.09	31.33	6.222	3.035
Bulindi 12	14184	7256	6513	10.36	110.3	69	39.25	18.67	4.462	2.464
Bulindi 12C	15977	7424	6278	15.43	146	92.67	55.93	30.33	6.128	3.189
Bulindi 14A	15311	7869	6978	11.42	141.7	83.67	59.75	27.67	6.936	3.01
Bulindi 14B1	16880	7711	6811	11.54	124	72	61.81	33	5.363	2.249
Bulindi 15-14	12099	6383	5319	15.93	122.3	66.33	43.65	32.67	4.773	2.133
Bulindi 15-2A	12488	6915	5834	14.79	101	53.67	54.78	30.67	5.636	1.804
Bulindi 15C	12892	6404	5561	12.89	153	82.67	65.22	32	5.473	2.722
Bulindi 15E	11659	5711	4934	13.59	144	83	59.27	28.67	6.697	2.896
Bulindi 16A	12824	6437	5386	16.02	181	103.33	69.24	29.67	6.589	3.657
Bulindi 18-4A	11694	6321	4935	20.46	107.3	74.67	64.95	30.67	5.881	2.472
Bulindi 18-6	13254	6754	5884	12.98	116.3	78	67.6	31.33	6.034	2.589
Bulindi 18.1A	12278	6252	5104	18.4	201	125	68.39	30	6.758	4.316
Bulindi 18.4	12141	5900	4929	16.45	145.7	105	77.17	31.33	6.383	3.339
Bulindi 18A	12599	6234	5606	10.12	83	55.67	81.49	31	5.45	1.772
Bulindi 18B	14455	7097	6044	14.83	142	96	65.51	32	6.073	3.065
Bulindi 19.1B	14691	7090	5804	17.89	180	128.33	74.45	30	6.368	4.209
Bulindi 19A	13770	7044	5895	16.11	194.7	104.67	53.01	30.33	6.537	3.524
Bulindi 21A	13042	6389	5184	18.88	149	100.67	67.65	30	6.682	3.36
Bulindi 22	12563	6571	5483	17.11	215.7	108.67	55.14	30.33	6.14	3.692
Bulindi 22B	12998	6427	5277	17.76	156	102.67	55.15	33.33	5.648	3.366
Bulindi 22C	12062	6337	6221	1.85	45.7	12.67	27.71	32.67	3.37	0.387
Bulindi 24	11099	5657	4666	17.39	206.7	93.33	53.25	33.33	5.657	2.942
Bulindi 24A-4A	14287	7095	5842	16.65	173.5	97.5	56.21	31.5	6.148	3.005
Bulindi 24B	12228	6029	5168	14.14	159	85	50.92	31	6.126	2.87
Bulindi 24C	13927	6971	6260	10.01	151.3	78.33	59.18	30.67	6.078	2.647
Bulindi 25B	13239	6657	5334	19.76	211.3	128.67	59.73	31	6.617	4.132
Bulindi 26	14193	7077	6267	11.44	142.3	81.67	49.01	33.33	5.135	2.653
Bulindi 27-1A	12039	5803	4851	16.28	158.7	85	52.83	33	5.802	2.567
Bulindi 27A	13054	6805	6112	10.33	106.3	71.33	60.99	29.33	5.867	2.372
Bulindi 27B	11122	5703	5141	10.03	79.7	54.33	67.84	37	4.226	1.713
Bulindi 29	11444	5765	4784	16.92	157.3	95	56.24	29.67	6.191	3.278
Bulindi 2A	12721	6185	5026	18.66	187	108.33	55.62	32.67	6.008	3.43
Bulindi 2B	12019	5977	5002	16.27	125.3	79.67	67.91	32.67	5.753	2.456
Bulindi 30.1	13758	6941	6187	10.95	121	70.33	79.34	36	4.138	2.372
Bulindi 31	12639	6496	4947	23.9	239.3	160	66.82	30.67	7.192	5.213
Bulindi 33B	13312	6735	5621	16.49	189.3	120	65.49	31.33	6.462	4.055
Bulindi 35A	12112	6041	5270	12.59	87.3	73.33	90.02	32.33	5.523	2.29
Bulindi 36B	14521	7199	6681	7.1	90	56.33	38.44	20.67	3.951	1.786
Bulindi 39	13106	6630	5221	21.42	226	117.67	62.06	30.33	6.613	3.925
Bulindi 39C	11411	5718	5163	9.51	81.7	54	75.28	32.67	5.175	1.691
Bulindi 3A	12996	6535	5724	12.4	105.7	73	68.44	30.67	6.067	2.388
Bulindi 3B	13849	6282	5084	19.04	216.3	129.67	56.18	32	6.26	4.274

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
Bulindi 3C	13119	6320	5154	18.4	134.7	111	82.29	31	6.605	3.605
Bulindi 40A	13323	6627	5905	11.06	133	77.33	65.24	31	5.726	2.635
Bulindi 43	12615	7070	5933	16	109	58.33	51.13	33.67	5.001	1.803
Bulindi 47B	13164	7737	5964	20.65	132.3	89.67	45.97	19	4.959	3.137
Bulindi 48C	12917	6495	6177	4.86	85.3	25	18.86	24	2.86	0.665
Bulindi 48D	12476	6375	5283	13.45	134.3	92.67	70.56	31.67	6.178	2.956
Bulindi 49A	14125	7041	5913	15.85	198.7	109.33	52.93	30.67	6.533	3.641
Bulindi 49B	12728	6641	5628	15.19	154.7	104	65.96	29.67	6.696	3.56
Bulindi 49C	11296	5772	4563	20.84	166.7	121.67	72.34	29.67	6.89	4.134
Bulindi 49D	14487	7170	6160	13.95	123	87	73.16	32	5.866	2.801
Bulindi 4A	12263	6161	5388	12.64	83.3	58.33	67.54	31	5.654	1.955
Bulindi 4B	13162	7549	5712	24.37	282.3	157.67	56.22	31	7.328	5.369
Bulindi 5.17B	13257	6246	5473	12.38	102.7	66.67	68.87	31	5.882	2.159
Bulindi 50C	13063	6533	6132	6.18	93.3	38.67	31.21	22	3.523	1.172
Bulindi 51	13123	6660	5834	12.23	143	75.33	50.56	32.67	5.337	2.488
Bulindi 52B	9572	6855	6082	11.82	130.7	40	21.99	33.33	3.496	1.23
Bulindi 54	12548	6691	5813	13.35	138.7	77.33	53.94	31.67	5.511	2.615
Bulindi 55-1A	13698	7083	6283	11.13	162.3	78.33	32.52	21.67	4.208	2.385
Bulindi 55B	12720	6268	5684	9.52	103.7	54.33	47.03	26.67	6.156	2.053
Bulindi 56	12422	6129	4702	23.21	229	151.67	66.19	30.33	7.147	5.047
Bulindi 56A	12233	6113	4977	18.63	131.3	89.33	66.95	32.33	5.932	2.794
Bulindi 57	12076	6128	5895	3.81	44.7	19	45.02	33	3.865	0.58
Bulindi 5C	14575	6870	6087	11.13	122.3	80.67	65.01	31.33	5.563	2.872
Bulindi 5P-22C	12733	6040	5056	16.14	131	78	54.36	28.67	6.193	2.74
Bulindi 61	12245	6565	5224	19.84	120.7	74.33	57.29	32	5.569	2.374
Bulindi 61A	*	6734	5117	21.14	149.7	91	59.39	30.33	6.356	3.131
Bulindi 61C	11457	5807	4806	17.21	157	92	57.64	29.33	6.599	3.125
Bulindi 62B	12093	5879	4878	16.27	196.7	106.33	50.38	31	6.338	3.636
Bulindi 63A	11151	5653	4668	17.54	185.3	99.33	51.44	30.33	6.437	3.373
Bulindi 63E	11185	6706	4942	24.05	130	72.33	53.3	30	6.085	2.441
Bulindi 64	11650	5688	4482	20.73	134.3	56.33	35.51	33.67	4.472	1.764
Bulindi 65	12465	6733	6205	7.9	77	46	63.21	33	4.675	1.4
Bulindi 67	13573	7387	6363	13.44	115	78.33	67.44	31.67	6	2.521
Bulindi 68	13218	6395	5945	6.98	75.7	43.33	45.99	36.33	3.99	1.342
Bulindi 6B	14366	6987	6731	3.68	25.3	19	75.76	35	3.404	0.583
Bulindi 6C	13278	6890	6607	4.14	76	26.33	43.87	32	4.317	0.821
Bulindi 7.2B	14417	7082	5563	21.52	247.3	160.33	63.45	31	6.993	5.209
Bulindi 70	13898	7161	5943	17.01	134.7	119	90.67	30.33	6.647	3.998
Bulindi 72-2	11569	5782	5019	13.34	138.7	66.33	57.58	32.33	5.376	2.124
Bulindi 74	12194	6193	5751	7.11	84.7	38.67	44.8	33.67	4.704	1.209
Bulindi 74C	15097	6793	5602	17.05	273.7	140.67	53.39	35	6.095	4.021
Bulindi 75B	13032	6655	5529	17.43	153.3	98.67	64.14	31.67	5.914	3.221
Bulindi 76C	13071	6536	5977	8.38	103.3	73.33	73.59	31	6.025	2.377
Bulindi 77	13001	6708	5847	12.89	143.7	77.33	47.78	30	6.004	2.696
Bulindi 77 C	12646	6799	5397	19.32	137.7	77.67	57.8	31.33	6.006	2.475

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
Bulindi 77A	12627	6918	5022	27.17	187.7	118.33	62.72	31.33	6.032	4.005
Bulindi 77B-1	13666	7033	5761	18.06	225	119	50.34	27.67	7.514	4.683
Bulindi 7B	13330	6573	6013	8.48	97.3	46	41.18	32.33	4.676	1.537
Bulindi 7E	10146	5199	4442	14.61	139.7	72	50.97	29.33	6.309	2.429
Bulindi 81-1A	13098	6598	5806	11.97	125	80	50.58	35.33	4.714	2.418
Bulindi 85	11230	6298	5211	17.14	114.7	61.67	48.31	33.33	5.112	1.999
Bulindi 8B	13556	7189	6185	13.95	99	50	45.35	32.33	4.894	1.616
Bulindi 8C	13122	6325	5038	20.46	214.7	124	64.76	31.33	6.634	4.001
Bulindi 9.2B	14654	7135	6591	7.68	101.7	54	48.23	35	4.628	1.483
Bulindi 9A	12023	5964	4978	16.24	188.3	92.33	55.65	32.33	6.062	2.989
Bulindi 9B	13223	6698	5544	17.04	184.7	111.67	62.95	31.67	6.331	3.622
Bulindi 9C	12935	6850	5894	14.32	213	93.33	39.1	34.33	5.487	2.957
Bulindi BT6	13681	6937	6428	7.52	70.7	43	50.85	24	3.352	1.194
Bulindi Q	12552	6171	5542	10.22	91	61	72.03	32	5.515	1.916
Bulindi R	13699	6277	5989	4.69	47	32.33	68.42	31.67	4.679	1.004
Bulindi SRB 18	14597	7311	6658	8.97	113.7	56.33	39.91	34.67	4.608	1.808
Bulindi XX	12973	6560	5284	19.4	195	112.67	59.19	30.33	6.769	3.724
Bulindi Y	12435	5985	5165	13.83	129.7	79.67	41.05	21	4.37	2.54
Duiker	16045	6866	6554	4.55	89.3	36.67	41.6	32	4.849	1.194
Duiker ZIM	15962	8052	7932	1.48	61.3	12.67	20.31	38	2.597	0.34
DXT SPS 15.6-2	18004	9298	8968	3.55	80.7	29.33	32.5	35.33	3.816	0.844
Elite Lines 4.11-11	14407	6824	6623	3.1	42.7	20.33	32.66	26.67	2.054	0.628
Elite Lines NII x GC 20.2	13772	6987	6516	6.7	127.7	53	41.29	32.67	5.068	1.664
G 7955	13031	6293	5839	7.08	131	35.33	44.27	25.67	1.821	0.954
G 7955 * Nam 4m	14499	7152	6946	2.83	88.7	25.33	27.64	33.67	4.058	0.757
G 7955*Nam 4m B	14638	7137	6495	9.04	128.7	60.67	40.87	32.33	5.063	1.986
GAZELLE	15593	7706	7584	1.6	108	10.67	8.78	39.67	2.396	0.298
GC 00138-29	16170	8119	7396	8.98	149	75.67	49.79	30.67	6.02	2.489
Introdn G 01	12911	6252	5860	6.27	163.7	52.67	37.71	32.67	5.199	1.624
Introdn G 02	6285	3050	2462	19.27	177.3	67	40.32	33.67	5.421	1.993
Introdn G 04	12080	5968	5650	5.54	92.3	35.33	31.61	35.33	3.746	1.08
Introdn G 07	17480	8389	7102	15.26	181.3	111.67	61.72	29.67	6.857	3.778
Introdn G 07B	14936	7996	7732	3.22	47.3	31	66.17	30	4.787	1.033
Introdn G 08	14603	6776	5748	14.77	194.7	62.33	31.8	32	5.42	1.971
Introdn G 09	13110	7177	6700	6.54	109.3	54	37.71	32.33	3.974	1.693
Introdn G 13	16	7934	7304	7.85	125.7	73.67	58.46	30.33	5.708	2.355
Introdn G 14	12822	6553	6195	5.52	112.7	39.67	33.43	31	5.389	1.473
Introdn G 15	14404	6910	6758	2.1	126	25.67	20.34	34.67	4.042	0.743
Introdn G 16	16781	8286	7865	5.02	164.7	46.33	26.93	32.67	4.896	1.498
Introdn G 17	10	5287	5147	2.65	35	13.33	25.77	22.67	2.54	0.391
Introdn G 19	12660	5968	5686	4.53	101.7	32	31	35	3.581	0.986
Introdn G 20	17905	8558	7823	9.61	136.7	42.33	30.52	31	5.105	1.388
Introdn G 21	14560	7454	6928	6.97	110.3	62.67	56.97	31.33	5.62	2.051
Introdn G 22	10028	4908	4145	15.66	190.3	84	46.63	31.33	5.946	2.78
Introdn G26	15819	8048	7403	8.09	186	66	31.37	35.67	4.86	1.978

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
Introdn G28	13676	6678	6238	6.61	101.7	50.67	66.41	31	4.287	1.634
Introdn G29A	6929	3400	2843	16.44	133.7	66.33	48.04	32.67	5.487	2.047
Introdn G29B	13942	6873	6158	10.27	137.3	82.67	53.26	34	5.12	2.703
Introdn G29B-2	15451	8366	8004	4.4	97.3	46.67	26.9	32.33	5.016	1.483
Introdn G30	11852	5605	4931	12.58	140.7	76	49.91	31	5.802	2.446
Introdn G31	12313	6082	5652	7.14	93.3	38	25.88	20.67	3.685	1.226
Introdn G32	17333	8962	8379	6.34	150.7	72.33	43.36	30.33	5.831	2.389
Introdn G33	11491	5896	4995	14.83	165.7	51.33	31.9	32.67	5.166	1.579
Introdn G34A	16299	8662	8006	7.98	178.7	72.33	42.74	31.67	5.707	2.424
Introdn G34B	4944	6668	6238	6.64	139.7	37.67	32.66	35.33	3.773	1.094
Introdn G37	16150	8293	7972	3.95	106.7	41.67	38.88	32.33	4.964	1.296
Introdn G38	17	8845	8417	5.02	98.7	42	37.56	34.67	4.283	1.329
Introdn G39	11829	5783	5325	7.75	138.3	55.33	36.44	35	4.71	1.606
Introdn G41	6255	3049	2383	22.26	202.7	86.67	42.8	31	6.074	2.775
Introdn G42	12791	6438	6283	2.54	85.7	21	48.27	33	2.917	0.707
Introdn G43	12563	5881	5833	0.78	34	9	29.64	36	2.338	0.253
Introdn G44	20384	10311	9611	6.72	157.3	76.33	44.6	34.33	5.318	2.319
Introdn G45	13668	6717	6221	7.41	149.3	45	38.23	33	4.949	1.381
Introdn G46	14706	7223	6468	10.21	139.7	85.33	60.39	31.67	5.943	2.757
Introdn G47	12784	6523	6198	4.83	113	14.67	12.15	38	2.907	0.395
Introdn G48	15269	7441	7039	5.47	143.3	47	30.74	33.67	4.679	1.51
Introdn G49	11105	6928	5759	13.86	76.3	11	11.03	38.67	2.154	0.336
Introdn G4B	6406	3126	2940	6.12	60.3	24.33	41.13	37.67	3.456	0.728
Introdn G50	14323	7301	7183	1.57	69.7	21.33	25.18	34.33	3.277	0.707
Introdn G52	15	7500	7136	4.82	70.3	39.67	53.01	34.67	4.448	1.231
Introdn G53	14	7019	6619	5.34	94.7	36.67	32.11	34.67	4.133	1.187
Introdn G54	20515	10430	10384	0.47	52.3	16.67	34.31	30	3.934	0.533
Introdn G55	14	7048	6664	5.44	136.7	36.67	28.34	33	4.585	1.109
Introdn G56	13704	6908	6567	4.73	123.7	47.33	48.04	32.67	4.043	1.443
Introdn G58	17543	9186	8603	6.41	165.3	72	40.83	31.33	5.666	2.25
Introdn G59	10477	5264	5168	1.83	134	18	17.69	32	3.751	0.559
Introdn G60	17049	9230	8721	5.63	216	58	32.57	34.67	4.206	1.907
Introdn G61	11490	7648	7541	1.74	71.3	40	31.62	23.33	2.795	1.173
Introdn G62	17057	8918	8583	3.96	159.7	40	24.65	33.67	4.173	1.192
Introdn G64	12293	6260	6106	2.48	115.7	25	16.51	36.33	3.032	0.766
Introdn G65	14766	7453	6437	13.63	179.3	111	62.14	30	6.836	3.776
Introdn G66	6973	3492	2821	19.29	137.7	64.67	48.17	30.67	5.823	2.122
Introdn G67	14188	7886	7138	9.25	158.7	83.33	45.95	31.67	5.319	2.708
Introdn G69	15263	8005	7770	2.92	108.7	30	51.23	33.33	3.567	0.907
Introdn G70	10109	5087	4740	6.68	73	40.33	48.07	35	4.067	1.194
Introdn G71	15279	7415	6635	10.57	193	78.67	37.49	31.33	5.797	2.676
Introdn G72	10297	5176	4708	8.81	192.7	48	21.05	36	3.963	1.342
Introdn G73	13687	6862	6372	6.99	85.3	57.33	42.96	21	3.97	1.918
Introdn G74	14462	6942	6739	2.94	101.7	26	21.32	36.33	3.581	0.818
Introdn G75	14034	6897	6537	5.21	163.3	40.33	24.39	34.33	4.602	1.201

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
Introdn G79	11259	5445	4640	14.75	231.3	92.33	41.36	30.67	6.389	3.018
Introdn G80	12456	6218	5751	7.49	162	45.33	26.55	34.33	4.683	1.322
Introdn G81	11159	6160	5390	11.09	117.3	16.67	15.4	35.33	3.348	0.484
Introdn G83	12261	6642	5410	17.67	136.3	82.67	40.62	22.33	4.163	2.465
Introdn G84	12055	6118	5473	10.39	122.3	68	36.14	32.33	4.696	2.262
Introdn G85	14593	7471	7044	5.61	89	44	50.52	33.67	4.806	1.369
Introdn G86	18135	9393	8668	7.7	157	84.67	69.68	33	5.31	3.08
Introdn G87	18477	9849	9495	3.65	81	47.67	62.53	32	5.047	1.541
Introdn G89	12137	6402	6081	4.46	26.3	4.67	23.86	43.33	1.667	0.124
Introdn G91	16085	8128	7411	8.72	155.7	85.33	53.53	30.33	6.201	2.847
Introdn G96	21861	10978	10157	7.65	142.7	82.67	58.11	29.33	6.281	2.761
K-Local	16137	7488	6984	6.57	93	55.33	56.58	31	5.401	1.788
Kab x UG 5	11934	5828	5382	7.64	151.7	52.33	34.91	29	5.892	1.765
KABI	11162	5399	4783	11.48	166.3	79	44.45	31.67	5.792	2.559
Kuntz	17194	8174	7723	5.59	108.3	49.33	47.57	34.67	4.678	1.38
Maksoy 1N	12912	6312	5833	7.6	112.7	9	7.99	36.00	2.338	0.253
Maksoy 2N	14594	6698	6401	4.95	104.3	40.33	36.45	19.67	4.048	1.358
Maksoy 3N	18061	8660	7749	10.5	186.3	90	48.22	31	6.359	3.018
Maksoy 4N	17555	8944	7981	10.7	135	89	65.47	30.33	6.457	2.997
Maksoy 5N	15742	7719	7364	4.57	73.3	39	48.05	32.67	4.637	1.23
MNG 11.2	*	6597	6011	8.52	164.3	71.67	42.05	32.67	5.521	2.198
MNG 12.4	13597	6583	6280	4.54	86.7	35.33	42.32	32.33	4.328	1.134
MNG 14.1 x NII F10	14191	7167	6233	13.01	153.7	77	47.58	32.33	5.749	2.51
NAM I	10438	6152	5253	12.61	93.3	28	28.65	33.67	4.013	0.831
NAM II	9254	4255	3406	22.48	181.3	86.33	40.56	32	5.402	2.736
Namsoy 3	11483	5639	5072	10.1	137	62.67	44.95	31.33	5.539	2.014
Namsoy 4M	14950	6954	6318	9.11	167	48.67	30.49	32.33	5.107	1.531
NG 14.1-6R	16661	8683	8059	7.07	99.3	59.67	57.56	30	5.826	2.045
NGDT 1.33-2	12338	6123	5260	14.02	306	76.33	24.69	32.67	5.785	2.383
NGDT 1.33-2B	12446	6195	5822	6	89	29.33	29.02	40	3.282	0.778
NGDT 1.35	14592	6493	5785	10.61	101	29.67	34.24	31.33	4.328	0.95
NGDT 1.4	15851	8214	7585	7.62	145	55	37.6	33	4.991	1.751
NGDT 10.1-3	15388	7658	7138	6.76	151	50	43.13	33.67	5.047	1.511
NGDT 10.10	16830	8592	7768	9.57	170	83.67	85.69	30.33	6.249	2.792
NGDT 10.13	12420	6644	5356	18.97	137.3	83.67	60.46	29.33	6.547	2.889
NGDT 10.4-2	12492	6112	5150	16.11	217	87.67	36.42	32	5.822	2.966
NGDT 10.4-4	13364	6688	6051	9.65	128.7	48	35.16	33	4.349	1.587
NGDT 10.4-5	15141	8183	6752	16.68	269.3	66.67	26.64	31.33	5.831	2.162
NGDT 10.6-2	13899	6603	5811	11.7	117.7	53	50.28	32	5.265	1.726
NGDT 11.11-6	11997	6041	5492	9.16	137.7	64.67	46.94	36	4.018	2.069
NGDT 16.16-2	10882	5206	4988	4.21	69.5	17	26.38	37	3.272	0.459
NGDT 2.12	13807	6840	6533	4.41	82.7	29	34.1	31.33	4.596	0.953
NGDT 2.15-10	14376	7475	7324	2.01	60.7	18.67	31.1	33.67	3.568	0.565
NGDT 2.15-13	10873	5557	5086	8.53	132	47	37.94	30.67	5.424	1.534
NGDT 2.15-1D	9805	4875	4253	12.8	163	57.67	34.56	34.67	4.981	1.681

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
NGDT 2.15-2A	11986	6115	5456	10.78	99.7	43	37.85	33.67	4.432	1.369
NGDT 2.15-2B	14947	7519	6474	13.91	151.3	92.33	62.36	29.33	6.693	3.144
NGDT 2.15-3	13620	6658	6109	8.24	106.3	45	28.22	38.33	3.873	1.429
NGDT 2.15-4	13122	6415	5798	9.57	117	61.67	52.36	32	5.601	1.958
NGDT 2.15-4A	17794	9124	8164	10.56	187	89.33	44.49	33.33	5.69	2.781
NGDT 2.15-5	12638	6577	5920	10.06	139.3	65	47.82	32.33	5.537	2.02
NGDT 2.15-6	14937	7135	6766	5.01	110.3	54.67	64.72	32.67	5.197	1.714
NGDT 2.15-7	12668	6641	6335	4.6	109.3	27.67	22.95	35	3.546	0.823
NGDT 2.15-8	13400	6849	5996	12.57	191.3	76.67	35.19	33.67	5.204	2.569
NGDT 2.15-9	11823	5661	5430	4.23	75.7	22	20.1	21	3.163	0.69
NGDT 2.15-9B	12399	6122	5765	5.83	80.7	30	39.49	35	4.058	0.898
NGDT 3.14-1	14106	7137	6268	12.24	151	79.67	56.3	28.67	6.565	2.783
NGDT 3.17-1	12038	6064	5737	5.43	70.3	35.67	29.49	24	3.008	1.056
NGDT 3.21B	15664	7717	7480	3.08	141	25	16.24	34.33	3.821	0.791
NGDT 3.24-2	12569	6781	6667	1.67	70.3	12.67	30.66	38	2.433	0.329
NGDT 4-11-2	18469	8601	7741	9.99	213.7	98	41.54	31	6.109	3.305
NGDT 4.11-10	17587	8690	8022	7.72	137	61.67	41.37	31.67	4.916	1.97
NGDT 4.11-21	15672	7346	6868	6.55	174.7	49.33	26.03	35.33	4.599	1.427
NGDT 4.11-3	18190	8120	7810	3.58	99.7	63.67	42.73	20.33	4.326	2.07
NGDT 4.11-4	17379	8829	8138	7.79	97.3	41.33	43.36	31.67	5.051	1.31
NGDT 4.11-7	16866	7775	7549	2.89	81	40	49.93	32	4.666	1.233
NGDT 4.17-4	14282	7021	6711	4.63	100.7	34.33	30.84	32.67	4.304	1.091
NGDT 4.20-1	12623	7637	6009	16.33	86.7	38	27.19	22	3.289	1.152
NGDT 7. 20-2	15289	7260	6891	4.87	56.7	37.67	68.35	31.33	5.037	1.223
NGDT 7.11-2	13314	6530	5501	15.85	184	93	60.58	31	6.306	3.06
NGDT 8.1	15835	8480	7696	8.65	256	76	27.18	31	5.248	2.581
NGDT 8.10-9	13552	6804	6469	4.88	90.7	39.67	41.28	34.67	3.497	1.229
NGDT 8.11-21	18412	8574	7832	8.64	118.3	51.67	41.32	33	4.917	1.62
NGDT 8.19	13957	6851	6242	8.98	142.3	58.67	37.5	32.33	5.223	1.837
NGDT 9.4	14370	7055	6866	2.7	57.3	16	29.36	33.67	3.41	0.467
NGDT BLP 12.4	15712	6513	5873	9.48	123.7	61.33	40.24	33.67	4.62	1.977
NII Ã— GC 28.2B	*	5648	5374	4.91	162.3	28.67	15.25	33.33	4.04	0.887
NII Ã— GC 35.3	*	6408	5542	13.46	187.3	91.67	48.09	31	6.293	3.035
NII x GC 1.15	12879	6011	5780	3.75	85.7	31.67	33.95	34.67	3.634	1.058
NII x GC 1.3	14417	7101	6870	3.3	119	26.67	24.72	34	3.974	0.783
NII x GC 11.1	12583	5943	5203	12.19	167	84.33	52.89	28.67	6.8	3.036
NII x GC 11.1B	12814	6551	5922	9.63	132	73	54.24	30.33	6.045	2.428
NII x GC 11.3	14015	6661	6392	3.98	91.7	41.33	43.32	31.33	5.04	1.435
NII x GC 13.1B	13197	7034	6460	7.92	126.3	68	42.03	31	4.878	2.251
NII x GC 14.4	13726	6824	5879	13.74	169.7	108	51.25	31	5.855	3.649
NII x GC 17.2	15016	7628	6759	11.41	188.7	90.67	48.11	30	6.507	3.027
NII x GC 17.2B	14853	7904	6753	13.82	129	58.67	55.75	32	4.966	1.854
NII x GC 17.3B	16357	8137	7480	8.08	143	66	24.3	20.67	3.871	2.324
NII x GC 17.3C	13837	7216	6693	7.2	117.7	43.33	35.59	31.67	5.014	1.352
NII x GC 17.4	14421	6213	6142	1.03	86.3	39.33	29.28	19	3.949	1.29

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
NII x GC 18.1A	15120	7321	6209	15.22	159.7	98.33	63.7	29.67	6.64	3.328
NII x GC 20.10-1	14858	7672	6898	9.95	230.7	89.33	35.47	30.67	5.995	2.982
NII x GC 20.2B	13867	6986	6615	5.23	146	41.67	25.71	32.67	4.622	1.289
NII x GC 20.6	15998	7945	7731	2.72	77.7	49.67	62.33	34	4.376	1.591
NII x GC 22.1	13133	6502	5719	11.93	245	86.33	35.87	31.33	6.076	2.808
NII x GC 22.10	17438	8297	7944	4.02	102.7	44.33	33.61	20.33	3.978	1.451
NII x GC 25.1	15471	7913	7176	9.19	150.7	70.33	42.24	31.67	5.578	2.329
NII x GC 28.2A	14171	7146	6763	5.42	72.7	35.33	67.41	34	4.12	1.172
NII x GC 3.3A	14270	7238	7171	0.97	66.7	11.33	10.76	31.33	2.672	0.328
NII x GC 3.3D	13373	6631	6334	4.48	124.3	36	27.13	31.33	4.705	1.107
NII x GC 30	13220	6673	6256	6.12	51.3	18.33	55.37	32.33	3.588	0.568
NII x GC 33.3	16656	8546	7870	7.93	115.7	61	51.4	31.33	5.638	2.092
NII x GC 34.1	14288	7079	6379	9.86	91	48	50	35	4.825	1.473
NII x GC 35.4	16672	8416	7671	8.71	160.3	75.67	37.03	33.67	4.871	2.479
NII x GC 38.1	13038	6543	6159	6.07	99	34.67	22.69	23	3.318	1.126
NII x GC 38.1A	13203	6704	6378	4.74	193	39.67	17.06	36	3.885	1.15
NII x GC 4.1-2	12433	6391	5769	9.78	164.3	51	32	31.33	5.335	1.678
NII x GC 4.4	13345	6941	6334	8.76	133.3	60.67	37.37	34	4.205	2.031
NII x GC 4.8B	16143	8057	7675	4.74	120.3	46.33	41.09	31.67	4.964	1.513
NII x GC 40.3	16025	8084	7026	13.06	219	100	46.38	30	6.68	3.346
NII x GC 41.2	13797	6956	6560	5.7	107.7	53.33	49.87	30	5.598	1.759
NII x GC 41.8	14379	7692	6577	14.1	111.3	68.67	60.42	29.67	6.086	2.376
NII x GC 42.5	12694	6903	6502	5.73	59.7	24	26.05	20	3.37	0.83
NII x GC 42.6	*	5880	5445	7.04	83	45	48.15	34	4.422	1.396
NII x GC 43.4	15384	7751	7341	5.27	74.3	45.33	48.55	32	3.884	1.4
NII x GC 43.6	15438	7571	6588	12.95	178.7	101	52.63	31.67	6.144	3.456
NII x GC 43.6B	15046	7509	7204	3.96	82.7	45.33	53.82	31.33	4.994	1.482
NII x GC 44.1	13437	6702	6130	8.41	156.3	64	42.98	29	6.098	2.305
NII x GC 44.3	15207	7629	7042	7.68	178	62	20.53	36.67	3.367	2.272
NII x GC 48.3	13842	6850	6063	11.57	150.3	49.33	39.72	33.67	4.896	1.507
NII x GC 53A	13687	6978	6244	10.65	165.7	82.67	25.08	22	3.619	2.574
NII x GC 53B	14503	7263	7130	1.78	69.3	19.33	16.94	24	2.447	0.511
NII x GC 6.0	13836	6586	6316	3.76	145.7	58.33	39.03	31	5.491	1.892
NII x GC 7.2B	16118	7913	7377	6.79	91.3	60.67	45.49	19.33	4.488	2.092
NII x GC 7.3A	16062	7952	7550	4.9	95	39	25.85	30.67	4.087	1.527
NII x GC 7.3B	15829	7984	7617	4.57	118.3	42.33	36.5	31.33	5.162	1.386
NII x GC 7.3D	14572	6939	6316	8.97	198.7	103.33	49.68	29.67	6.595	3.423
Obs 101	13891	7468	6712	9.09	91	54.5	39.23	32	5.376	1.703
Obs 102	14534	7423	6386	14.19	196	96.67	51.51	29	6.794	3.358
Obs 103	15638	7973	7681	3.6	69.3	21.33	20.67	21.33	3.128	0.671
Obs 104	12995	6471	5380	16.29	265.7	110	35.43	32.33	5.585	3.741
Obs 106	15130	7718	6655	13.86	135.3	99.67	74.78	31	6.402	3.22
Obs 108	13889	7080	5562	21.4	274.3	136.33	49.15	30.33	6.996	4.557
Obs 109	18770	8854	7511	14.9	233.7	127.67	53.17	30	6.839	4.411

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
Obs 110	18325	8896	8524	4.2	82	25.67	33.41	33.67	3.878	0.782
Obs 111	12988	6548	5363	18.13	216.3	97	45.03	30.67	6.473	3.176
Obs 112	13000	5707	5425	4.83	145.3	35.67	23.03	31	4.841	1.186
Obs 113	14194	7273	6804	6.52	91.3	40.67	43.57	33	4.895	1.286
Obs 115	14900	7644	6378	16.65	172.3	109.67	42.6	19	5.186	3.851
Obs 116	14757	7381	5848	20.78	312.3	146.33	47.08	29.67	7.313	4.945
Obs 117	13913	6771	5926	12.27	80	48	67.43	31	5.299	1.526
Obs 118	13016	6547	5452	16.71	211.3	112	53.7	31.33	6.322	3.735
Obs 119	17489	8397	7279	12.12	126	85	73.45	30.33	6.024	2.871
Obs 120	15066	7070	6858	2.89	124	21.67	18.18	35	3.249	0.631
PYT NaCRRI Plot 111	13743	6724	6181	8.13	73	52	46.84	21.33	3.914	1.625
ROAN	16452	7813	6779	12.84	232.7	108.33	44.54	34	5.3	3.488
S- Lines 13.2	7137	3490	3145	9.79	104.7	38.33	35.6	36.67	4.249	1.06
S- Lines 4.21	14240	6290	5992	4.35	126.7	34.33	24.29	33.33	4.483	1.189
S-Lines 1.19	10391	5161	4487	13.05	120.3	71.67	60.71	30.67	6.072	2.385
S-Lines 10.4	8490	4257	3940	7.37	105.3	26.33	21.57	39.67	3.103	0.711
S-Lines 11.1	11094	5335	5071	4.9	98.7	30.33	31.86	33.33	4.466	0.919
S-Lines 12.17	13987	7315	6770	7.37	103.3	30.33	24.49	35	3.278	0.932
S-Lines 13.12	8535	4947	3677	23.39	104.3	55	32.63	32.67	3.827	1.87
S-Lines 13.14	7500	3759	3047	19.07	123	71.33	57.67	30	6.161	2.417
S-Lines 13.2A	8455	3977	3842	3.44	140	12	7.18	23.67	2.12	0.357
S-Lines 16.2	12056	6447	5949	7.21	71.7	44	53.07	35	4.058	1.35
S-Lines 3.16	10254	5023	4475	10.94	159	52.67	32.62	33.67	4.952	1.577
S-Lines 3.17	10368	7526	5974	20.66	272.7	149.67	69.45	30	7.243	5.004
S-Lines 3.7	13271	7350	6254	14.43	161.3	69.67	44.34	28.67	6.171	2.456
S-Lines 5.18	12618	6572	5317	19.05	234	127.67	54.86	30	6.843	4.305
S-Lines 6.22	12086	6031	5672	6.15	92.3	44	36.66	33.67	3.913	1.438
S-Lines 7.11	7006	3560	3003	15.72	153.7	60.67	39.51	34	5.205	1.791
S-Lines 9.2	9566	6411	4926	15.94	52	14	17.58	24.33	2.123	0.38
SAFARI	15307	7049	6805	3.43	102	20	13.28	32.67	3.804	0.583
SAGA	19234	9178	8412	8.42	66.7	35	30.86	20.33	3.505	1.16
Santa	15454	6697	6375	4.73	131	25.67	14.71	25.33	2.76	0.672
SEMEKI	17895	8448	7986	5.4	118	60	42.82	32.67	4.937	2.029
SERENADE	19391	9162	8688	5.27	143	59	36.21	31	5.271	1.905
Siesta	15978	8364	7863	5.85	121.3	51.67	43.33	33.33	5.084	1.583
Soprano	11901	5955	5091	13.99	121.3	55.33	43.04	34.33	4.81	1.621
SQUEL	16137	8278	7759	6.43	113	70.67	59.98	34.33	5.113	2.138
SQUIRE	17234	7565	7195	5.19	72	25.33	26.39	34.67	3.201	0.79
UG 5	18346	9323	9026	3.2	88.3	32.33	41.08	34.33	4.421	0.953
USA 10	14103	7288	6438	11.59	95.7	63.67	65.25	30.67	5.306	2.165
USA 10B	14208	7126	6059	14.8	146.7	87.33	64.45	32.33	5.834	2.738
USA 11(B1)	13423	6653	5922	11.29	159.7	85.67	47.97	31.67	5.657	2.794
USA 12	13527	6691	5743	14.19	150.7	76	51.35	31.67	5.944	2.412
USA 12(B2)	12004	5928	4986	16.03	168	86	50.79	34.67	5.568	2.528

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
USA 13	13704	6451	5226	18.68	167.7	114.33	68.66	31	6.608	3.705
USA 13B2	17506	8637	7404	14.34	217	117.67	55.86	31.67	6.514	3.745
USA 14	6552	3337	2817	16.08	71	43	62.76	34.33	4.213	1.318
USA 15(B1)	12332	6071	4814	21.05	196.3	108.67	54.15	32	6.265	3.478
USA 16	13442	6353	5265	16.74	134	93.33	68.83	30	6.22	3.198
USA 18/81	9989	4814	4181	13.39	96	67.33	66.19	35	5.096	1.967
USA 1B2	12969	6662	5348	20.04	204	111.33	55.33	31.67	6.413	3.69
USA 2	14889	7605	6705	12.05	178.7	75	39.9	34.67	5.301	2.339
USA 20	15882	8131	7190	11.51	139	82	57.62	32.5	5.752	2.585
USA 207	12866	6175	5194	15.84	262.7	104.67	36.46	34.33	5.7	3.16
USA 22	15547	7253	6265	12.71	124	85	64.3	32.33	5.657	2.763
USA 26	14080	6994	6022	13.88	98.7	65	64.84	32.67	4.759	1.993
USA 28	12412	6150	5181	15.42	131.7	78.67	56.75	31.33	5.814	2.608
USA 29	11818	6677	5428	18.96	213	88.67	34.38	33.33	5.233	2.851
USA 3	15998	8420	7124	15.22	173	97.67	50.78	29	5.871	3.52
USA 30	9955	4758	4028	15	102.7	74.33	75.54	37.33	4.163	2.411
USA 31	10231	5278	4743	10.05	105.7	44	47.59	36	4.555	1.222
USA 32	12851	6605	5059	23.45	152.7	91	65.5	30.33	6.451	2.985
USA 33	5225	2713	2309	57.47	102.7	42.67	43.95	35	4.668	1.228
USA 34	16308	7830	6918	11.67	122.3	69.33	55.5	37	4.667	1.959
USA 36	10861	5093	4306	15.38	141	67.33	45.57	32.67	5.438	2.088
USA 38	12310	6312	5188	17.71	160	83.33	51.58	32.67	5.72	2.607
USA 3B1	13782	6832	5411	20.83	220	104.33	46.55	31.67	6.345	3.361
USA 3B2	13713	6915	6241	9.74	119.7	44.67	37.19	33	4.961	1.353
USA 4(B1)	12083	6015	5237	12.78	122.7	68.67	54.41	33.33	5.325	2.178
USA 40	17131	8558	7874	8.2	89.3	44	49.33	36	4.361	1.252
USA 46	15422	7111	5984	15.66	173	104	61.63	32.67	5.707	3.335
USA 4B2	12540	6283	5412	13.87	121	66.67	67.69	29	6.043	2.333
USA 5	8505	4194	3677	12.38	117.3	29.67	23.66	36.33	3.703	0.848
USA 53	11624	5712	4787	15.9	156.3	89.67	86.78	35	4.942	2.886
USA 54 (serenade)	10888	5794	5179	10.05	104.3	53.33	42.59	33.33	4.795	1.74
USA 57	10830	5451	4349	19.87	207.7	106	54.3	32.67	6.103	3.322
USA 6	13532	6254	5729	8.36	90	37	25.61	36.33	2.953	0.974
USA 6(B1)	15912	7791	6372	17.91	191	106	53.18	32.67	5.906	3.332
USA 6(B2)	15727	7886	6672	15.43	146	98.67	65.57	30	6.571	3.317
USA 60	11148	6229	4796	21.24	117.7	63.33	45.27	37	4.248	1.836
USA 61A	12533	6717	5458	18.54	206.3	63.67	29.61	34	5.137	2.02
USA 64	12703	6560	5730	12.6	123.3	46.67	40.78	33	5.077	1.43
USA 65	14580	7003	6418	8.31	41.7	21.67	56.93	35.67	3.195	0.635
USA 66	10283	5231	4104	21.61	184.7	102.67	55.93	32.67	6.154	3.142
USA 7	13662	6708	4896	27.18	290.7	172.33	60.47	30.33	7.314	5.75
USA 72	17505	9013	8199	9.02	147.7	74.33	49.3	32	5.593	2.379
USA 7B1	16373	7232	6159	13.94	204.3	99	49.88	31.33	6.339	3.17
USA 8	14871	7410	5976	19.29	220.3	117	51.43	30.67	6.697	3.915
USA 80	14434	6901	5761	16.43	166	96.33	59.27	32.67	5.938	3.002

Genotype	100 seed wt	Initial wt	Final wt	% wt loss	Eggs	Adults	% IE	MDP	DSI	GI
USA 82	8887	4582	3641	20.44	191.7	95.67	49.06	34	5.812	2.92
USA 83	12462	6457	5663	12.45	88.3	53.67	60.44	31	5.164	1.792
USA 88	12346	6843	5831	13.23	40.3	16	42.33	31.67	3.198	0.486
USA 9	13728	6523	5880	9.8	75.7	43.33	43.69	20.33	3.944	1.417
USA 9(B2)	15417	7281	6174	15.12	149.3	78.67	51.77	33	5.644	2.36
USA XX	13291	6714	5330	20.57	188.7	128	60.56	33	6.002	3.991
Sig (F.pr)	<.001	<.001	<.001	<.001	0.595	0.001	<.001	<.001	<0.001	<.001