Genetic Studies on Host Plant Resistance to Mexican Bean Weevil (*Zabrotes subfasciatus* Boheman) in Ethiopian Common Bean (*Phaseolus vulgaris* L.) Germplasms

By

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Thesis Abstract

The common bean (*Phaseolus vulgaris* L.) is the second most important food legume in Ethiopia. It is the most important cash crop for the smallholder farmers and has a significant impact on the national economy. However, the productivity of the crop is hampered by many biotic and abiotic stress factors. In recent years, the Mexican bean weevil (*Zabrotes subfasciatus* Boheman), hereafter also called bruchid, has been causing significant grain losses in storage. Therefore, the objectives of the present study are as follows: (a) to evaluate the Ethiopian common bean landrace collections, commercial varieties, advanced breeding lines and elite resistant lines for resistance to the Mexican bean weevil; (b) to assess the magnitude and pattern of genetic diversity in Ethiopian common bean landraces, commercial varieties, advance breeding and exotic resistant lines for the response to infestation by bean bruchid, using phenotypic and SNP markers; (c) to examine the population structure among common bean genotypes collected from different breeding status, seed colours and sizes, and to identify genomic regions that are associated with bean bruchid resistance, using SNP markers distributed across common bean genome; (d) to assess the agronomic performance of common bean genotypes selected for their response to bruchid infestation, using yield and yield components under different agro-ecologies; (e) to identify suitable parental genotypes that are useful for breeding for bruchid resistance and to identify the farmers' selection criteria for choosing varieties; and (f) to interogress arcelin genes into commercial varieties and an advanced breeding line. For this study, a total of 300 common bean genotypes were phenotyped for bruchid resistance under laboratory conditions, and they were genotyped, using Illumina BARCBean6K_3 SNP BeadChip. Data on insect and seed traits were used for genetic diversity and genome-wide marker-trait association analysis. One-hundred and-forty-four genotypes were selected, based on their level of resistance, population structure and genetic distances and they were evaluated under three different agro-ecological field conditions, for yield and yield-related traits. Participatory variety selection (PVS) was also conducted for the selected genotypes. Subsequently, six female parents and seven donner lines were selected, based on the farmers' traits of interest, level of resistance and suitable agronomic traits, of which one commercial variety and one advanced breeding line were crossed with one donor Marker Assisted Zabrotes (MAZ) resistant lines. The segregating population was phenotyped for bruchid resistance at the F_4 generation.

The laboratory screening of the genotypes revealed that a wide range of variation was recorded among the landraces, commercial varieties, advanced breeding and resistant lines for all the parameters studied. Absolute resistance was recorded only from the resistant lines, namely RAZ-11, RAZ-36, RAZ-2, RAZ-44, RAZ-120, RAZ-40, MAZ-200 and MAZ-203, while

the majority of the local germplasm was found to be susceptible. Some of the local germplasm showed a partial resistance to bean bruchid and two promising entries were identified, namely, SCR-11 (breeding line) and NC-16 (landrace).

The genetic diversity analysis, using phenotypic and SNP markers, revealed that considerable variation was existed among the Ethiopian common bean genotypes. High phenotypic diversity indices among phenotypic traits were recorded. The principal component analyses identified four PCs that explained 82% of the total phenotypic variation among genotypes. The polymorphic information content (PIC) ranged from 0.21 to 0.38, with a mean 0.34, reflecting the relatively high discriminating ability of the SNP markers. More than 70% of the gene diversity was recorded within the common bean population that were classified according to their breeding status and seed size. The four and two populations that were based on breeding status and seed size, respectively, were highly differentiated. Both the SNP and the phenotypic markers grouped the 297 common bean genotypes into two major distinct clusters and three sub-clusters, irrespective of their geographic origin.

The population structure analysis, based on Bayesian genotyping clustering approach, classified the common bean genotypes into two populations, namely, the Middle American and Andean gene pools. Similar population patterns were also observed by using the principal coordinate analysis (PCoA). The genome-wide association study (GWAS) identified 24 single-nucleotide polymorphism (SNP) markers on nine chromosomes, with a significant ($P < 0.05$) association with a percentage adult emergence (PAE) and a percentage seed weight loss (PSWL). However, only 13 SNPs located on Chromosomes 4 and 7 were significantly ($P < 0.001$) associated with the two traits. Other significant SNPs were identified on other chromosomes of the common bean, but none of them were above the cutoff point (1.00×10^{-4}).

Based on the above analyses, 144 diverse genotypes were selected and evaluated at three sites. Six principal components (PCs) were identified that explained 84% of the total variation among the genotypes. The 15 agro-morphological traits classified the genotypes into three distinct major clusters and sub-clusters. The clustering patterns of the genotypes were according to the seed size, in which small and medium beans were distinctly separated from the large seeded beans. The study established the existence of considerable genetic variation among common bean genotypes. Unique genotypes, such as Nasir, Awash Melka and RAZ-36 from Cluster I, RAZ-2, RAZ-11 and RAZ-42 from Cluster II and SER-125, SCR-15, MAZ-200, MAZ-203 and RAZ-120 from Cluster III were selected, based on their distinct agronomic performance and their response to the Mexican bean weevil infestation.

The participatory variety selection revealed that farmers used complex and diverse selection criteria in different agro-ecologies. The selection criteria varied among agro-ecologies and gender groups. Yield and yield-related traits were ranked as the most important selection criteria in all the locations and gender groups. Women ranked the taste and cooking time as the top criteria for varietal choice, while men were more interested in marketability, seed size and colour. In all three agro-ecologies, both farmer groups were able to select the top 10 best genotypes, although varietal preferences across locations and gender groups were diverse. The top set of selected genotypes matched the breeders' selection, with only minor difference.

The phenotyping of the F_4 families derived from SCR-15 X MAZ-200 crosses showed highly significant differences ($P < 0.001$) among the entries, parents and offspring for all of the susceptible parameters, except the number of eggs. Based on the percentage adult emergence, 34.6% of the progeny genotypes were categorized as highly resistant, 12.0% were resistant, 21.6% were moderately resistant and 32.7% were susceptible. The study observed considerable phenotypic variation among the offspring and parental lines for the susceptibility parameters. The levels of broad sense heritability ranged from 68.5% – 93.9% for all the traits, suggesting that selection may be useful to improve bruchid resistance. In general, the study has identified absolute resistant lines among the exotic germplasm, while partial resistance genotypes among the Ethiopian genotypes signifies the possibility of the introgression of the resistance genes. The information reported in this study could serve as an important benchmark for future common bean breeding and conservation programs.

Declaration

I, Tigist Shiferaw, declare that

1. the research reported in this thesis, except where otherwise indicated, is my original research;
2. this thesis has not been submitted for any degree or examination at any other university;
3. this thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;
4. this thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted: t
    a. their words have been re-written, but the general information attributed to them has been referenced; and
    b. where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. this thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed

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Tigist Shiferaw Girsil

As the candidate's supervisors, we agree to the submission of this thesis:

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Prof. Rob Melis (Supervisor)

……..……

Dr. Julia Sybia (Co-Supervisor)

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Dedication

This thesis is dedicated to my beloved husband and best friend, Dr. Daniel Bekele, for his love, all-round support, encouragement, understanding, and enthusiasm during this long process.

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Thesis Introduction

Common bean production

The common bean (*Phaseolus vulgaris* L.) is the most important edible food legume and it constitutes 50% of the grain legumes consumed worldwide (McClean et al., 2008). It is the most important source of calories, proteins and minerals in the human diet (http://www.cgiar.org/our-research/crop-factsheets/beans/). In 2010, out of the total beans produced, countries in Latin America and the Caribbean constituted 24.4%, followed by Africa (17%) (FAOSTAT, 2014). The total annual worldwide common bean production is about 12 million metric tons. Of which, 8 million tons are from Latin America and Africa. The contribution of Africa is about 2.5 million tons annually that makes the continent second major bean producer http://grainlegumes.cgiar.org/crops/common-bean/. The crop has a major role in household food and nutritional security. In Africa, the common bean can transform traditional subsistence farming to a market-oriented modern sector and makes a substantial contribution to the continent's economy. In eastern and southern Africa, the common bean is the most commonly-grown and consumed-grain legume and a good source of calories and dietary protein (Hillocks et al., 2006; Buruchara et al., 2011). Ethiopia is the fourth largest common bean producer in eastern Africa, after Tanzania, Uganda and Kenya (FAOSTAT, 2017).

In Ethiopia, the common bean ranks second, after the faba bean, both in area coverage and production. The major production regions are Oromiya (152,152 ha), the Southern Nations, Nationalities and Peoples (SNNP) (96,200 ha) and Amahara (65, 918 ha) (CSA, 2015). The area under common bean significantly increased from 181,600 to 330,000 ha between 2004 and 2012. Besides this, the total production tripled to 387,000 tons and the average yield increased from 0.62 to 1.50 ton per hectare. Accordingly, the average farmer's income also increased from 120 to 750 US dollars per ton of beans (CIAT, 2013). Among the legumes, the Ethiopian farmers like to grow beans because of their short lifecycle and as they generates hard cash to buy food and household materials (Legesse et al., 2006). Different bean types are produced in the country, but small white and red beans are the most common.

In Oromiya, and specifically in the Central Rift Valley lowland areas, white beans are the most dominant types. In these areas, 95% of the farmers grow the common bean and the production constitutes about 50% of the total bean production of the country (Legesse et al., 2006). In SNNP, although a wide range of bean varieties of different seed colour, size and shape are grown, red

beans are the most predominant and make up 80-90% of the area allocated for beans (Ferris and Kaganzi, 2008). Among the agricultural commodities exported from the country, the common bean ranks third, after coffee and sesame, and contributes about 33% of the exported commodities (FAOSTAT, 2017).

Common bean export has a long history in Ethiopia and farmers have been exporting white beans for more than 30 years. Initially, farmers sold their beans to the neighbouring countries at low prices, while in the early 1970s the export process advanced to more specialised markets and the level increased to 40,000 tons (Shaun et al., 2012). In 2008, the bean exports increased to 74,800 tons and the revenue from this commodity was US$49,651 million (FAOSTAT, 2009). This made Ethiopia the eighth major bean exporter worldwide, after China, Myamar, the United States of America, Argentina, Canada, Nicaragua and Colombia (FAOSTAT, 2009). According to Ferris and Kaganzi (2008) and Rubyogo et al. (2010), about 10,000 tons of red cooking types have been also exported through informal channels to neighbouring countries, particularly to northern Kenya.

Constraints limiting bean production in Ethiopia

Although promising progress has been made in recent years, towards increasing common bean production in the country, there is still a large gap between the potential and the actual yield (Rubyogo et al., 2010; CIAT, 2013). This is mainly attributed to a number of abiotic and biotic stress factors. Low soil fertility, soil acidity and drought are the major abiotic stress factors (Wortmann et al., 1998; Rubyogo et al., 2011), while diseases and insect pests are among the biotic factors that play a significant role in the reduction of bean production in the country. The most important and widely-distributed common bean fungal and bacterial diseases include rust (*Uromyces appendiculatus*), angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), common blight (*Xanthomonas campestris* pv *phaseoli*) and halo blight (*Pseudomonas syringae* pv *phaseolicola*). A large number of insect pests have also been recognized in the common bean, but only a few of them are of economic importance (Kemal et al., 2008). The bean stem maggot (*Ophiomyia* spp.) is the most important field pest of beans (Abate and Adhanom, 1981b) in the country, especially in moisture-stressed areas. The African bollworm is another important field pest of the crop in the Rift Valley and other drier parts of Ethiopia (Abate and Adhanom, 1981a; Abate et al., 1985). The bruchid beetles *Acanthos celidesobtectus* (Say) and *Zabrotes subfasciatus* (Boheman) are the most recognized storage insect pests (Schoonhoven and Cardona, 1986; Parsons and Credland, 2003).

The bean bruchids

The two bruchid species that attack beans are distributed worldwide in all bean-growing areas, but their prevalence is affected by the weather conditions, especially the mean temperature of the storage facilities. The *Zabrotes subfasciatus* predominates in the low altitudes of tropical and subtropical areas, whereas *Acanthoscelides obtectus* is found at a higher altitude and in temperate areas (Cardona et al., 1989; Myers et al., 2001). These species can cause an estimated dry weight loss of 10 - 40% (Khamala, 1978; Kiula and Karel., 1985); however, under on-farm storage conditions, and without postharvest management, the damage can increase to 50-70% dry weight loss (Khamala, 1978; Lima, 1987). Moreover, the grain damage caused by bruchids leads to low market prices of the grains and reduces the amount of cash flow at household level. This, in turn, forces the farmers to sell their beans at very low prices immediately after harvest. Storage losses are strongly correlated with the length of the storage period and the moisture content of the grains. The longer the storage period and the higher the grain moisture content, the greater the loss (Abate and Ampofo, 1996; Wortmann et al., 1998). Damage, due to bean bruchids, is defined by the insect emergence holes, and leads to considerable quality and quantity loss. This leads to market price loss, whereby every hole per 100 beans can cause a 2.3% decrease in the bean price (Mishili et al., 2011).

In eastern and central Africa, *Z. subfasciatus* is the dominant species of storage insect pests (Nchimbi and Misangu, 2002). In Ethiopia, although the two species are present, *Z. subfasciatus* is the most prevalent species (Negasi, 1994). This may be related to the favourable weather conditions and unsuitable traditional storage facilities, which lead to a high infestation of the insect. In the warm areas of the central Rift Valley, the prevalence of the insect is very high and the storage losses are large (Wortmann et al., 1998). The damage, due to the storage pests can reach up to 38%, with an equivalent weight loss of about 3.2% (Negasi, 1994). Getu et al. (2003) reported a 60% grain loss recorded in the third to the sixth month of the storage period, highlighting the significance of the insect.

Management of bean bruchids

Farmers in Ethiopian apply several bean bruchid management practices to keep these pest population below the damage level, while the beans are in storage. These practices are cultural, biological, physical and/or chemical control methods (Tadesse et al., 2008). The cultural practices include the sun drying the grains, the mild roasting the grains that are not used as seeds, the

removal of infested pods at harvest, mixing the grains with ash and other small grains, such as tef and finger millets, the use of botanicals (mixing grains with inert dusts, plant powders and vegetable oils) and coating the seeds with mud, the use different storage methods and granary hygiene (Abate and Ampofo, 1996; Tadesse et al., 2008). A wide range of these pest management options have been tested for the postharvest protection of the common bean grain. However, the efficacy of the traditional practices is very low for longer periods of storage. As a result, farmers currently rely mostly on chemical pesticides. However, discrepancies related to application time and rates, environmental contamination, improper application and risk to the health of the user, as well as concerns of insecticide use for small lots of seed, puts the effectiveness of pesticides in question (Songa and Rono., 1998; Paul et al., 2009). Moreover, the labor intensity of cultural and physical practices limits their effectiveness for the control of the common bean bruchid when in storage (Songa and Rono 1998; Paul et al., 2009). Breeding for host plant resistance and biological control options are the components of integrated pest management (IPM) options that are used to attain more feasible and sustainable effects on storage pests (Kananji, 2007; Tadesse et al., 2008). The main focus of this thesis is breeding for host plant resistance for the control of bean bruchid (*Z. subfasciatus*).

Breeding for bean bruchid resistance in Ethiopia

An effort has been made to develop bruchid-resistant varieties of the common bean, and this has resulted in the development of “Resistance to Zabrotes” (RAZ) lines. These lines were developed by the International Centre for Tropical Agriculture (CIAT). The resistance in these lines is due to the presence of the arcelin gene, which is inherited as a monogenic dominant trait (Cardona et al., 1990; Kornegay et al., 1993). The screening of landraces, breeding populations and commercial Ethiopian cultivars has been done to get the sources of resistance to bruchids. None of the Ethiopian accessions were resistant to bean bruchid. As an attempt to get suitable sources of resistance, the National Bean Breeding Program introduced the RAZ lines in the early 1900s. These lines revealed a high and consistent level of resistance (Negasi and Tsedeke., 1992; Negasi, 1994; Firdissa et al., 2000; Assefa, 2010). However, these lines lack the famers’ preferred traits, especially with regard to yield, and the adoption rate has been low (Assefa, 2010).

Even though bean bruchid is the major storage pest in Ethiopia, very little effort has been made to breed for bruchid resistance. Assefa (2010) evaluated the yield performance and level of resistance of some Ethiopian commercial lines and advanced lines of common bean developed at CIAT. The author reported that all the advanced lines showed a good level of resistance, while

the commercial lines were susceptible to bean bruchids. However, the yield of the resistant lines was very low compared to the commercial varieties. The local germplasm has not yet been adequately evaluated as a potential of source of resistance to the bean bruchid. Starting in the 1970s, the National Bean Breeding Program has developed and released more than 55 common bean varieties. The majority of these released varieties have been adopted by farmers, seed producers and exporters and are widely grown in different bean-growing agro-ecologies of the country. However, all of these commercial varieties are susceptible to bean bruchids. The identification and incorporation of stable sources of resistance into the National Bean Breeding Program, from either local or exotic germplasm, is vital. The resistance to Zabrotes (RAZ) lines, the recently- developed Marker Assisted Zabrotes (MAZ) lines and the resistant Malawian landraces were proven to be good sources of resistance to *Z. subfasciatus*. The screening of the local germplasm together with the resistant lines for bean bruchid and the evaluation of the agronomic suitability of the genotypes, is of paramount importance. The focus of this thesis was on the Mexican bean weevil (*Z. subfasciatus*).

Research objectives

The specific objectives of the study were as follows:

(a) to evaluate the Ethiopian common bean landrace collections, commercial varieties, advanced breeding lines and elite resistant lines for resistance to the Mexican bean weevil;
(b) to assess the magnitude and pattern of genetic diversity in Ethiopian common bean landraces, commercial varieties, advance breeding and exotic resistant lines for the response to infestation by bean bruchid, using phenotypic and SNP markers;
(c) to examine the population structure among common bean genotypes collected from different breeding status, seed colours and sizes, and to identify genomic regions that are associated with bean bruchid resistance, using SNP markers;
(d) to assess the agronomic performance of common bean genotypes selected for their response to bruchid infestation, using yield and yield components under different agro-ecologies;
(e) to identify suitable parental genotypes that are useful for breeding for bruchid resistance and to identify the farmers' selection criteria for choosing varieties; and
(f) to introgress arcelin genes into commercial varieties and an advanced breeding line

Outline of this thesis

The thesis consists of seven distinct chapters in accordance with, the number of activities related to the specific objectives mentioned above. Chapters 2-7 are written as discrete research papers intended for publication. For this reason, there may be some overlapping and unavoidable repetition of the contents and references among chapters.

Chapter	Title
-	Thesis introduction
1	A review of the literature.
2	Evaluation of Ethiopian common bean (*Phaseolus vulgaris* L.) genotypes for resistance to the Mexican bean weevil (*Zabrotes subfasciatus* Boheman).
3	Genetic diversity analysis of common bean (*Phaseolus vulgaris* L.) genotypes for resistance to Mexican bean weevil (*Zabrotes subfasciatus*), using single nucleotide polymorphism (SNP) and phenotypic markers.
4	Population structure and genome-wide association analysis of bruchid resistance in Ethiopian common bean (*Phaseolus vulgaris* L.) genotypes.
5	Assessment of diversity and performance of Ethiopian common bean (*Phaseolus vulgaris* L.) genotypes for yield and yield components.
6	Participation variety selection of common bean genotypes in the Oromiya region of central Ethiopia.
7	Introgression of arcelin gene into commercial and advanced breeding lines of common bean.
8	An overview of the research findings.

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CHAPTER 1

Review of Literature

1.1 Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes worldwide. According to data published in the Food and Agriculture organization (FAO) in 2014, the world dry bean production was estimated at 26.5 million tons that are produced from a cultivated area of 30.6 million hectares (FAOSTAT, 2014). It is a primary source of dietary protein for people in the lower income class (Wortmann et al., 2004). The common bean is cultivated in a broad range of agro-ecologies and cropping systems, ranging from high potential to marginal and drought prone areas. The diverse bean growing conditions couples with specific preferences for particular bean types, seed colour and shape resulted in wide genetic diversity. In Ethiopia, common bean is the most widely-grown pulse crop, after the faba bean and an important source of income for many Ethiopian farmers (Asfaw et al., 2009). However, the productivity of the crop is below its potential, due to a number of abiotic and biotic stress factors. Most importantly, the Mexican bean weevil is the most destructive insect pest, inflicting significant post-harvest losses of stored grain in Ethiopia. Different control options have been used to manage the losses caused by the insect. However, the development of cost-effective, environmentally-safe, sustainable and feasible control measures is the best option to manage bean bruchids in the common bean, particularly for the smallholder farmers. This chapter presents a review of literature from a number of related studies and elaborates on the theoretical and practical aspects of the research. The first section provides information about the origin, domestication, biology, ecology and diversity of the common bean. The second section covers the biology, importance and control strategies of the bean bruchid. The third section comprises information about past research endeavors on breeding for bruchid resistance, the inheritance of the resistance, marker-assisted breeding and participatory plant breeding.

1.2 Common bean origin and domestication

A knowledge of the origin, domestication and diversification of cultivated and wild common bean species is useful for the characterization, conservation and deployment of available genetic resources. The genus *Phaseolus* belongs to the family Leguminosae and the subfamily Papilionoideae. The common bean is among the five domesticated *Phaseolus* species that are native to South America (Gepts and Debouck, 1991). *Phaseolus vulgaris* and the majority of the cultivated and wild *Phaseolus* species have a diploid genome (2n=2x=22). Among all the species in the Leguminosae family, the common bean has a relatively small genome size (521.1 Mb) (Schmutz et al., 2014). Although the common bean is a self-pollinated species, the hybridization and the introgression of genes, from wild to cultivated beans species is easily to produce fertile and viable progenies (Singh, 2001; Zizumbo-villarreal et al., 2005). However, incompatibility has been reported in some inter-gene pool crosses (Singh and Gutiérrez, 1984; Burle et al., 2011).

The Common bean originated in the neo-tropics, and two independent centres of origin and domestication are reported (Gepts, 1998). The multiple centres of domestication of the crop have resulted in two distinct major domestication gene pools, namely, the Mesoamerican and Andean gene pools (Singh et al., 1991b; c; Bitocchi et al., 2013). The two gene pools are distinguished by their seed size and biochemical characteristics, including polymorphism in the phaseolins (seed-storage globulin proteins) (Gepts et al., 1988; Singh et al., 1991c, 1998; Haley et al., 1994; Velasquez and Gepts, 1994a). The Mesoamerican gene pool can be further divided into three different races, namely, Mesoamerican (all small-seeded), Durango (medium-seeded semi-climber), and Jalisco (medium-seeded climber). Similarly, the Andean gene pool (all large-seeded) can be further divided into three races, namely, Nueva Granada, Peru and Chile (Singh et al., 1991a; Beebe et al., 2000; Blair et al., 2009, Bitocchi et al., 2012). From its centres of origin, the crop has been disseminated to other parts of the world, such as Africa, Asia, Europe, and Oceania (Gepts and Bliss, 1988). Common beans are believed to have been introduced to Africa in the 16th and 17th centuries, together with maize (Greenway, 1945; Gentry, 1969). The adaptation of the crop to different geographic regions, other than its centre of origin and domestication has led the crop to evolve different morphological, physiological and biochemical characteristics that have endowed the crop with abundant genetic variation (Gepts and Debouck, 1991; Gepts, 1998).

Since the introduction of common beans in Africa, farmers have developed and preserved important genotypes that are adapted to their local environments and their specific needs and this has led to the evolution of diverse morphological variants, called landraces (Wortmann et al., 1998; Sperling, 2001). In addition, the national bean research programs in many Africa countries, have been introducing a large number of new germplasms from different parts of the world (CIAT, 2005). Consequently, the East African highlands have become the second centre of biodiversity for the common bean, due to its wide range of landrace diversity (Allen and Edje., 1990; Wortmann et al., 1998; Sperling, 2001; Asfaw et al., 2009). Several researchers have reported on the co-existence of Andean and Mesoamerican genepools in Africa (Martin and Adams, 1987; Asfaw et al., 2009; Blair et al., 2010a; Okii et al., 2014). Ethiopia and Kenya are among the major bean- producing countries in the sub-Saharan Africa, with highly diverse bean production systems (Hillocks et al., 2006; Asfaw et al., 2009).

1.3 Biology and ecology of common bean

The common bean is a warm-season, annual legume, involving different types and growth habits. According to Singh (1982), the growth habits of the common bean are classified into four major classes, based on the type of terminal bud, stem strength, climbing ability and fruiting pattern. These include the Type I (determinate upright bush), Type II (indeterminate upright bush), Type III (indeterminate, prostate, non-climbing or semi-climbing) and Type IV (indeterminate, strong climbers) growth habits. The common bean has a fibrous root system with a clear tap or main root and nodules distributed on the lateral roots. The main stem derives from the axis of the seed embryo, from which the hypocotyl and the epicotyl emerge. The two unifoliolate leaves borne above the cotyledonary node are opposite each other, followed by one trifoliolate leaf at each node, in an alternate phyllotaxy (CIAT, 1986; Miklas and Singh, 2007).

The common bean has cleistogamous flowers which are highly self-pollinated with a <1% outcrossing rate (Miklas and Singh, 2007). However, outcrossing rates ranging from 0.0 to 10.2%, with a mean of 6%, have been reported in the common bean (Ibarra-Perez et al., 1997). The inflorescence is a pseudoraceme with several flowers, of which only the basal few bear pods. The corolla consists of two petals that are fused to form a spirally-twisted, prolonged keel, with a two winged and a standard petal. The flower can be pink, purple, white, or bicolour, with or without stripes at the outer base of the flower. In the keel, there are nine fused stamens and one free stamens on the upper side of the keel, each with a bilobed anther sac borne on a long filament

that is united into a long sheath or tube around the style. The pistil (gynoecium) is in the centre of the flower and consists of an ovary that usually contains usually from 5-8 ovules. The flattened stigma tends to extend around the tip of the style and is covered with a sugary fluid. The style is coiled and the stamen follows the stylar coil in such a way that the anther sac is appressed to the stigma. Pollen dehiscence and stigma receptivity occur at the same time, before the flower is fully open, and pollen is shed directly to the stigma. Anthesis often occurs in the early morning hours and the stigma can remain receptive for up to two days, before and one day, after normal anthesis. Crosses can be made prior to anthesis, with or without the emasculation of the anthers. The flowers that are used as the male parent can be removed from the male plant and used immediately in the morning, they can be placed in a plastic bag and kept in the refrigerator for use in the afternoon (CIAT, 1986, 1987). More than 50% of the pods are borne on branches. Mature pods are straight to slightly curved with 5-8 seeds. Considerable variation was recorded in the seed and pod size, as well as the shape and colour. Germination is epigeal, with the cotyledons dropping off a couple of few weeks after emergence.

The common bean is a short-day crop that requires less than 12 hours of daylight, in order for flowering to occur (White and Laing 1989). The growth and development of the common bean is highly favoured by mildly cool environments. Under non-stressed conditions, with a mean growing temperature range from 18 - 22^{0}C and about 12 hours' day-length, most bean cultivars complete their growing cycle in 70 to 120 days. The climbing cultivars require more than 250 days to mature in the highlands (above 2000 meter above sea level), while these cultivars mature within 150 days in the humid highlands (Miklas and Singh, 2007). The bushy cultivars (Types I, II, and III) are grown as a monoculture or under different cropping systems (relay, strip, and intercropping) in all bean-producing areas (Singh, 1982). Although the common bean is grown in a wide range of soil types, light loamy soils with a neutral pH are more favourable (Miklas and Singh, 2007).

1.4 Genetic diversity of common bean

Genetic diversity is the variability of a specific trait or combination of traits that is observed in a given crop species and that can be attributed to the underlying genes (Acquaah, 2007). The degree of expression of the genes is influenced by the environment. The genetic variability that is expressed in individuals within a given crop species can be transmitted from one generation to the next. In nature, genetic variability can result from genetic recombination, a mutation, and/or a variation in the chromosome number. A plant's genetic resources are essential for maintaining agro-ecosystem stability in relation to soil structure, nutrient cycling, balancing diseases and pests

populations and retaining hydrological systems (Saad et al., 2013). A plant's genetic resources provide an extensive range of materials that are fundamental for food, fiber, medicine, and industry (Saad et al., 2013). Genetic diversity is fundamental for achieving high productivity and yield stability (Tilman et al., 2005) for breeders to develop improved varieties (Buanec, 2005).

Understanding of the genetic variability of crops is essential for the efficient utilization and effective conservation of genetic resources (Mohammadi and Prasanna, 2003a). Genetic variability can be detected at morphological, biochemical or molecular level. Some genetic variations are manifested as visible morphological traits (de Lima et al., 2012). Morphological traits are agronomically important traits that are measured directly from the population. Mohammadi and Prasanna (2003) noted that the analysis of genetic variability of accession in crop plants, with respect to agro-morphological traits is the most important precursor for determining genetic diversity. However, the expression of these traits is liable to morphological plasticity (Garcia et al., 1997). Morphological characterization is time-consuming, it requires phenotyping skills, as well as multi-locations and multi-years of experimentation, to account for environmental variations (Spooner et al., 2005). Other genetic variations, on the other hand, are compositional or biochemical and requires various tests for evaluation (Shechter, 1975). Several studies have been conducted to assess the genetic diversity of the common bean, using agro-morphological traits (Rodino et al., 2001; Horňáková et al., 2003; Oscar et al., 2004; Chacon et al., 2005; Stoilova et al., 2005, 2013; Ahmed, 2013; Awan et al., 2014). The most frequently-used morphological traits in characterizing common bean germplasm are the growth habit, the pod number and length, seed dispersal, seed weight and photoperiod sensitivity. These traits have been used to differentiate wild, weedy and domesticated species (Gepts et al., 1999; Papa and Gepts, 2003; Zizumbo-Villarreal et al., 2005). Biochemical markers, such as isozymes and storage proteins have been used to characterize a plant's genetic resources (Vallejos and Chase, 1991; Delaney and Bliss, 1999; Chacon et al., 2005; Gonzalez et al., 2010). Biochemical analysis is based on the separation of proteins into specific banding patterns and it requires only a small amount of biological material. The storage protein, phaseolin, has been the most commonly-used protein marker in genetic diversity assessments of wild and domesticated bean types (Gepts et al., 1986; Gepts, 1988; Gepts and Bliss, 1988; Singh et al., 1991c; Cattan-Toupance et al., 1998; Chacon et al., 2005; Papa et al., 2006; Logozzo et al., 2007). However, the application of protein markers to genetic diversity analysis has been limited by the relatively low levels of polymorphisms, and it has been highly subject to environmental influences and gene interactions (Ince and Karaca, 2011).

The availability of molecular markers enables breeders to examine genetic diversity at a molecular level (Mondini et al., 2009). The markers systems, namely, Restriction Fragment Length Polymorphism (RFLP) (Chase et al., 1991; Velasquez and Gepts, 1994b; Freyre et al., 1998), Random Amplified Polymorphic DNA (RAPD) (Beebe et al., 2000; Duran et al., 2005; Szilagyi et al., 2011), Inter Simple Sequence Repeat (ISSR) (González et al., 2005; Dagnew et al., 2014), Amplified Fragment Length Polymorphism (AFLP) (Kumar et al., 2008), Simple Sequence Repeat (SSR) (Blair et al., 2007, 2009, 2010a; Asfaw et al., 2009; Kwak and Gepts, 2009; Burle et al., 2010; McClean et al., 2012; Fuente et al., 2013; Mercati et al., 2013; Okii et al., 2014; Fisseha et al., 2016) and Single Nucleotide Polymorphism (SNP) (Cichy et al., 2015) have been used in common beans. Although, these marker systems provide different types of information, they are polymorphic, stable and effective in differentiating genotypes, as compared to morphological and protein markers. The choice of the markers depends on the objective(s) of the study, the level of resolution required, the availability of technological infrastructure, and the operational and time constraints (Karp et al., 1997; Mohammadi and Prasanna, 2003). The release of the two common bean whole genome sequences (Mesoamerican and Andean; each about 600 Mbp) provides breeders, with the freedom to develop more suitable markers, with a wider genome coverage (Schmutz et al., 2014). The sequence data are often used for the development of molecular markers, specifically the single nucleotide polymorphism (SNP) markers.

The application of molecular markers for genetic diversity analysis requires ideal molecular markers that are co-dominant, reproducible, highly polymorphic, widely and evenly distributed across the genome, and cost-effective (Blair et al., 2013). Although SSR markers fulfil most of these properties, their application is limited, due to the high cost, resulting from labour and time investment. Single nucleotide polymorphism (SNP) markers satisfy the above criteria, together with the potential for high throughput and low cost genotyping (Galeano et al., 2009a; b; Hyten et al., 2010). SNPs can be used for linkage map construction (Galeano et al., 2012; Song et al., 2015), genetic diversity analysis (Corte´s et al., 2011; Blair et al., 2013; Goretti et al., 2013; Cichy et al., 2015a; Rodriguez et al., 2016) and marker–trait association (Kamfwa et al., 2015a; Kamfwa et al., 2015b; Cichy et al., 2015b; Kamfwa et al., 2015b; Moghaddam et al., 2016; Hoyos-villegas et al., 2017). In the common bean, SNP markers have been used to perform synteny analysis (Galeano et al., 2009a; McConnell et al., 2010).

Understanding the pattern and level of genetic diversity of common bean landraces, breeding lines and commercial varieties and their relationships to the two gene pools (Andean and Mesoamerican) provide information on the level of gene flow, for future bean breeding and

conservation programs in Ethiopia. The study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analysed by a specific method or a combination of methods. Generally, the use of two or more different methods helps to better understand the genetic diversity and the relationships within and among the genotypes of crop species (Burle et al., 2011). A necessary condition for any genetic diversity study is the availability of adequate genetic resources. Genetic diversity in specific crops includes weedy and wild relatives, traditional varieties, exotic germplasms, and modern commercial varieties (Mondini et al., 2009). To date, few diversity assessments have been made of the germplasm of the Ethiopian common bean.

1.5 The bean bruchids

1.5.1 Distribution and biology

The bean bruchids belongs to the order Coleoptera and the family bruchidae. There are two types of bruchids that commonly cause severe damage on stored beans, namely, *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say) (Cardona, 2004). Both bruchid species are distributed worldwide in all bean-growing areas, but their prevalence is highly affected by the ambient temperature. *Zabrotes subfasciatus* originated in the tropical and sub-tropical regions of South and Central America, and they are prevalent in many other tropical and sub-tropical regions, especially East and Central Africa (Singh, 1979; Abate and Ampofo, 1996; Wortmann et al., 1998; Alvarez et al., 2005). *Zabrotes subfasciatus* is more common in the low altitude areas, whereas *A. obtectus* is more frequent in the higher altitude areas (Cardona et al., 1989; Myers et al., 2001). The widespread occurrence of the pest in Ethiopia has also been reported by Negasi (1994) and Wortmann et al. (1998). Climate change will influence the patterns of the insect, with respect to their incidence and intensity. In the warmer areas of the country, *Z. subfasciatus* is the most important storage pest that causes serious grain losses.

The species *Z. subfasciatus* starts infestation in the stored seeds and adult longevity is relatively short (about 11 days). The females lay their eggs onto the dry seed and the eggs hatch on the seed coat. The first-instar larvae penetrate the seed coat and complete the life cycle inside the seed. The larvae of the species moult four times before pupating. During the last larval instar, the feeding and pupation cell becomes visible as a circular window in the seed, where the larvae feed on the lower testa surface. The male and female adult *Z. subfasciatus* can easily be differentiated by their colour and size. The female has cream-coloured spots on her elytra and are longer in size, while the male is short, with a pure grey colour. The insect completes its life cycle within 25-

47 days i.e. 5-6 days for the egg, 14 days for the larva and 6-7 days for pupal stages. The females lay 36 eggs on average, and the adult life span is 10-13 days (Schoonhoven and Cardona, 1986; Cardona et al., 1989).

1.5.2 Importance

Storage insect pests cause both quantitative and qualitative losses. Quantitative losses include the number of seeds damaged by the insect and the seed weight loss, whereas the grains that are contaminated by excrement or insect bodies cause qualitative losses (Schoonhoven and Cardona, 1986; Jones, 1999). The relative importance of bruchid in sub-Saharan Africa is presented in Figure 1.1. The grain moisture content is directly correlated with bruchid infestation, where a seed moisture content of greater than 17% favors the rapid development of storage insects and fungi (*Aspergillus* spp., *Penicillium* spp. and *Phomopsis* spp) (Schoonhoven and Cardona, 1986). The extent of the seed weight losses caused by bean bruchids depends on the storage period and storage conditions. On average, a 10-40% dry weight loss was reported, as a result of bean bruchid damage (Khamala, 1978; Kiula and Karel., 1985; Singh and Schwartz, 2011) and the dry weight loss can reach up to 50-70% in most of the on-farm storage facilities, due to the lack of postharvest management practices (Khamala, 1978; Lima, 1987). Several researchers have reported on the extent of dry seed weight loss by bruchid in various countries in Africa. A mean of 30% stored bean damage, due to bean bruchids, has been reported in Rwanda, Burundi and Tanzania (Karel and Autrique., 1989; Nahimana, 1992). Similarly, a mean of 23% and 38% stored bean damage have been recorded in Uganda and Malawi, respectively (Karel and Autrique., 1989; Kananji, 2007). However, the highest bean damage of 73%, due to bean bruchids has been reported in Kenya (Karel and Autrique., 1989). In Ethiopia, bean bruchids have caused an average of about 38% bean damage and 3.2% seed weight loss under farmer storage condition (Negasi, 1994). Getu et al. (2003) and Araya and Getu (2009), on the other hand, reported that bean bruchids caused a grain weigh loss of up to 60% for beans stored from 3-6 months. The marketability, nutritional value, germination and seedling vigour of grains damaged by bean bruchid are significantly reduced (Singh and Schwartz, 2011).

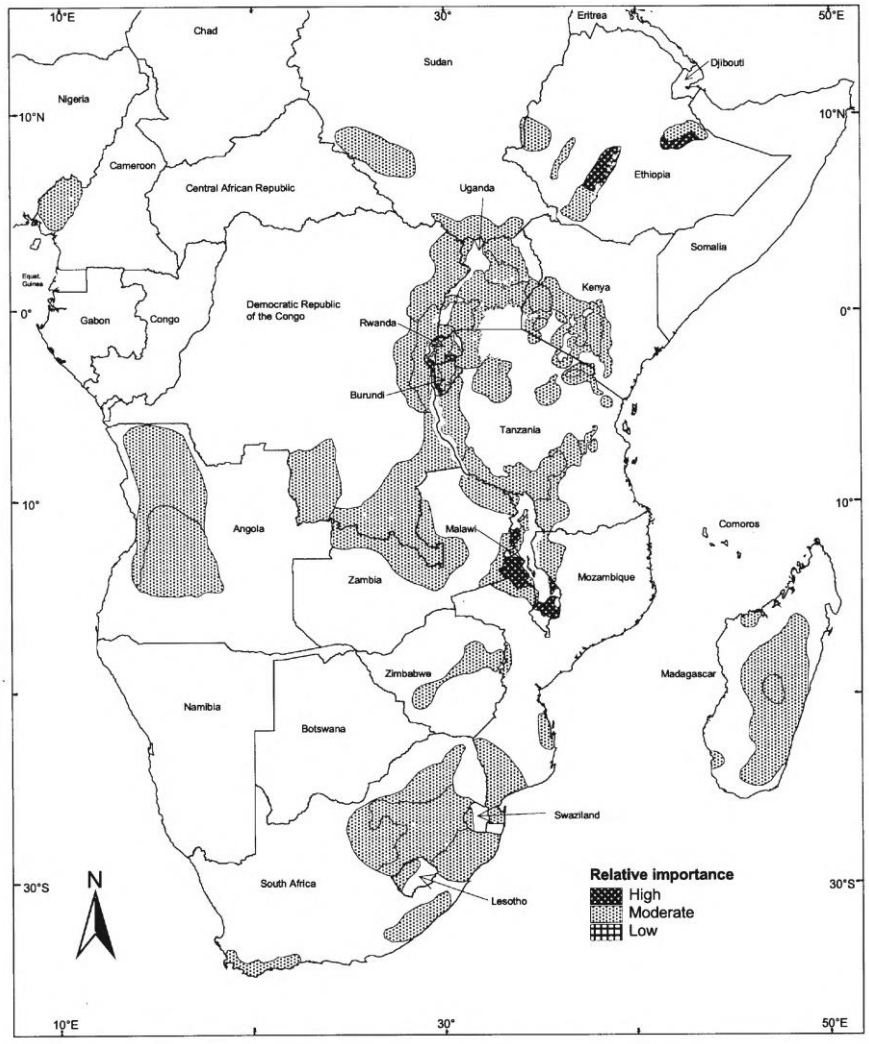

Figure 1.1 Map showing relative importance of bruchids in sub-Saharan Africa (Wortmann et al., 1998)

1.5.3 Management of bean bruchids

Different types of control options have been used by farmers to keep the pest population below economic damage level. These include the sun-drying of the grains before storage, to reduce the grain moisture content, the cleaning and repairing of storage facilities, storing the grains with botanical pesticides or mixing them with small cereals, such as tef or ash, treating them with chemical insecticides and smoking the beans over a fire (Abate and Ampofo, 1996; Tadesse et al., 2008). Nowadays, however, the cultural control practices are not used as often by farmers, because they now use chemical pesticides. Although insecticides are effective for bruchid control, smallholder farmers do not have separate storage structures for the fumigation of food grains and seeds. The development of environmentally-safe, sustainable, feasible and integrated pest

control measures i.e. cultural, biological, host resistance and chemical, is vital for the control of bean bruchids.

Cultural and physical control method

Farmers use different cultural methods to reduce the initial insect population. Proper drying before storage, the removal of all residues, the repair of storage structures and hygienic measures are the most common cultural practices for the control of bruchids (Tadesse and Eitecha, 2000). Unlike *A. obtectus,* other pre-harvest cultural practices are not useful for controling *Z. subfaciatus* because the infestation begins while it is in storage. According to Quentin et al. (1991), shaking or tumbling the bean seeds several times per day controls bruchids by disrupting the larvae inside the seed and reducing the number of adults that emerge. Storing unthreshed beans is also practised, in order to reduce the damage caused by bean bruchids, as *Z. subfaciatus* prefers to lay their eggs on the seed coats (Abate and Ampofo, 1996). The mixing of bean seeds with ash or small cereals, like tef and sorghum is also reported as one of control options for bean bruchids, as this affects insect's mobility and oviposition. However, these practices are effective if they are applied before infestation has taken place. The sun-drying of the grains followed by, sieving is a good technique to use against the storage pests of beans (Giga and Chinawda, 1996). According to Songa and Rono (1998), this method has proved to be quite effective in reducing bruchid infestation, with no or minimal, effect on seed quality or germination. The use of cultural control measures are easy to implement, with minimal cost and limited labor. However, to be effective, long-term planning and careful timing is vital (Kananji, 2007).

Biological control methods

Biological control is a useful and safe control option for storage pests, but research has been limited to date. *Dinarmus basalis* (Rondani) has been proved to be a promising parasitoid for *A. obtectus* and *Callosobruchus chinensis* (L.), which significantly reduces the population of bruchids (Islam and Kabir, 1995; Schmale et al., 2001). Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were found to be effective for the control of the maize weevil and cowpea bruchids in Ethiopia, under experimental conditions (Adane et al., 1997a; b, 1998). The use of *B. bassiana* as an endophyte to control common bean bruchids, is under investigation (Parsa, CIAT, Colombia, personal communication). Although promising results were recorded with parasitoids, its implementation under small-scale farmer storage conditions is difficult. Moreover, the incompatibility of the parasitoids with chemical control practices (Tadesse et al., 2008) has made the biological control of bean bruchid less appealing. Besides, the lack of knowledge and

resources for rearing the parasitoids and the difficulty to parasitize the larva, as the larval are found inside the seed, limit the application of biological control for bruchid management (Kananji, 2007).

Chemical control

Chemical insecticides are commonly used against storage pestes, either in the form of fumigation or dust formulations (Rai et al., 1987). Organophosphates and pyrethroid insecticides are the most commonly used chemicals for the control of storage insects. Smallholder farmers use dusts, while for large-scale storage facilities fumigation is more effective (Tadesse et al., 2008). In Ethiopia, there are many recommended insecticides against storage pests, but the most commonly, used insecticides are pirimiphos-methyl and malathion dust (Tadesse et al., 2008). It is important to have a knowledge of the application of insecticides and an awareness of the potential dangers caused by the chemicals (Jones, 1999). However, most of the farmers in developing countries are exposed to insecticide toxicity, due to the improper handling and application of insecticides and the lack of awareness of the potential dangers in storage facilities. The other disadvantages of chemicals are environmental pollution and their effect on beneficial insects. In addition, the insects can develop a resistance to the chemicals. Chemical residues remaining in the grain of common bean affect the health of human being (Pacheco et al., 2015).

1.6 Breeding for bean bruchid resistance

Host plant resistance is the basic component of integrated pest management, and it is a cheap, effective, sustainable and environmentally-safe method. Progress has been made on bruchid resistance breeding by scientists at CIAT. Schoonhoven and Cardona (1982) reported that almost all cultivated common bean cultivars and landraces lack resistance to *Z. subfasciatus.* However, several resistance genes were found in a few wild common bean accessions (Schoonhoven et al., 1983). One mechanism of resistance is believed to be antibiosis, which is conferred by the seed storage proteins produced by the APA (arcelin, phytohemagglutinin and α-amylase inhibitor) gene family (Schoonhoven et al., 1983; Acosta-Gallegos et al., 1998). Antibiosis resistance extends the time of adult emergence, insect growth and lifecycle. Reducing adult emergence, especially in the first and second instars larvae, in turn, results in reducing the weight of surviving adults (Osborn et al., 1988; Minney et al., 1990; Dorn et al., 2007). Although the APA proteins differ in their biochemical and physiological properties, their expressions show similar patterns

(Moreno et al., 1990). Based on its protein size and electrophoresis patterns, arcelin is different from all the other APA proteins (Romero-Andreas et al., 1986).

Different variants of arcelin genes were identified and each variant was found to have a different effect on *Z. subfasciatus*. Currently, eight variants of arcelin (Arc-1 to Arc-8) have been identified (Osborn et al., 1986; Lioi and Bollini, 1989; Santino et al., 1991; Acosta-Gallegos et al., 1998; Zaugg et al., 2012). These arcelin variants are clustered in three groups, with the first group being composed of Arc-3 and Arc-4. These variants were found to be the progenitors of the rest of the variant genes. The second group consists of Arc-5 and Arc-7, while the third group includes Arc-1, Arc-2 and Arc-6 (Lioi et al., 2003). Arc-5 and Arc-1 confer the highest level of resistance to *Z. subfasciatus* in common bean accessions, followed by Arc-4, Arc-2 and Arc-3 in their order of importance (Cardona et al., 1990). The mode of action of arcelin is not well understood; however, some studies suggest that it might be due to a disruption of epithelial cells in the gut of the insect. Others have hypothesized that arcelin might provide the insects with a source of poorly digestible protein (Minney et al., 1990; Paes et al., 2000; Carlini and Grossi-de-Sa, 2002). All the RAZ lines have been developed through backcross breeding by CIAT, using Arc-1 variant lines (Cardona et al., 1990).

Different national research programs have verified the resistance of RAZ lines. In Ethiopia, the screening of the RAZ lines and commercial bean cultivars has been done and it has been confirmed that most the CIAT accessions reveal high levels of resistance, compared to the commercial varieties. The resistant lines, such as RAZ-1, RAZ-7, RAZ-8 and RAZ-11, showed a stable resistance to Zabrotes (Negasi and Abate., 1992; Negasi, 1994). Assefa (2010) studied the yield performance and the level resistance of advanced breeding resistant lines of the common bean, which was developed by CIAT under field conditions in Ethiopia. All the advanced lines exhibited a high resistance to bean bruchids, but the yield performance of the lines was very poor, compared to the commercial varieties. According to Cardona (2005), the introgression of arcelin into commercial cultivars was effective against Zabrotes, but most of the RAZ lines had lower yields than their respective recurrent parents. Recently, different RAZ lines were evaluated across different environments and they showed improved potential for yield. These lines will be released into the national evaluation program in 2017. The candidate genotypes are RAZ-42 and RAZ-11 (Negash et al., 2014). The transfer of genes resistant to *A. obtectus*, from a genotype of tepary bean (*P. acutifolius*) into an African bean cultivar has also been reported (Mbogo et al., 2009; Kusolwa and Myers, 2011). Even though, successful breeding efforts have been made to

develop arcelin-derived resistant cultivars (Cardona et al., 1990; Cardona and Kornegay, 1999; Myers et al., 2001; Cardona, 2004; Beneke, 2010), no resistant commercial bean cultivar has been released to date. Hence, it is essential to search for additional sources of bruchid resistance from landraces that are easier to use in a breeding program.

Using a different approach, Kananji (2007) collected a large number of landraces from farmers in Malawi and screened them for bruchid resistance. Malawian landrace K35 showed a high level of resistance to both bruchid species, while K25 exhibited a good resistance only to *Z. subfasciatus*. These two landraces showed resistance levels that were even better than the lines with arcelin (SMARC lines) (Kananji, 2007). This is an indication that sources of bruchid resistance may also exist within the Ethiopian landraces, which will be useful for resistance breeding programs.

1.7 Inheritance of resistance to bean bruchids

Understanding the inheritance of resistance to bean bruchids is crucial for developing a successful breeding program. Osborn et al. (1986) and Suzuki et al. (1995) studied the inheritance of resistance conferred by arcelin, using single F_2 seeds from crosses between lines that carried the arcelin gene and cultivated lines that lack arcelin. The results confirmed that the resistance is genetically inherited in a simple Mendelian manner (Osborn et al., 1988). Kornegay et al. (1993) reported that arcelin is inherited as a monogenic dominant trait, which provides a higher level of resistance to bruchids when it is in the homozygous (Arc^+/Arc^+) state than in its heterozygous (Arc^+/Arc^-) state. This indicates that the transfer of the *Z. subfasciatus* resistant gene to commercial cultivars, through backcrossing would be easy. Resistance controlled by a single gene is liable to break down at some stage of the breeding cycle. Therefore, several resistance genes and/or Quantitative Trait Loci (QTLs) from various sources could be used in resistance gene stacking to form a more stable and long-lasting resistance. The inheritance of the resistance gene to *A. obtectus*, which is obtained in the Malawi landraces was controlled by many genes (Kananji, 2007).

1.8 Marker-assisted common bean breeding

Marker-assisted selection (MAS) is a procedure that has been developed to avoid the effects associated with the environment when selection is conducted based on phenotypic traits. The efficiency of phenotypic selection can be enhanced by the selection of genes through MAS (Francia et al., 2005). The selection based on linked molecular markers, which are not influenced by the environment and the marker can be detected at any stage of the plant's development. The

application of these tools in the breeding programs increases the rate of genetic gain twofolds compared to, the rate of gain by phenotypic selection (Ragot and Lee, 2007; Xu and Crouch, 2008).

Traditionally, selection for resistant lines can be done by using laboratory screening, which is tedious, time-consuming and requires large laboratory space and a large amount of seed, to undertake replicated trials. Selection can also be achieved by analysing the presence of the active arcelin gene, using either an immuno essay or electrophoresis. Biochemical markers have been used to detect the presence of arcelin in small quantities of ground seed tissue. Protein based screening requires protein electrophoresis and arcelin-specific antibodies (Blair et al., 2002). However, these methods are time-consuming and expensive, due to the demanding protein extraction protocols. The MAS, on the other hand, is a simpler and more efficient tool in the development of bruchid resistant cultivars, thus it is essential to find more cost-effective and technically simpler resistance screening methods (Miklas et al., 2006). Marker-assisted breeding has been shown to be a valuable tool in the development of resistant cultivars.

DNA-based markers have been applied, to monitor the expression of the arcelin protein in breeding programs (Miklas et al., 2006). Several attempts have been made in different national and international research institutes to identify molecular markers that are tightly linked to the arcelin gene (Miklas et al., 2006; Blair et al., 2010b). The arcelin genes were mapped on Chromosome 4 of the common bean genome (Nodari et al., 1993). A total of sixty-eight genotypes, consisting of seven wild accessions, each representing the seven arcelin variants, were identified (Blair et al., 2002). Based on populations developed by crossing the resistance to Zabrotes (RAZ) lines and susceptible varieties, several Simple Sequence Repeat (SSR) markers associated with the arcelin gene were identified (Blair et al., 2002, 2010b). Currently, new SNP markers that are linked to the arcelin loci are being developed by the International Center for Tropical Agriculture (CIAT) (CIAT, unpublished report). These markers have not yet been validated in different populations. These newly-developed markers will be useful for marker-assisted selection and the introgression of arcelin, to develop bruchid resistant lines. However, relying on one resistance gene is not prudent for the suitable control of bean bruchid. The identification of new resistance genes and/or QTLs from different sources and mapping of these QTLs is therefore very important.

1.9 Participatory plant breeding

Participatory Plant Breeding (PPB) and Participatory Variety Selection (PVS) are similar concepts, but the difference hinges on the degree and stage of the farmers' involvement in the plant breeding process (Weltzien et al., 2003). In PPB, the farmers' involvement starts with the identification of desirable traits and the selection of parental material for crossing, and its continues with the evaluation of segregating populations for those traits for the targeted geographic areas and end-uses. The most comprehensive review of farmers participation in plant breeding, which was conducted in 40 developing countries, was published by Weltzien et al. (2003). In this review, the positive aspects of PPB for generating new and improved varieties, facilitating adoption, enhancing in-situ conservation, expanding genetic diversity and empowering farmers, have been presented in detail.

The conventional breeding approach has mainly focused on generating crop varieties with improved yield potential and resistance/tolerance to biotic and/or abiotic stress factors, under controlled environments. The breeders are fully responsible for making decisions at each and every step of the breeding program. In this system, the farmers' preferred traits, such as adaptation to variable agro-ecologies and cropping systems, the value of post-harvest and socio-economic traits such as taste, aroma, cooking time and marketability, have received little or no attention. However, farmers have a defacto varietal preference for the different uses, agro-ecologies and farming systems (McGuire, 2008). In many crop-breeding programs, a large number of crop varieties have been released; however, the released varieties have had low adoption rates. McGuire (2008) reported that the main reason for the poor adoption rate and the low impact of the improved varieties is a lack of the breeders' awareness of the traits farmers desire. The farmers' participation in setting up research priorities and technology evaluation is crucial to scientists, in order to design, test and recommend appropriate new crop technologies. This can be achieved through participatory research and evaluation that allows the incorporation of the farmers' indigenous and technical knowledge, the identification of the farmers' criteria and priorities, and the definition of the research agenda. The application of this information will accelerate the uptake and diffusion of novel technologies. This will justify the importance of the participation of farmers in any technology development activities.

Another advantage of PPB is its relationship to genetic diversity. A PPB provides a means of conservation and an evaluation of the usefulness of some traditional germplasm used in the breeding programs. However, in conventional plant breeding, the potential of traditional varieties as a sources of genes for breeding programs, has not yet been exploited, specifically in stress environments. The advantage of traditional varieties is that they are well-adapted to the local conditions and they have the farmers' preferred attributes, despite their low productivity. Ceccarelli (1989) reported that breeding for a wide adaptation, using exotic material reduces genetic diversity and increases genetic vulnerability. The PPB has also has a large positive effect on diversity, because different farmers in different locations select different materials (Ceccarelli et al., 2001). In addition, farmers grow a mixture of varieties within a field, which allows them to have a high buffering capacity in stress environments (Cleveland et al., 1999). Breeders, however, often try to minimize the amount of variation over space, by breeding for broad adaptation (Ceccarelli and Grando, 1999).

The concept of PPB in bean breeding was developed and proposed in the early 1900s, but little or no efforts has been made to include farmers and consumers in a variety of selection programs. Understanding the bean production problems and research priorities of local farmers in various agro-ecologies and socio-economic conditions is vital for the selection of varieties that will be adopted long term by farmers (Sperling and Loevinsohn, 1993). Farmers are aware of their problems and needs; however, their perceptions and approaches are often different from those of the breeders (Ashby and Lilja, 2004). Farmers have been using numerous characteristics, such as yield, seed colour and size, taste, marketability, storability, growth habit, disease and insect resistance as well as drought tolerance to choose a given variety (Mekbib, 1997). Assefa et al. (2005) reported that farmers had applied up to 40 distinct selection criteria for evaluating the bean lines in eastern Ethiopia, indicating the complexity of the user needs and production conditions. In southern Ethiopia, on the other hand, farmers used very few criteria that are related to drought tolerance, culinary attractiveness and marketability (Asfaw et al., 2012). In all these cases, participatory selection was quick, efficient, and accurately revealed farmers' preferences (Mekbib, 1997; Assefa et al., 2005, 2013; Asfaw et al., 2012).

1.10 Conclusion

Although the common bean is an important crop both for food and export in Ethiopia, its production and productivity is low because of various yield-limiting factors, including bean bruchid infestation. Various control options, such as cultural, biological and chemical, have been used to control the insect. However, the above options have been found to be less appealing, due to issues related cost, health and environmental pollution, to technical aspects under smallholding farming systems. Therefore, the use of resistant varieties integrated, with other control methods has proved to be the best option, as it is an environmentally safe, sustainable and feasible control option.

The National Bean Breeding program in Ethiopia has been characterized by its conservative breeding strategies that are designed to adhere to consumer preferences, mainly market qualities and resistance to diseases, that affect common bean production in the country. However, traditional varieties (landraces) are available that possess enormous genetic potential. This may be useful in the common bean breeding program, which has mainly used exotic germplasm sources for hybridization. This has reduced the genetic basis of the crop and limited the variability of the available germplasm for breeding. Hence, the evaluation of the landraces as sources of valuable genes for many agronomic, physiological and pest resistance traits would be important.

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CHAPTER 2

Evaluation of Ethiopian Common Bean (*Phaseolus Vulgaris* L.) Genotypes for Resistance to the Mexican Bean Weevil (*Zabrotes Subfasciatus* Boheman)

Abstract

The common bean (*Phaseolus vulgaris* L.) is among the most important grain legume crops in Africa, in general, and Ethiopia, in particular. The common bean grain is heavily attacked by the Mexican bean weevil (*Zabrotes subfasciatus* Boheman). A total of 300 common bean entries were subjected to a “no-choice” test, under ambient temperature and relative humidity conditions at the Melkassa Agricultural Research Center, Ethiopia, using a randomized complete block design with three replications. Data were collected on insect-related traits namely, the number of eggs per female, the number of adults emerged, the percentage of adults emerged, the days to adult emergence and the adult dry weight. Seed-related traits, including the percentage of infested seeds, the total number of holes, the number of holes per seed, percentage seed damage, seed weight loss, seed size (g per 100 seed), seed coat weight (as a percentage of total seed weight) and seed density were recorded. A significant level of variation of all the parameters measured, was recorded among the genotypes. The relative resistance was recorded in landraces, improved genotypes and breeding lines, but from the resistant genotypes, only RAZ-11, RAZ-36, RAZ-2, RAZ-44, RAZ-120, RAZ-40, MAZ-200 and MAZ-203 showed consistent and complete resistance, with a zero index of susceptibility (IS) value. Two other promising entries were also identified from the breeding lines (SCR-11) and landrace collections (NC-16) of Ethiopia. Stratified ranking diagrams showed that accessions from different eco-geographical origins in Ethiopia, and those with different colours showed different patterns of response to infestation. The resistant genotypes should be included by the Ethiopian bean breeding program for comprehensive yield trials at a national level and their possible release as commercial varieties. The incorporation of bean weevil resistance genes found in this study into otherwise adapted varieties, through backcross breeding techniques supported with marker assisted selection, seems to be the best strategy, not only in terms of time saving, but also in terms of effectiveness and efficiency.

Key words: Common bean, host plant resistance, Mexican bean weevil, *Phaseolus vulgaris*, *Zabrotes subfasciatus*

2.1 Introduction

The common bean (*Phaseolus vulgaris* L.) is among the most important food legumes, representing 50% of the grain legumes consumed worldwide (McClean et al., 2008). It is a strategic crop that provides not only household food and income, but also a cheap source of protein that confers nutritional security to poor people in eastern and southern Africa (Katungi et al., 2009). As a major legume crop in Ethiopia, it contributes to the income and nutrition of more than 3.3 million smallholder farmers (CSA, 2014). In addition to the direct economic advantages, the common bean also plays a significant role in soil fertility replenishment, by fixing biological nitrogen in the soil (Herridge and Danson, 1995), thereby providing sustainability to the farming system, particularly when grown in rotation with cereals.

Ethiopia is the third largest common bean producer in east Africa, after Tanzania and Kenya (FAOSTAT, 2012). In 2014, for instance, 520,121 hectares of land were allotted to the common bean and a total of 621, 665 tons of grain was produced in Ethiopia (CSA, 2014). The common bean is the second major food legume crop in Ethiopia, after the faba bean (*Vicia faba* L.) in terms of the area cultivated and the volume of production. However, it is the most important legume for export to countries in Europe and the Middle East (Shaun et al., 2012; CSA, 2014). Despite its importance, the potential of common bean production is rarely attained in tropical and sub-tropical Africa, including Ethiopia, because of several biotic and abiotic constraints.

Storage insect pests are among the most serious problems constraining common bean production in the field and in storage at, the global level, particularly in the humid tropics and subtropics (Cardona, 2004; Keneni et al., 2011). Some reports indicate that, without postharvest management, storage insect pests may cause an estimated dry weight loss of 10-40%, and up to 70% grain damage in less than six months under on-farm storage conditions (Kiula and Karel, 1985; Paul et al., 2009). Two species of bean bruchids, namely, *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman), are the most important pests worldwide (Schoonhoven and Cardona, 1986; Parsons and Credland, 2003), the latter causing the most significant qualitative and quantitative losses to stored common bean seeds, not only in Ethiopia (Tadesse et al., 2008), but also elsewhere in eastern and central Africa (Nchimbi and Misangu, 2002). In the warmer region of the Central Rift Valley of Ethiopia, for instance, this species is known to cause immense damage (Wortmann et al., 1998). It is estimated at 38%, with equivalent weight loss of about 3.2% (Negasi, 1994). Getu et al. (2003) reported a grain loss of 60% only after 3-6 months in storage. In addition to the direct losses, the bean seeds are also liable to indirect losses

as, once damaged by bruchid, the common bean seeds are neither fit for planting, due to poor germination nor for food or feed (Aslam, 2004; Haile, 2006). Losses result in an indirect loss of profit, because farmers are forced to sell their beans at low prices immediately after harvest, or at considerable price discounts for damaged seeds (Uebersax et al., 1996).

Different control options have been attempted by Ethiopian farmers, including the drying of the seed before storage, the cleaning of storage structures, the admixing of grain with botanical pesticides, seeds of small cereals or ash, the storing of beans in smoke over fire and treating the seeds with insecticides (Abate and Ampofo, 1997; Tadesse et al., 2008). However, most of these practices are not used as often by farmers nowadays, due to a move towards chemical insecticides (Tadesse et al., 2008). Insecticides can confer effective bruchid control, but the use of chemicals is not a method of choice for smallholder farmers, because of supply shortages, the cost and concerns related to environmental hazards and food safety. Farmers also lack separate storage structures that are conducive for fumigation (Keneni et al., 2011). The development of environmentally-safe, sustainable and feasible control measures, like host plant resistance is obviously the best option for managing storage bruchids in the common bean, particularly amongst smallholder farmers.

The International Center for Tropical Agriculture (CIAT) has developed Mexican bean weevil resistant lines, using a few wild common bean accessions as sources of resistance (Schoonhoven et al., 1983; Acosta-Gallegos et al., 1998). Antibiosis expressed through the adverse effects of seed protein arcelin in the cotyledons of these wild accessions, was found to extend the time to adult emergence, as well as the growth and lifecycle of the insects (Osborn et al., 1988; Cardona et al., 1989; Minney et al., 1990; Velten et al., 2008). Even though promising results were achieved from past breeding efforts in terms of, developing genotypes with arcelin based resistance (Cardona et al., 1990; Cardona and Kornegay, 1999; Myers et al., 2001; Cardona, 2004; Beneke, 2010), such efforts have not yet resulted in the release of any commercial variety for wider production. It is, therefore, essential to search for additional sources of Mexican bean weevil resistance from the local landraces, which are prefered in the local breeding program. Some reports have indicated that Ethiopia has a wealth of genetic diversity in the common bean (Asfaw et al., 2009), but these genetic resources have not yet been systematically assessed for resistance to Mexican bean weevil. The objective of this study was, therefore, to evaluate the Ethiopian common bean landrace collections, as well as the released varieties, breeding lines and elite genotypes for resistance to the Mexican bean weevil.

2.2 Material and methods

2.2.1 Plant materials

A total of 300 common bean entries (204 landrace collections, 34 released varieties, 27 breeding lines and 35 genotypes, with a known resistance to the Mexican bean weevil) were evaluated in this study. Out of the landrace collections, 148 were obtained from the Ethiopian Biodiversity Institute (EBI) and the remaining 56 were collected from the major bean-growing areas in Ethiopia. The released varieties and breeding lines were obtained from the Melkassa, Sirnka and Areka Agricultural Research Centers and the Haramaya University in Ethiopia, whereas the Mexican bean weevil resistant genotypes were obtained from the International Center for Tropical Agriculture (CIAT) in Uganda, the Pannar Seed Company and the Malawian National Bean Improvement Program. The passport data of the genotypes used in the study are presented in Table 1 of Appendix A and the seed colour, size and shape are shown in Figure 1 of Appendix A. The map showing the collection sites of the Ethiopian materials is presented in Figure 2.1.

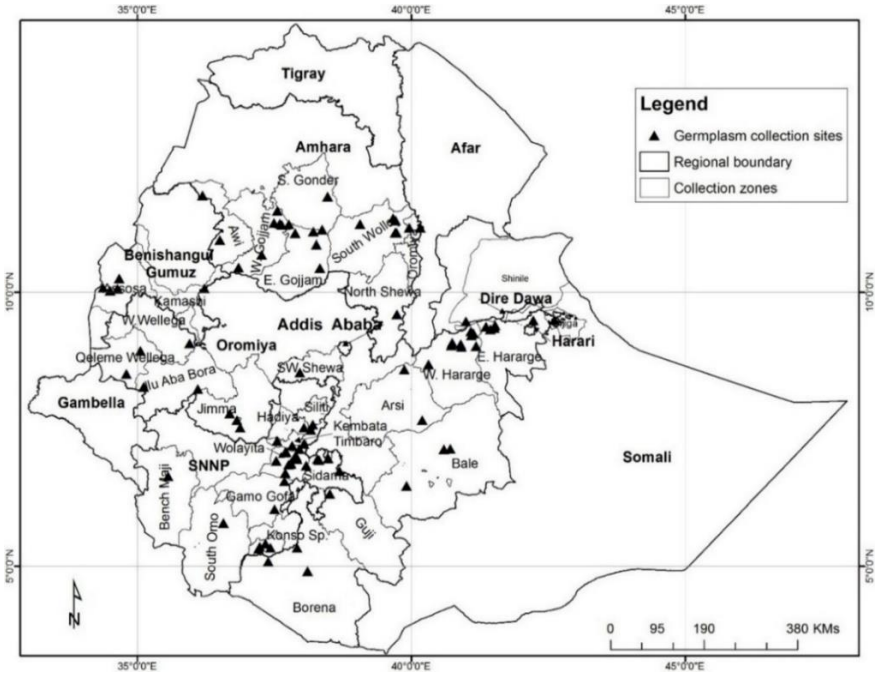


Figure 2.1 A map of Ethiopia showing the geographical positioning of the specific collection sites of 204 common bean landrace collections

The genotypes were highly variable and contrasting for many features, such as seed colour (red = 114, white = 75, black = 30, carioca = 13, speckled = 9, yellow = 12, cream = 19, pinto = 5, mottled = 16 and brown = 7), and seed size, based on hundred seed weight (g) (small <25 g, medium = 26-40 g and large >40 g) (Singh et al., 1991). Small-seeded genotypes were 200, medium-seeded were 73 and large-seeded were 27 in number. The genotypes were grown under the same conditions at Melkassa during the off-season of 2013 (February-May), under irrigation for seed increase, and to offset any differences in seed age and the effects of the preceding growing conditions (Liao et al., 2008).

2.2.2 Bruchid resistance evaluation protocol

The experiment was conducted in the Entomological Research Laboratory at the Melkassa Agricultural Research Center, Ethiopia, under ambient temperature and relative humidity conditions, as presented in Figure 2.2. Adult bruchids were collected from stored bean seed, from which a culture of bean bruchids was developed to supply bruchids of a similar age for the experiment. A susceptible variety, Batu, was used for the mass rearing of bruchids, at an average room temperature of 27°C and a relative humidity of 70%. The experiment was intentionally conducted between March and May, 2014, which is the period of the year when higher storage losses are commonly expected in the area, because of high temperatures.

Freshly harvested seeds were cleaned and placed in a deep freezer (-20^0C) for four weeks, to destroy any prior bruchid infestation, as suggested by Dobie (1977). The seeds were subsequently acclimatized, under experimental conditions for seven days. Twenty grams of seeds were placed in transparent plastic jars (6 cm x 7 cm), with an opening at one end for free air circulation. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each jar was infested with five pairs (five females and five male) of newly-emerged bruchids, which were kept in the jars for 10 days to allow oviposition, after which they were removed. The seeds were observed daily to record any bruchid emergence. The removal and counting of emerged adult bruchids was done every second day, starting from the first emergence and continuing until the last emergence.

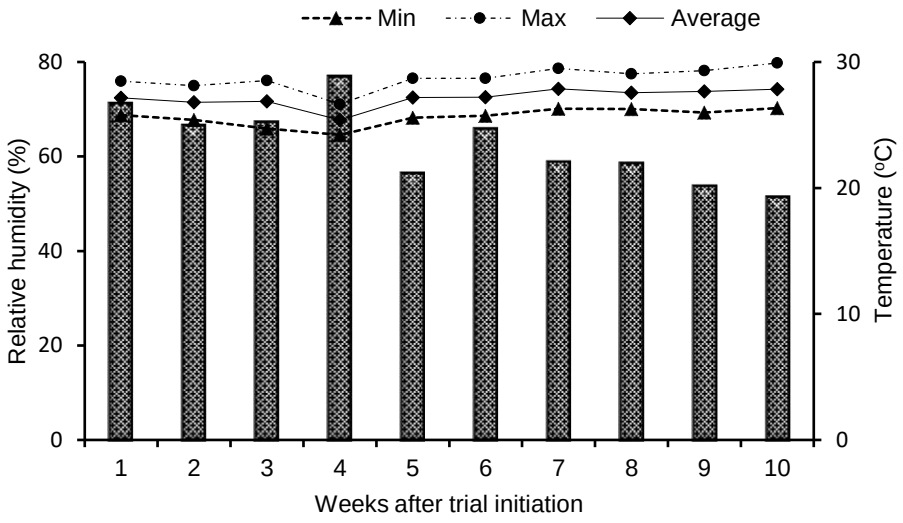


Figure 2.2 Temperature (°C) and relative humidity (%) at the Entomological Research Laboratory of Melkassa Agricultural Research Center during the study period

2.2.3 Data collection and analysis

Data were collected on insect-related traits, including the number of eggs per female, the number of adults emerged, the percentage of adults emerged, the days to adult emergence (DAE) and the adult dry weight. Seed-related traits, including the proportion of infested seeds (%), the number of holes, the number of holes per seed, the percentage of seed damage, seed weight loss, seed size (g/100 seed), seed coat weight (as percentage of total seed weight) and seed density, were also recorded. All the data were collected on the first progeny, except for the percentage damage (1st and 2nd progeny) and seed weight loss (%) (2nd progeny). The Index of Susceptibility (IS) scores were calculated, as previously described by Cardona et al. (1990) and used by Hartweck et al. (1997) for the Mexican bean weevil. The IS values were calculated as:

Index of susceptibility $= log {}^{F}/_{DAE} * 100$, where F = progeny per infesting female and DAE = days to adult emergence. The genotypes were classified, based on Dobie's (1974) indices of susceptibility as resistant (0-3), moderately resistant (4−7), susceptible (8−10) and highly susceptible (11). Grain damage was calculated as the percentage of damaged seeds in each jar. The percentage seed weight loss of each genotype was calculated by separating damaged and undamaged seeds from each jar at the end of the experiment, as suggested by Shaheen et al. (2006).

$$\text{Percentage weight loss} = \frac{\text{Initial weight} - (\text{Weight of damaged} + \text{Undamaged seed})}{\text{Initial weight}} \times 100$$

Data based on numerical and percentage values were first log (count) and arcsine (percentage) transformed, in order to ensure the homogeneity of variance. The Analysis of Variance (ANOVA) was done with the SAS software Version 9.2 (SAS Institute, 2003), using the following model: $p_{ik}=\mu+b_k+g_i+e_{ik}$, where p_{ik} = phenotypic observation on genotype i block k, (i = 1…G, k = 1… B) and G and B = number of genotypes and block, respectively, μ = grand mean, g_i = the effect of genotype i and b_k = the effect of block k and e_{ik} = error. Tukey's honestly significant difference test was used for comparing the mean values. The best 10% of the genotypes were identified, their means were independently calculated for each character and comparisons were made between the mean performances of the best 10% of the genotypes and the original population, using the Z-test (Singh, 2001) as follows:

$$Z = \frac{\overline{Y} - \overline{X}}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

Where: $\overline{Y}$ = mean of original population, n_1 = number of individuals in original population, $\overline{X}$ = mean of selected individuals, n_2 = number of individuals in selected sample, S_1 = standard deviation of original population and S_2 = standard deviation selected sample.

A comparison was made of the pattern of frequency distributions and a stratified ranking diagram for some important attributes of response of the 300 original entries and the best 10% of the selected genotypes showed the improvements of the selected genotypes over the original population, using the statistical software MINITAB Release 14 for Windows® (Fox et al., 1990; Minitab Inc., 2010).

2.3 Results

2.3.1 Genotypic performance

The analysis of variance showed highly significant differences (P < 0.01) among the genotypes for all characters (Tables 2.1 and 2.2). The most resistant genotypes, with no adult emergence were from the MAZ and RAZ lines, including MAZ-200, MAZ-203, RAZ-2, RAZ-36, RAZ-44, RAZ-11, RAZ-120 and RAZ-40, as expected. On the other hand, the most susceptible genotypes, with the highest number of adults emerged and included Awash-1, Acc. No. 223329, Acc. No. 215719, NC-46, Acc. No. 214678, SCR-20, Beshbesh and MAZ-217. Even though some common bean genotypes (like Awash-1, NC-46, Acc. No. 215719 and Acc. No. 223329) with the highest number of eggs also had the emergence of the highest number of adults, a few others (like MAZ-217, NC-20 Acc. No. 214678, NC-18 and SCR-28) had the lowest number of eggs, but the highest proportion of emergence into adults. All RAZ lines, with no exception, as well as some MAZ lines prolonged the period to adult emergence to over 40 days, which may indicate the existence of certain inhibiting factors. Similarly, other entries had the highest (like AFR-702, MAZ-151, SMARC-4, ETHAW-01-L-1-7A and MAZ-200) and the lowest (MAZ-180, MAZ-153, RAZ-114 and RAZ-9) number of eggs, but they did not support adult emergence.

Table 2.1 Analysis of variance and range of the 300 common bean genotypes for six insect traits after infestation by bean bruchid

		Mean squares					
Source of variation	**DF**	**NE**	**DAE**	**NAE**	**ADW**	**IS**	**PAE**
Rep	2	1916.5	20.0	239.9	0.0001	2.3	400.8
Genotype	299	1716.5**	20.2**	1408.5**	0.0064**	12.9**	1662.7**
Error	598	462.2	1.5	279	0.001	1.2	167.7
Range		8.0-138.0	21.0-40.0	0.0-110.0	0.0-0.95	0.0-11.0	0.0-100.0
Mean ± SE		53.1±17.6	22.6±6.5	39.6±13.6	0.04±0.03	6.63±0.9	74.65±10.6
CV (%)		11.8	5.4	13.0	24.6	16.7	17.5

Genotypes with no adult emergence were excluded from the calculation of the analysis of variance and the range of days to adult emergence; DF = degrees of freedom; NE = number of eggs; DAE = days to adult emergence; NAE = number of adult emerged; ADW = adult dry weight; IS = index of susceptibility; PAE = percent adult emergence; SE = standard error; CV = coefficient of variation; ** significantly different at p < 0.01 probability level

Table 2.2 Analysis of variance and range of the 300 common bean genotypes for nine seed traits after infestation by bean bruchid

Source of variation	DF	Mean squares								
		INS (%)	NH	NH/S	FPD (%)	SPD (%)	SWL (%)	SCW (%)	SD	HSW
Rep	2	221.9	577.2	0.07	217.4	7.2	324.7	0.01	0.001	0.75
Genotype	299	786**	1516.5**	0.79**	780.9**	2236.2**	523.1**	3.66**	0.011**	321**
Error	598	143.1	303.8	0.25	106.8	42.5	63.3	0.24	0.002	1.54
Range		3.7-85.5	0.0-114.0	1.0-4.0	0.0-75.4	0.0-100	0.0-50.3	6.2-15.7	1.2-1.6	10.7-50.8
Mean ± SE		35.3±9.8	41.1±14.2	1.6±0.3	28.8±8.4	86.7±5.3	24.0±6.5	8.9±0.4	1.4±0.04	26±0.01
CV (%)		21.3	13	19.8	22.1	7.5	20.9	5.5	3.6	4.6

DF = degrees of freedom; INS (%) = percent infested seeds; NH = number of holes; NH/S = number of holes per seed; FPD (%) = first progeny damage; SPD (%) = second progeny damage; SWL (%) = seed weight loss; SCW (%) = seed coat weight; SD = seed density; HSW = hundred seed weight; SE = standard error; CV = coefficient of variation; ** significantly different at $p < 0.01$ probability level

Based on the index of susceptibility, the performance of genotypes varied between zero, for CIAT varieties with arcelin-based resistance to *Zabrotes subfasciatus* and 11, for the most susceptible landraces. All RAZ lines, some MAZ lines and genotype SCR-11 showed resistance, with the index of susceptibility values ranging from 1 to 3. There was no landrace in the resistant class, based on the index of susceptibility but 44.1% were in the moderately resistant category (Table 2.3).

Table 2.3 Distribution of 300 genotypes from different origin into different bruchid susceptibility classes, as categorized by the index of susceptibility

Class	Landraces		CIAT-BL		CIAT-RL		MNBIP		ENBIP		Overall total	
	No	%	No	%	No	%	No	%	No	%	No	%
Resistant (0-3)	-	-	1	3.7	24	72.7	-	-	-	-	25	8.3
Moderately resistant (4-7)	90	44.1	18	66.7	9	27.3	1	50	14	41.2	132	44
Susceptible (8-10)	113	55.4	8	29.6	-	-	1	50	20	58.2	142	47.3
Highly susceptible (11)	1	0.5	-	-	-	-	-	-	-	-	1	0.3
Total	**56**		**27**		**33**		**2**		**34**		**300**	

CIAT-BL = CIAT breeding lines; CIAT-RL = CIAT resistant lines; MNBIP = Malawian National Bean Improvement Program and ENBIP = Ethiopian National Bean Improvement Program

Based on the index of susceptibility, 8.3% of the 300 genotypes were categorized as resistant, 44.0% as moderately resistant, 47.3% as susceptible and 0.3% as highly susceptible. All genotypes were classified into two groups (moderately resistant and susceptible), except the CIAT varieties, with known arcelin-based resistance, of which 72.7% fell into the resistant category and only 27.3% into the moderately resistant categories. Based on the percentage seed damage, the genotype NC-38 had 50.31% damage, whereas no seed damage was recorded on CIAT varieties with arcelin-based resistance, including MAZ-203, RAZ-2, RAZ-36, RAZ-44, RAZ-42, RAZ-11, RAZ-120 and RAZ-40.

Simple measures of variability, including range, arithmetic mean and standard error also showed the existence of a level of high variation among the genotypes (Tables 2.1 and 3.1). The number of eggs laid per female ranged from 8-138, the number of days taken to adult emergence ranged from 21-40 (considering only genotypes in which adults had emerged), the number of emerged adults ranged from 0-110 (0-100%) and the adult dry weight ranged from 0.00-0.95 g. Wide ranges were also recorded for other characters, including the index of susceptibility, the number of holes per seed, the percentage damage, seed weight loss (%), seed coat weight (%), seed size and seed density (Tables 2.1 and 2.2). The standard errors of almost all parameters recorded in this study were low.

2.3.2 Comparison of selected genotypes with the original population

A comparison of mean performances of the best 10% selected genotypes, with mean performances of the whole population for different characteristics is presented in Table 2.4. The best 10% of the selected genotypes exhibited a significantly lower number of eggs, a number of infested seeds, a number of adults emerged, a lower adult dry weight, a number of holes per seed and an index of susceptibility, longer days to adult emergence, and a lower percentage of seed damage and seed weight loss. They also had small seed sizes, thick seed coats and a higher seed density, compared to the overall population (Table 2.4).

The Z- test showed significant differences ($P<0.01$) between the mean of the original population ($\bar{Y}$) and means of the selected 10% best genotypes ($\bar{X}$) for all the response characteristics considered in this study. From the top 10% selected genotypes, MAZ 203, RAZ-2, RAZ-36, RAZ-44, RAZ-42, RAZ-11, RAZ-120, RAZ-40, RAZ-119, RAZ-114, RAZ-138, MAZ 174, RAZ-19, RAZ-111, RAZ-11-1, MAZ 153, MAZ 200, MAZ 179, MAZ 151 and RAZ-9, were completely resistant, based on the percentage of adult emergence, the index of susceptibility and percentage of seed weight loss. There was no complete resistance for the other landraces, the improved varieties and the rest of the breeding lines, although genotypes SCR-11, NC-16, Acc. No. 208705 and ETAW-01-L-1-7A were among the top 10% for different parameters of resistance. Statistically significant differences ($P<0.05$) were recorded for the percentage adult emergence, days to adult emergence, the index of susceptibility and seed weight loss of the released varieties, but none of them exhibited complete resistance. Two of the released varieties, namely AFR-702 and Awash Melka, were among the top 10% best performers, particularly for percentage adult emergence and percentage seed weight loss, respectively. Significantly higher percentage seed weight losses in released varieties were recorded on genotypes Nasir (42.82 %), Ayenew (43.61%) and Awash-1 (45.85%). On the other hand, percentage seed weight losses were lower in two other released varieties, namely, Awash Melka (3.74 %) and Red Wolayta (7.47 %).

Table 2.4 Comparison of the mean performances of the selected 10 % best genotypes and the overall population for response characters to infestation by bean bruchid

Character	$(\bar{Y})$	$(\bar{X})$	Change through selection /$\bar{Y}$-$\bar{X}$/	Change as % of population parameter ($\bar{Y}$)	Z
Number of eggs	53.06	20.00	33.06	62.30	26.16**
Infested seeds (%)	35.34	11.90	23.44	66.30	33.33*
Days to adult emergence	27.57	33.91	6.343	23.00	85.41*
Number of adult emerged	39.63	4.00	35.63	89.90	36.72*
Percent adult emerged	74.65	12.12	62.54	83.80	82.89*
Adult dry weight (gm)	0.038	0.003	0.035	91.00	26.88*
Index of susceptibility	6.63	1.57	5.06	76.32	77.95*
Number of holes	41.09	4.00	37.09	90.30	36.17*
Number of holes per seed	1.63	1.00	0.63	38.70	41.77*
Percent damage (1st progeny)	28.80	4.00	24.80	86.10	40.89*
Percent damage (2nd progeny)	86.70	11.40	75.30	86.90	197.04*
Hundred seed weight (gm)	26.01	14.40	11.61	44.60	159.13*
Seed weight loss (%)	23.99	1.50	22.49	93.70	48.23*
Seed coat weight (%)	8.95	11.10	2.15	24.10	75.05*
Seed density (g/ml)	1.352	1.460	0.108	8.000	36.84*

* = significant (P < 0.05); $\bar{Y}$ = mean of the 300 population and $\bar{X}$ = mean of the 10% best genotypes

A comparison of the frequency distributions of the 10% most resistant genotypes, with the frequency distribution of the overall population clearly revealed a significant level of improvement through selection (Figures 2.3 a-f).The average number of eggs was reduced from 53.2 in the overall population, to 20.0 in selected individuals, the number of adults emerged from 39.6 to 4.0, the percentage adult emerged from 74.7 to 12.1, the index of susceptibility from 6.60 to 1.57, the percentage seed damage from 86.7 to 11.7, the percentage seed weight loss from 23.9 to 1.5, the seed coat weight from 11.10 to 8.95 g and the seed density from 1.46 to 1.35 g/ml Similarly, a comparison of the frequency distributions of the selected 10% most resistant genotypes with the frequency distribution of the overall population, using a stratified ranking diagram showed a significant level of improvement through the selection of the best genotypes for different resistance traits (Figures 2.4 A-D).

Genotypes from different sources had different patterns of response to infestation, in terms of the four most important insect and seed related traits (the number of eggs, the percentage adult emerged, the index of susceptibility and seed weight loss). The resistant genotypes exhibited clear superiority, as most of them ranked in the bottom 1-10%, for the above mentioned traits in the original population. However, almost all categories (landraces, CIAT breeding lines, the improved varieties released in Ethiopia, the CIAT resistant lines and introductions from Malawi) showed possibilities for selecting individuals with a less relative number of eggs, percentage adult emerged, index of susceptibility and seed weight loss. The original and the selected population of landraces from different eco-geographical origins, based on a stratified ranking diagram had different patterns of response for the four traits (Figure 2.5 A-D). The common bean accessions collected from the central parts of the country resulted in a higher number of genotypes ranked in the lower part (1-10%) than those collected from the rest of the country, for all the traits in the overall population. Except for some inconsistencies in the western, northern and southern parts of the country, almost the whole country showed potential for selecting individuals with a less relative number of eggs, the percentage adult emerged, the index of susceptibility and seed weight loss.

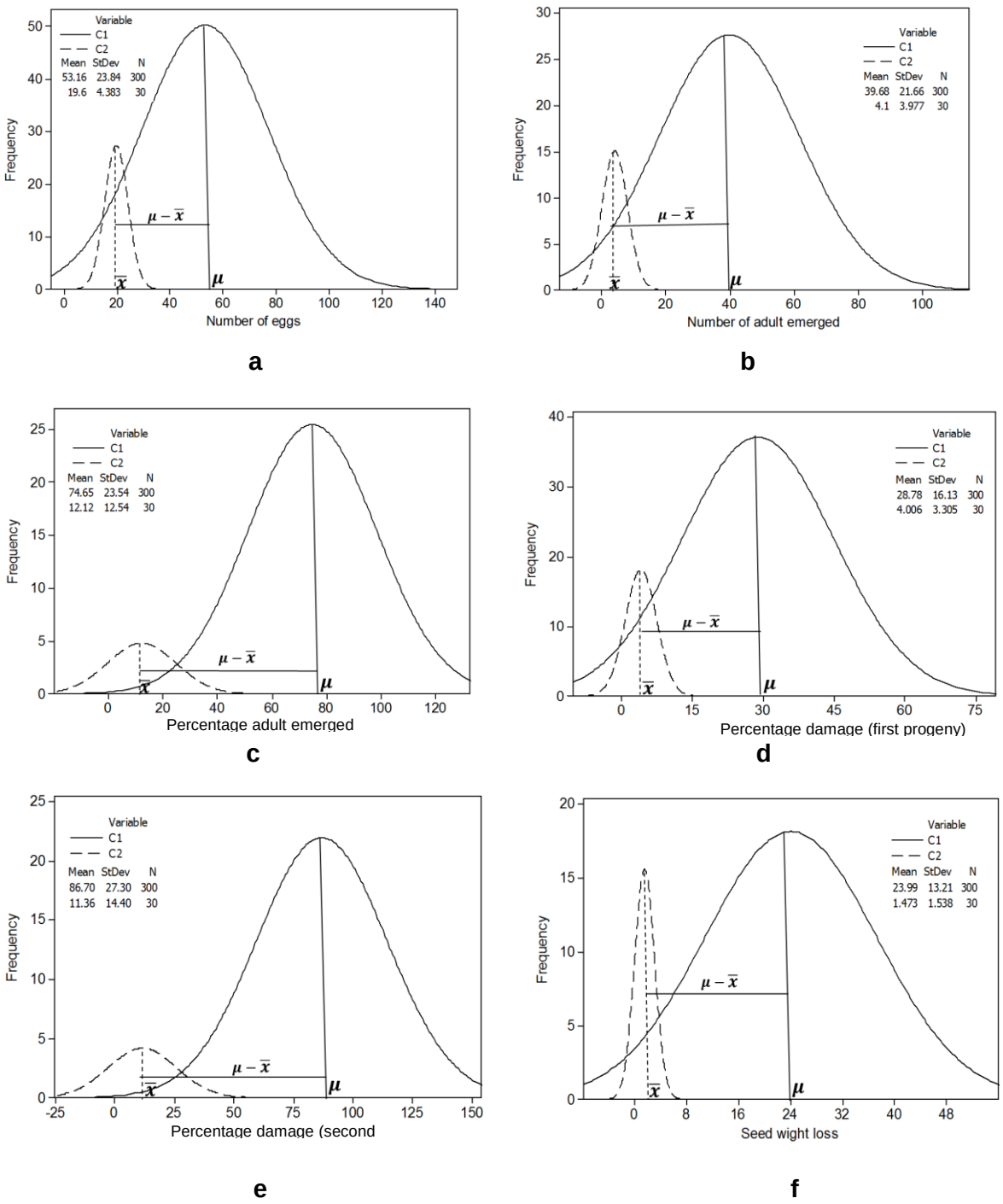
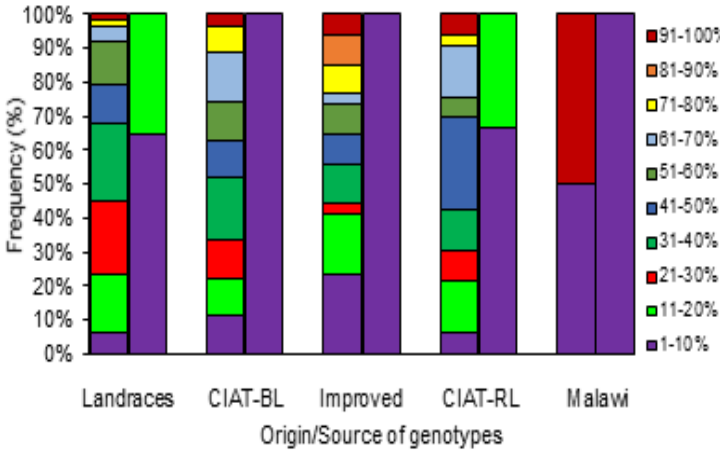
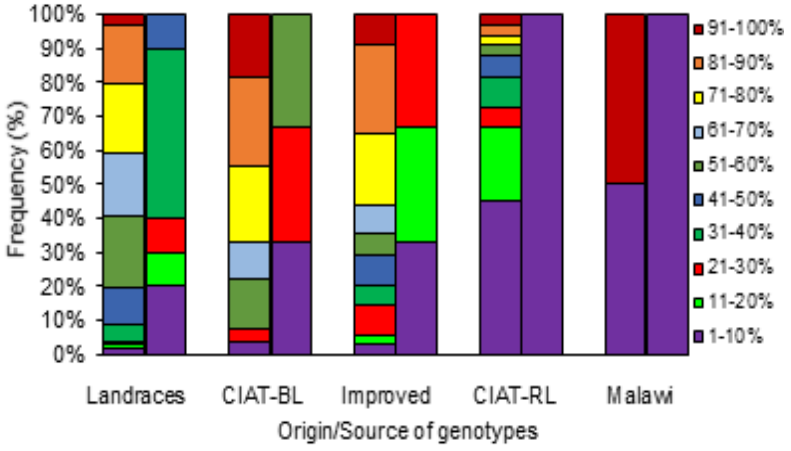


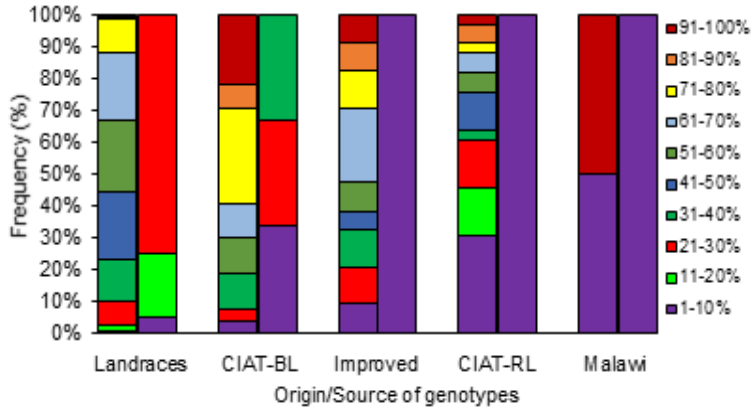
Figure 2.3 Comparison of the distribution of the best 10% of the genotypes ($\bar{x}$) with the distribution of the whole population (μ) for different characters related to response to infestation by bean bruchid.



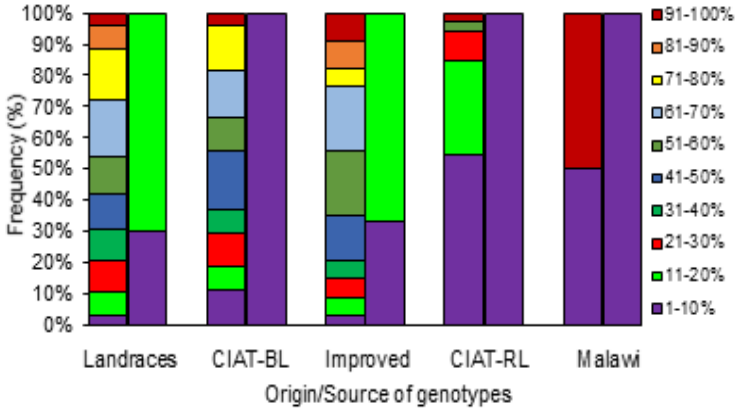
(A) Number of eggs



(B) Percentage adult emerged

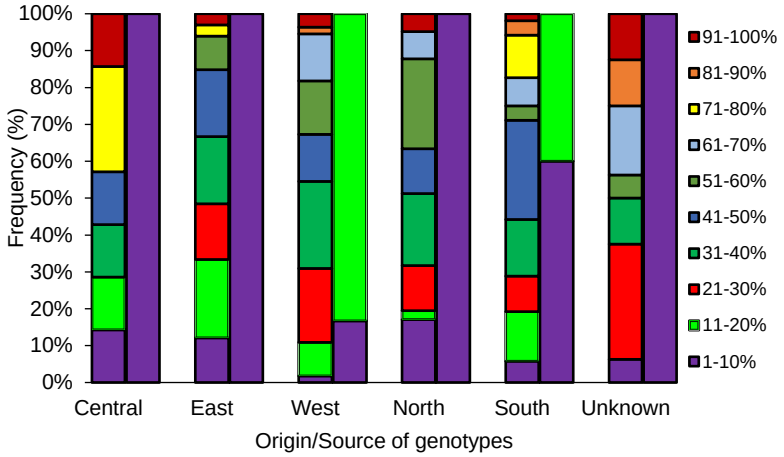


(C) Index of susceptibility

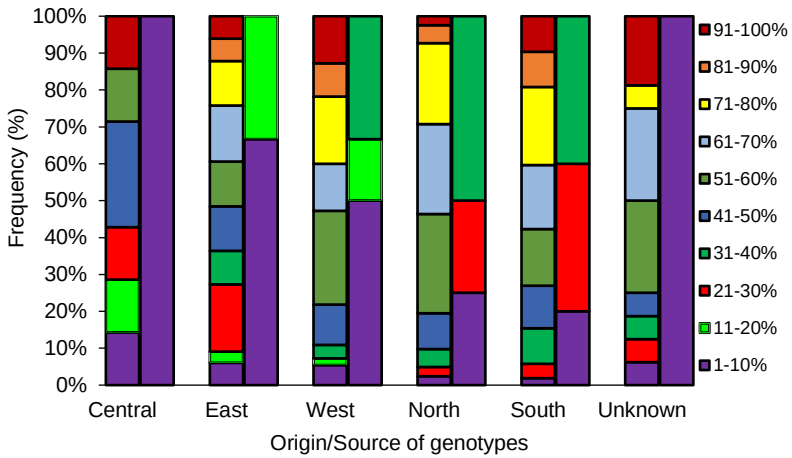


(D) Seed weight loss (%)

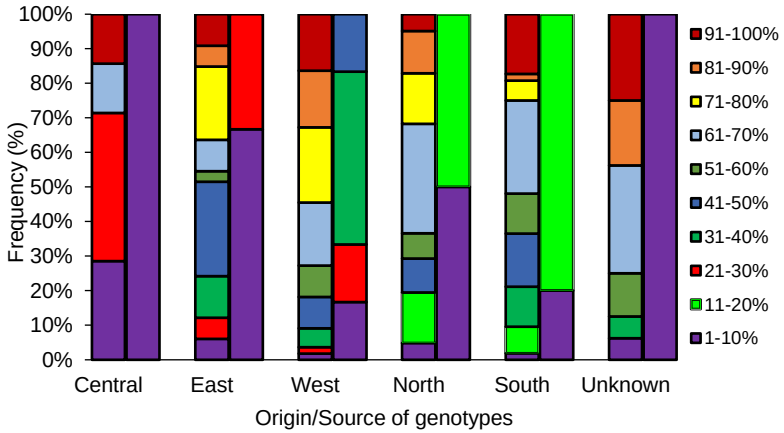
Figure 2.4 Stratified ranking diagram showing the relative distributions for four different important traits in the original (1st bar) and 10% best (2nd bar) populations of common bean landraces, CIAT breeding lines, improved varieties released from Ethiopia, CIAT resistant lines and introduction from Malawi



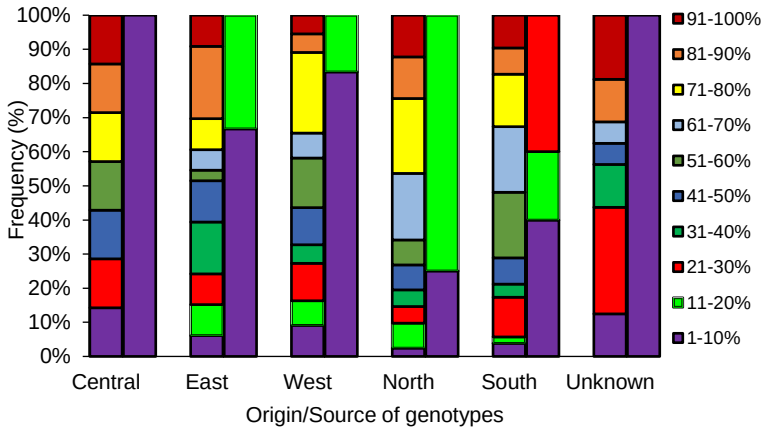
(A) Number of eggs



(B) Percent adult emerged



(C) Index of susceptibility



(D) Seed weight loss (%)

Figure 2.5 Stratified ranking diagram showing the relative distributions for four different important traits in the original (1st bar) and 10% best (2nd bar) populations of common bean landraces collected from different eco-geographic regions of Ethiopia

2.4 Discussion

The significant genotype mean squares for percentage adult emergence, the days to adult emergence and the index of susceptibility showed that the 300 genotypes exhibited genetic variations for bruchid resistance. Apart from the genotypes with known resistance (33), only one breeding line (SCR 11) was found to be resistant, based on Dobie's index of susceptibility. Indicators of the presence of antibiosis, such as reduced adult emergence, prolonged life cycle and reduced adult dry weight were recorded in the resistant materials (RAZ and MAZ lines). The results indicated that the above susceptibility parameters (percentage adult emergence, days to adult emergence and index of susceptibility) were able to differentiate the genotypes, based on their variations in bruchid susceptibility (Schoonhoven et al., 1983; Redden and McGuire, 1983; Cardona et al., 1990). In this study, the highest and lowest number of eggs did not support the proportion of adult emergence, indicating that at least some mechanisms of host resistance, including antixenosis (non-preference) for ovi-positioning and antibiosis (deterrence) for adult emergence, might be involved, which was also indicated by De Morais and Pinheiro (2012). Various authors also reported that, during the no-choice test experiments, bruchids oviposited on all accessions and a high number of eggs may not necessarily result in correspondingly high number of progeny, thus a lower number of eggs laid per female may not be a good indicator of resistance, as reported by others (Negasi and Abate, 1992; Shiferaw, 2004).

Genotypes were grouped based on the index of susceptibility as a measure of resistance. The index of susceptibility is linearly correlated with the intrinsic rate of increase and the logarithm of the number of insects that emerge over a given time of period and, hence, it provides a reliable estimate of resistance levels (Dobie, 1974). Genotypes with a low index of susceptibility values are ranked as highly resistant and those with high values are ranked as susceptible (Cardona et al., 1989). A zero index of susceptibility in this study indicated that no bruchids had emerged over the test period, which was the case for varieties with arcelin-based resistance to *Zabrotes subfasciatus* (Singh and Singh, 1992). The index of susceptibility ranged from 0-11 among the tested genotypes, which indicated that there was a remarkable variation among the genotypes for this index. No complete resistance was observed in any of the landraces, breeding lines and improved varieties, as previously reported (Schoonhoven et al., 1983; Acosta-Gallegos et al., 1998, Acosta-Gallegos et al., 2008), but partial resistance was observed in a few genotypes in this study. The index of susceptibility of all the improved genotypes ranged from 5 to 9, indicating that all the released varieties from the national program fall into moderately resistant (Awash Melka, Hundene and Fidise) to susceptible categories. This may be because more priority was given to the productivity of the varieties,

at the expense of storage insect resistance, by the hitherto common bean breeding in Ethiopia. Seed weight loss ranged from 0-50.3 g (Table 2.2) in two generations and the insect completes up to 6-7 generations per year, showing that the damage level can be very high within a short period. A sixty percent seed weight loss has been reported in bean seed stored for 3-6 months in Ethiopia (Getu et al, 2003).

Arcelin, a lectin like protein, is able to confer resistance against *Z. subfasciatusis* and is found in the cotyledons of wild common beans. It also affects the survival and development of *Z. subfacsiatus* (Osborn et al., 1988; Cardona et al., 1989). CIAT resistant lines, i.e. RAZ-11, RAZ-36, RAZ-2, RAZ-44, RAZ-120, RAZ-40, MAZ-200 and MAZ-203, exhibited complete resistance to *Z. subfacsiatus*. Assefa (2010) tested genotypes of the common bean (30 RAZ and 10 susceptible commercial varieties) for their resistance against *Z. subfacsiatus* and found that the arcelin 1 variant (present in RAZ 4, RAZ 120, RAZ 42, RAZ 101, RAZ 173, RAZ 119, RAZ 44, RAZ 174, and RAZ 151) had a good level of resistance for *Z. subfasciatus*. The arcelin variants Arc-5 and Arc-1 showed the highest resistance to *Z. subfasciatus* in common bean accessions, followed by Arc-4, Arc-2 and Arc-3, in their order of importance (Harmsen, 1989; Cardona et al., 1990). The difference in the resistance level is believed to be due to the protein differences or carbohydrate content (Harmsen et al., 1987).

2.5 Conclusions

The results from the present study showed that, despite the existence of significant relative differences in response to infestation by the bean bruchid, the Ethiopian common bean accessions did not show complete resistance. The commercially-improved bean varieties released in Ethiopia were susceptible to *Z. subfasciatus*. On the other hand, genotypes with known arcelin-based resistance (RAZ-2, RAZ-36, RAZ-44, RAZ-42, RAZ-11, RAZ-120 RAZ-40, MAZ-200 and MAZ 203) were completely resistant in this study. A breeding line with a relatively better resistance to bruchids, namely SCR-11, can be used in breeding programs, in order to exploit not only its relative resistance to the bruchid, but also its adaptation to the local conditions. Incorporation of bruchid resistance gene into well-adapted or released common bean varieties by employing backcross techniques supported with Marker Assisted Selection (MAS) seems to be the best strategy, not only in terms of time-saving, but also in terms of effectiveness and efficiency.

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CHAPTER 3

Genetic Diversity Analysis of Common Bean (*Phaseolus Vulgaris* L.) Genotypes for Resistance to Mexican Bean Weevil (*Zabrotes Subfasciatus*), Using Single Nucleotide Polymorphism (SNP) and Phenotypic Markers

Abstract

The Mexican bean weevil is an important storage pest that causes quantitative and qualitative losses to stored common bean grains. The wealth of genetic diversity of the common bean germplasm available in Ethiopia has not yet been systematically assessed and documented. The objective of the present study was to examine the genetic diversity present among 297 common bean genotypes in their response characters to infestation by the Mexican bean weevil, using 2254 single nucleotide polymorphism (SNP) markers and phenotypic markers. The studied genotypes consisted of landraces, released varieties, breeding lines and Mexican bean weevil resistant lines. Twelve insects and seed-related phenotypic traits were used for the assessment, under laboratory conditions using a randomized complete block design with three replications. The genotyping was conducted by using the Illumina BARCBean6K_3 SNP BeadChip. Data on insect and seed traits were collected and the phenotypic diversity was determined, using diversity indices, principal components, discriminants and cluster analyses. High phenotypic diversity indices among phenotypic traits were recorded, ranging from 0.87 to 0.96, with a mean of 0.98. The principal component and discriminant analyses identified four PCs and three discriminant functions, which explained 82% and 100% of the total phenotypic variations among genotypes, respectively. The Polymorphic Information Content (PIC) ranged from 0.21 to 0.38, with a mean of 0.34 reflecting the relatively high discriminating ability of the markers used. The mean gene diversity among genotypes ranged from 0.24 to 0.50, with a mean of 0.44. The mean genetic diversity was highest among the common bean population when the genotypes were grouped, based on their breeding status and seed size. The Jaccard's genetic distance, which was measured by using SNP markers, ranged from 0.19 to 0.82, with a mean of 0.62, while the Jaccard phenotypic distance among genotypes ranged from 0.00 to 1.00, with a mean of 0.64. The analysis of molecular variance revealed highly significant differences ($P < 0.001$) among individuals within the population, within individuals and among populations, in their order of importance. Both the SNP markers and the phenotypic markers grouped the 297 common bean genotypes into two major distinct clusters and three sub-clusters, irrespective of their geographic origin. A weak, but positive

correlation was observed between the phenotypic and genotypic data, suggesting that both the SNP and phenotypic markers were effective in discriminating the genotypes. However, SNPs were more powerful in differentiating the genotypes into their respective gene-pools and breeding status. Based on the diversity analysis, 144 genotypes were selected as parental genotypes and further field performance evaluation was conducted. The availability of adequate genetic diversity in Ethiopian common bean genotypes is useful for future breeding and conservation activities.

Key words: common bean, genetic diversity, Mexican bean weevil, phenotypic diversity, single nucleotide polymorphism

3.1 Introduction

The common bean (*Phaseolus vulgaris* L.) is the second most widely-grown grain legume crop in Ethiopia, after the faba bean. It is also the third most important export crop, after coffee and sesame (Shaun et al., 2012; CSA, 2015). The crop contributes to the growth of the national economy and to the incomes of millions of smallholder farmers. Common bean supplements the traditional cereals that dominate the human diet, enabling a more efficient protein uptake, particularly for the poor, who cannot afford to buy animal products. Like many other legumes, the common bean significantly restores soil fertility through nitrogen fixation and improved mineral solubilization (Herridge and Danso, 1995). It also serves as a "cycle-breaker" to many crop-specific pests and diseases, when grown in rotation with cereals (Malhotra and Singh, 2004; Kirkegaard et al., 2008).

Despite its importance to the national and household economy, there is a huge gap between the actual yield and the biological yield potential of the crop in tropical and sub-tropical Africa. This may be attributed to several biological and environmental stress factors, among which storage insect pests are the most important in Ethiopia (Schoonhoven and Cardona, 1986; Wortmann et al., 1998; Tadesse et al., 2008). The Mexican bean weevil, *Zabrotes subfasciatus* (Boheman), is the most important post-harvest insect pest, which causes both qualitative and quantitative losses worldwide (Schoonhoven and Cardona, 1986; Wortmann et al., 1998; Parsons and Credland, 2003; Tadesse et al., 2008). Several control options have been used for the management of this pest. However, the manipulation of the storage environment by using chemical insecticides and the genetic manipulation of the crop, through breeding for resistance to bean bruchid are the most commonly-used management practices. In countries like Ethiopia which are dominated by resource-poor farmers, the breeding of common bean genotypes resistant to bean bruchid is considered to be an economically-feasible and ecologically-sound practice (Assefa, 2010).

The common bean was introduced into Africa in the 16th century, together with maize (Greenway, 1945; Gentry, 1969). Since its introduction, African farmers have preserved and discovered important genotypes that are adapted to their local environments and needs, which has led to the evolution of morphologically-diverse landraces (Wortmann et al., 1998; Sperling, 2001). In addition, the national bean research programs in Africa have been introducing a large collection of new germplasm from different parts of the world (CIAT, 2005). The success of any breeding or conservation program largely depends on the proper characterization and documentation of variation within and among populations (Negahi et al., 2014). In order to facilitate the wider use of the available genetic resources of the common bean, it is necessary to have a better understanding of its genetic diversity. Several diversity studies have been conducted to characterize the common bean grown in South America (Singh et al., 1991b; Beebe et al., 2000, 2001; Santalla et al., 2004; Chaco´n et al., 2005; Díaz and Blair, 2006; Blair et al., 2007; Kwak and Gepts, 2009; Burle et al., 2010), North America (McClean et al., 1993), Europe (Rodiño et al., 2001; Santalla et al., 2002; Sicard et al., 2005; Stoilova et al., 2005; Logozzo et al., 2007; Angioi et al., 2010; Mercati et al., 2013), Asia (Zhang et al., 2008; Sharma et al., 2013), Central Africa (Blair et al., 2010) and East Africa (Asfaw et al., 2009; Dagnew et al., 2014; Okii et al., 2014a; b). However, the majority of these studies were designed to understand the organization of the diversity within the species.

To determine genetic variation among the germplasm of various crop species, morphological, biochemical and molecular markers are commonly used (Miklas and Singh, 2007). The advantage of the two former markers is that the traits can be directly related to the fitness of the populations and their usefulness for plant breeding (Miklas and Singh, 2007). However, the application of phenotypic and biochemical markers are highly influenced by environment factors. Moreover, with these marker systems, the isolation of unique genotypes that consistently express the desirable traits is a function of luck, persistence, as well as skill. DNA-based markers, on the other hand, are more stable, efficient in detecting even subtle changes within a genome and effective in genetic diversity studies and varietal comparisons (Wang et al., 2010). The integration of molecular marker data with phenotypic data has been used to examine the diversity within species, to ascertain the coherence between the phenotypic and genotypic data and to identify parental genotypes (Burle et al., 2011).

Following the domestication of the common bean, two distinct major domestication gene-pools were recognized, namely, in the Andes and Mesoamerica (Gepts et al., 1986; Singh et al., 1991a; b; c; Bitocchi et al., 2013). The two gene-pools can be distinguished by biochemical markers, such as phaseolin (seed storage protein) and allozymes, and morphological traits, such as the seed size and shape, the shape and size of the flower bracteoles, the number of

nodes of pod-bearing inflorescence and the pod beak position (Singh et al., 1991a; Becerra and Gepts, 1994; Gepts, 1998). The presence of both Andean and Mesoamerican gene-pools was also reported in Africa, a continent that has become a second center of genetic diversity for the common bean (Allen and Edje., 1990; Wortmann et al., 1998; Sperling, 2001; Asfaw et al., 2009; Blair et al., 2010). In East Africa, a high level of genetic diversity in common bean is found in the highlands of Ethiopia and Kenya, which are among the top ten major bean-growing countries in sub-Saharan Africa (Hillocks et al., 2006; Asfaw et al., 2009). A wide range of common bean genetic diversity is found in Ethiopia (Dagnew et al., 2014; Fisseha, 2015); however, the small-seeded Mesoamerican genotypes are the most predominant types (Asfaw et al., 2009).

The common bean breeding in Ethiopia focusing mainly on developing high yielding varieties with enhanced levels of resistance to diseases that affect the common bean in the country (Negash, 2006). However, breeding for resistance to bean bruchid has been given less attention. In recent years, due to the high temperatures caused by climate change, storage pests have become the most important biotic factors in the common bean, to the extent that they threaten the production of the crop. A large germplasm collection of the common bean is held by the Ethiopian Common Bean Breeding Program. However, only a few studies have been conducted thus far, to analyze the diversity of the crop (Asfaw et al., 2009; Dagnew et al., 2014; Fisseha et al., 2016). In addition, the magnitude and pattern of the genetic diversity, for response to infestation to the Mexican bean weevil has not yet been systematically studied. Elite exotic lines, such as Marker Assisted Zabrotes (MAZ) and Resistance to Zabrotes (RAZ) lines have been developed for bruchid resistance by CIAT. However, the varieties have not yet been exhaustively studied for their resistance to bean bruchid, under Ethiopian conditions, especially the MAZ lines. This information is essential for developing resistant genotypes with desirable agronomic traits. Therefore, this study aimed at assessing the magnitude and pattern of genetic diversity in Ethiopian common bean landraces, as well as the released varieties, breeding lines and exotic resistance genotypes, for responding to infestation, by bean bruchid using phenotypic and SNP markers.

3.2 Materials and methods

3.2.1 Plant materials

Out of 300 common bean (*P. vulgaris*) genotypes that have been phenotyped (Chapter 2), 297 genotypes were used in this study since the remaining three genotypes were not able to be genotyped due to unavailablty of seed during the laboratory experiment. Among the genotypes, 202 were landraces and 95 were released varieties and breeding lines. The landraces were collected from four administrative regions (provinces) of Ethiopia, namely, Amhara (68), Oromiya (54), the South Nations, Nationalities and Peoples (SNNP) (49) and Benshangul-Gumuz (13), and 18 genotypes with an unknown origin were also included. The 95 genotypes consisted of 27 breeding lines, 33 released varieties and 35 lines resistant to the Mexican bean weevil. The released varieties and breeding lines were obtained from the Ethiopian Institute of Agricultural Research (EIAR) and the Haramaya University, Ethiopia. The Mexican bean weevil resistant lines were obtained from the International Center for Tropical Agriculture (CIAT), the Uganda, Pannar Seed Company, South Africa and the Malawi National Bean Improvement Program, Malawi. The passport data of the genotypes used in the study are presented in Table 1 of Appendix A and the seed colour, size and shape are shown in Figure 1 of Appendix A.

3.2.2 Phenotyping

The phenotyping of all the 297 genotypes was conducted in the Entomological Research Laboratory at the Melkassa Agricultural Research Center, Ethiopia. Twenty grams of seed was placed in transparent plastic jars (6 cm x 7 cm), with an opening at one end for free air circulation. The experiment was laid out in a randomized complete block design (RCBD), with three replications. Each jar was infested with five pairs (female and male) of newly-emerged bruchids. The insects were kept in the jars for 10 days, to allow oviposition after which the insects were removed and the number of laid eggs was counted. The jars were observed daily, to record any bruchid emergence. The removal and counting of emerged adult bruchids were done every other day, starting from the first emergence till the last emergence. The maximum/minimum temperatures and relative humidity for the 10-weeks period; are indicated in Figure 2.2.

Data were collected on insect-related traits, such as the number of eggs per female, the number of adults emerged, the percentage of adults emerged, the days to adult emergence (DAE) and the adult dry weight. Similarly, seed associated traits, such as the proportion of infested seeds, the number of holes, the number of holes per seed, the percentage seed damage, seed weight loss, hundred seed weight (g/100 seed), seed coat weight (as

percentage of total seed weight) and seed density, were recorded. All the data were collected from the first progeny, except for the percentage damage (1st and 2nd progeny) and percentage seed weight loss (2nd progeny). The detailed procedure for data collection and analysis are presented in Chapter 2. The phenotypic data were subjected to statistical analysis by using GenStat Version 18 (Payne et al., 2017) and SAS Version 9.2 statistical software (SAS Institute, 2003).

The Shannon-Weaver diversity index (H') was estimated for each variable over all the genotypes, and within the collection sites and breeding status (Perry and McIntosh, 1991), as follows:

$$H' = 1 - \sum_{i=1}^{n} pi \log_e pi$$

Where: *H'* = Shannon diversity Index; p_i = the proportion of accessions in the i^{th} class of an n–class character; n = the number of phenotypic classes of traits.

Each diversity index value was divided by its maximum value ($\log_e n$) and normalized, to keep the values between 0 and 1. The diversity index for each character was computed from the complete data set, while the average diversity index was computed for each character, for the collection sites and breeding status. In addition, the proportions of diversity were partitioned as the within and between populations variations, in relation to the total variation.

The data were also subjected to a Principal Component Analysis (PCA) and Discriminant Analysis (DA) procedures, using the same Genstat Version 18. The canonical discriminant analysis was used to provide a reduced dimension model that indicates the measured differences among groups, based on the collection sites and their breeding status (Zhao and Maclean, 2000). A hierarchical cluster analysis was performed to examine the grouping patterns of the genotypes, based on their dissimilarity matrix with respect to the corresponding means of all twelve characters (Mohammadi and Prasanna, 2003). The dissimilarity matrix was calculated, using the Dice similarity index the cluster analysis was made, using unweighted pair group method and the arithmetic mean (UPGMA), using DARwin 6.0 software (Perrier and Jacquemoud- Collet, 2006). A dendrogram was then generated on the dissimilarity matrix and bootstrap analysis was performed for node construction, using 10000 bootstrap values.

3.2.3 Genotyping

DNA extraction, purification and quantification

The DNA from 297 genotypes was extracted from the young leaves of bean plants grown in the Michigan State University (MSU) greenhouse, USA. Genomic DNA was extracted by using hexadecyltrimethyl ammonium bromide and following the method described by Rogers and Bendich (1985). The concentration of the extracted DNA was determined by using a Nanodrop Spectrophotometer (ND-8000; NanoDrop Products, Wilmington, DE). The quality of DNA was observed on a 1% agarose gel in a 0.5x TBE buffer at 70 V for 45 minutes. The DNA of the genotypes was sent for genotyping to the USDA/ARS Soybean Genomics and Improvement Laboratory (Beltsville MD, USA) for genotyping using the Illumina (Illumina Inc., San Diego, CA) BARCBean6K_3 Single Nucleotide Polymorphism (SNP) marker series. About 5,398 SNP markers, distributed across the 11 pairs of the common bean chromosomes were used to scan the genotypes, using the Illumina BeadStation 500G. To aid the identification of nucleotide polymorphism and to determine the position of the variation, compared to a reference sequence, SNP calling was performed by using the Illumina's Genome Analyzer Module V2011.1, as described in Cichy et al. (2015).

Genetic diversity analysis

Data filtering procedures were performed in order to filter high quality SNPs. Monomorphic SNPs and SNPs with minor allele frequency of less than 2% were filtered out and only 47% (2554) SNPs were used for diversity analysis. Genetic diversity parameters, such as Shannon diversity index (I), gene diversity (H_e), heterozygosity (H_o), polymorphic information content (PIC) and inbreeding coefficient (F_{IS}), were calculated, using GenAlEx Version 6.5 (Peakall and Smouse, 2012). An analysis of molecular variance (AMOVA) was performed with 100 permutations, using the same software. Genetic relationships within and among the genotypes were assayed with a neighbour-joining algorithm, using the unweighted pair group method (UWPGM) in DARwin 6.0 software (Perrier and Jacquemoud-Collet, 2006). Pairwise dissimilarity matrices were obtained from the Dice coefficient and a dendrogram was generated. For the node construction, a bootstrap analysis was performed, based on 10000 bootstrap values, using DARwin. The distinctiveness of the clusters were checked, using the cophenetic correlation coefficient. The grouping of the population was done by using a morphological marker, hundred seed weight (HSW), as described by Singh et al. (1991a). Consequently, the genotypes were grouped as small-seeded (< 25 g/100 seed), medium-seeded (25-40 g/100 seed) or large-seeded (> 40 g/100 seed). The other grouping was based on geographic origin and breeding status as landraces, varieties, breeding and resistant lines.

3.3 Results

3.3.1 Phenotypic diversity

The Shannon-Weaver diversity index was used to compare the phenotypic diversity among characters, collection sites and breeding status. The estimates were done for each trait and pooled across characters, sites and breeding status (Table 3.1). The phenotypic traits in this study were highly polymorphic and the H' values for each trait ranged from 0.87 to 0.96, with a mean diversity index of 0.92 for all the genotypes across all sites and breeding status. Traits, such as the number of holes per seed (0.96), percentage adult emergence (0.95), second progeny percentage damage (0.94) and index of susceptibility (0.94) were highly polymorphic. Seed density had the lowest diversity index, followed by the percentage seed coat weight and the number of eggs. The average diversity index (Hcl) of the traits pooled across the different populations, based on their breeding status showed variations ranging from 0.88 to 0.98, with a mean of 0.94. The lowest and the highest average diversity index (Hcl) were scored for seed density and second progeny percentage damage, respectively. All insect- related traits, except for the number of eggs, exhibited a $\geq$ 0.95 average diversity index. Most of the seed related traits showed relatively low diversity for all the genotypes across the breeding status. The proportion of diversity within breeding status was significantly high; however, all traits revealed no significant variation among the different populations, based on breeding status. The proportion of variation within the population groupings was more prominent than that between the population variation.

The population diversity values ranged from 0.88–0.99, 0.89–99, 0.86–0.90 and 0.84–0.98 for landraces, breeding lines, resistant lines and varieties, respectively. The diversity values of the resistant lines and the varieties was generally smaller than the landraces and the breeding materials. No significant variation was observed among landraces, breeding lines and varieties for all the traits, except the hundred seed weight and percent seed coat weight. Breeding lines showed a significantly high variation for the hundred seed weight and varieties revealed a significantly low variation for the seed coat weight. However, the resistant lines were the least diversified of all the traits (Table 3.1).

3.3.2 Principal component and discriminant analysis

Table 3.2 presents the principal components and percentage contribution of each component to the total variation of the common bean genotypes. Eigenvalues are often used to determine the number of major components that significantly explained the total variation. The first four principal components, with eigenvalues greater or equal to one explained 81.94% of the total variation among the studied genotypes for 12 insect and seed-related characters. The first

principal component (PC1) retained 49.96% of the total variation among 297 common bean genotypes. PC1 had a significant positive association with the number of adults emerged (0.39), the index of susceptibility (0.39) and the number of holes (0.38). The additional three components explained 13.08%, 11.22% and 7.68% of the total variation, respectively. PC2 and PC4 had a highly significant positive association with the hundred seed weight (0.61) and seed density (0.74), respectively. PC3, on the other hand, was due to the contrast between the number of eggs (-0.50) and the percentage of adult emergence (0.45). The discrimination of the genotypes and their grouping into different clusters was dictated by the cumulative effects of a number of characters.

Table 3.1 Estimates of Shannon-Weaver diversity index (H') for twelve phenotypic characters related to bruchid infestation response among 297 common bean genotypes

Trait[†]	Diversity within collection site					Diversity with breeding status				Overall diversity			
	AMH	BGM	ORO	SNNP	UNK	LD	BL	RL	VR	H'	H_{cl}	H_{cl}/H'	$(H'-H_{cl})/H'$
NE	0.86	0.89	0.91	0.92	0.93	0.89	0.91	0.89	0.87	0.89	0.90	1.04	-0.01
NAE	0.95	0.98	0.97	0.97	0.97	0.96	0.97	0.91	0.96	0.93	0.96	1.00	-0.03
PAE	0.99	1.00	1.00	0.99	0.99	0.96	0.97	0.89	0.97	0.95	0.97	1.01	-0.03
IS	0.98	0.99	0.98	0.99	0.98	0.98	0.97	0.90	0.98	0.94	0.97	0.99	-0.03
NH	0.95	0.99	0.97	0.97	0.98	0.96	0.96	0.91	0.96	0.93	0.96	1.00	-0.03
NHPS	0.98	0.97	0.97	0.97	0.97	0.97	0.97	0.90	0.98	0.96	0.97	0.99	-0.01
PD1	0.95	0.97	0.96	0.98	0.97	0.96	0.96	0.88	0.97	0.93	0.95	0.99	-0.03
PD2	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.91	0.98	0.94	0.98	1.00	-0.04
SWL	0.96	0.96	0.94	0.97	0.95	0.95	0.95	0.92	0.96	0.92	0.95	0.99	-0.04
SCW	0.91	0.94	0.92	0.93	0.85	0.91	0.90	0.89	0.84	0.89	0.90	1.06	0.00
SD	0.85	0.92	0.86	0.93	0.90	0.88	0.89	0.86	0.86	0.87	0.88	1.03	-0.01
HSW	0.95	0.95	0.90	0.95	0.89	0.93	0.97	0.92	0.90	0.91	0.93	1.04	-0.02
Overall	0.94	0.96	0.95	0.96	0.95	0.94	0.95	0.90	0.94	0.92	0.94	1.00	-0.01

H' = diversity index; H_{cl} = average diversity index of each character for the population within the populations based on breeding status; H_{cl}/H' = proportion of diversity within breeding status; $(H'-H_{cl})/H'$ = proportion of diversity between breeding status; NE = number of eggs; NAE = number of adult emerged; PAE = percent adult emergence; IS = index of susceptibility; NH = number of holes; NHPS = number of holes per seed; PD1 (%) = first progeny damage; PD2 (%) = second progeny damage; SWL (%) = seed weight loss; SCW (%) = seed coat weight percentage; SD = seed density; HSW = hundred seed weight; AMH = Amhara; BGM = Benshangul-Gumuz; ORO = Oromiya; SNNPR = South Nations, Nationalities and People; UNK = Unknown origin; BL = breeding lines; LD = landraces; RL = resistant lines; VR = Varieties

In this study, three discriminant functions were identified that explain 100% of the variations recorded in the 12 phenotypic characters (Table 3.2). The first discriminate function accounted for 89.8% of the total variation and were found to be correlated well with the second progeny percentage damage and index of susceptibility. Each additional function further explained the 10.2% and 0.80%, respectively. The second and third functions were found to be well

correlated, with hundred seed weight, first progeny percentage damage, the number of adults emerged and the number of holes per seed.

Table 3.2 Principal component (PC) and discriminant function (DA) scores, eigenvalues and the contribution of component axes and functions to variation in common bean genotypes

Character	Principal component				Discriminant function		
	PC1	PC2	PC3	PC4	DA1	DA2	DA3
Number of eggs	0.28	0.27	-0.50	-0.08	-0.57	-0.19	0.04
Number of adult emerged	0.39	0.07	-0.21	-0.02	0.19	0.49	0.08
Percent adult emergence	0.29	-0.30	0.45	0.08	0.28	-0.74	0.52
Index of susceptibility	0.39	-0.11	0.10	0.03	0.37	0.28	0.75
Number of holes	0.38	0.08	-0.21	-0.03	0.23	-0.79	-0.41
Number of holes per seed	0.20	0.24	0.36	0.13	0.01	0.47	-0.80
1st progeny percent damage	0.34	0.31	-0.01	-0.08	0.17	0.60	-0.48
2nd progeny percent damage	0.33	-0.22	0.34	0.06	1.50	0.84	-0.21
Percent seed weight loss	0.34	-0.03	0.01	0.02	-0.13	-0.20	0.22
Proportion of seed coat weight	0.08	-0.39	-0.16	-0.64	0.17	-0.23	0.09
Seed density	0.05	-0.31	-0.35	0.74	-0.04	0.32	0.41
Hundred seed weight	-0.05	0.61	0.22	0.05	-0.49	0.87	0.49
Eigenvalues	6.00	1.57	1.35	0.92	2.82	0.30	0.03
% total variance	49.96	13.08	11.22	7.68	89.80	9.40	0.80
% cumulative variance	49.96	63.04	74.26	81.94	89.80	99.20	100.00

3.3.3 Genetic characterization using SNPs

From the initial 5,398 BARCBean6K_3 SNP markers, only 2554 SNP loci, with no missing values and minor allele frequency of >2% were used in the current genetic diversity assessment study. The distribution and genetic diversity parameter estimates of the 2554 SNPs used in this study are presented in Table 3.3. The number of SNPs on each chromosome ranged from 141 on Pv09 to 325 on Pv05, with a mean of 232 SNPs per chromosome. SNPs located on Pv06 had relatively large polymorphic loci (64%), followed by SNPs on Pv11, 5 and 8, with 58%, 57% and 55% of polymorphic loci, respectively. The observed heterozygosity of the SNP loci ranged from 6% to 11%. The PIC values varied from 0.21 to 0.38, with a mean of 0.34. Approximately 89% of the markers used in this study had PIC values exceeding 0.30, demonstrating the high discriminatory power of the markers. The gene diversity values ranged from 0.24 to 0.50, with a mean gene diversity of 0.44; however, the majority of the values (76%) fell between 0.40 and 0.50. However, no significant differences were observed in PIC and gene diversity values among the eleven chromosomes.

Table 3.3 Distribution and genetic diversity parameters of 2554 SNPs measured in a set of 297 common bean genotypes

Chromosome No.	No SNPs used	Polymorphic SNPs	% P	Ho	He	F_{IS}	PIC
Pv01	492	243	49.39	0.08	0.45	0.81	0.35
Pv02	573	310	54.10	0.06	0.45	0.87	0.33
Pv03	352	145	41.19	0.09	0.43	0.81	0.34
Pv04	522	206	39.46	0.08	0.43	0.82	0.34
Pv05	567	325	57.32	0.09	0.44	0.79	0.34
Pv06	300	193	64.33	0.07	0.45	0.85	0.35
Pv07	439	183	41.69	0.08	0.44	0.82	0.34
Pv08	566	312	55.12	0.08	0.45	0.81	0.35
Pv09	408	141	34.56	0.07	0.44	0.85	0.34
Pv10	440	179	40.68	0.11	0.43	0.74	0.34
Pv11	544	318	58.46	0.11	0.44	0.74	0.35
Mean	473	232	48.76	0.08	0.44	0.81	0.34
SE	28.01	21.72	2.923	0.005	0.002	0.012	0.002

%P = percentage polymorphic markers; H_o = Observed heterozygosity; He= gene diversity; F_{IS}= inbreeding coefficient; PIC= polymorphic information content

3.3.4 Genotypic diversity within and among population groups

The 297 common bean genotypes were categorized into 3, 4 and 5 populations, based on seed size, breeding status and geographical origin, respectively. The genetic parameter estimates of the populations, based on seed size, breeding status and geographic origin are presented in Table 3.4. Large-seeded genotypes (HSW > 41gm) had a significantly low Shannon diversity index (I = 0.50) and gene diversity (H_e = 0.34), compared to medium and small-seeded genotypes. However, medium and small-seeded genotypes revealed a relatively low inbreeding coefficient, suggesting that 19% and 21% of the alleles were not fixed. Gene diversity was much higher in medium-seeded genotypes (H_e = 0.44), followed by small-seeded genotypes (H_e = 0.41). In addition, the large-seed genotypes were found to be monomorphic for 19 SNPs, while the small-seeded genotypes had unique alleles in 50% of the loci. A relatively highly differentiated population was observed when the genotypes were classified, based on their breeding status. Resistant lines revealed significantly high values for most of the genetic diversity parameters, whereas breeding lines gave the lowest values. The resistant lines had unique alleles in 24% of the loci, whereas the breeding lines had 52 missing alleles. The varieties and landraces had similar values for all the genetic parameters. A comparison with landraces, based on the geographic origin revealed a wide range of diversity among the populations. Landraces collected from Amhara and Oromiya had a significantly

higher Shannon diversity index of 0.61 and 0.60, respectively. Similarly, the highest gene diversity values were recorded from landraces collected from the Amhara and Oromiya regions. The landraces with unknown origin were relatively more diverse than those landraces collected from the South Nations, Nationalities and People (SNNP) and Benshangul-Gumuz regions. The overall mean observed heterozygosity was 0.08 and all the genotypes showed very low values (H_o < 0.1), suggesting that more than 90% of the loci were homozygous. Similarly, the overall mean inbreeding coefficient (F_{IS}) was 0.81, suggesting that 81% of the loci were fixed.

Table 3.4 Genetic parameter estimates based on 2554 SNPs among common bean populations

	Genetic parameter				
Population	N	I	H_o	H_e	F_{IS}
Seed size					
Large > 41gm	26	0.50	0.05	0.34	0.84
Medium 25 - 41gm	71	0.62	0.08	0.44	0.81
Small < 25gm	200	0.61	0.09	0.41	0.79
Mean		0.58	0.07	0.40	0.82
Breeding status					
Breeding line	27	0.54	0.09	0.37	0.79
Landraces	202	0.60	0.09	0.42	0.80
Resistant lines	35	0.67	0.07	0.45	0.83
Variety	33	0.60	0.08	0.42	0.79
Mean		0.60	0.08	0.41	0.81
Geographic origin					
Amhara	68	0.61	0.09	0.421	0.78
B. Gumuz	13	0.47	0.08	0.332	0.78
Oromiya	54	0.60	0.08	0.421	0.80
SNNP	49	0.50	0.08	0.331	0.80
Unknown origin	18	0.54	0.09	0.374	0.79
Mean		0.54	0.08	0.376	0.79
Overall mean		0.64	0.08	0.442	0.81
SE		0.001	0.004	0.001	0.009

N = number of genotypes within population; I = Shannon diversity index; H_e = gene diversity; H_o = observed heterozygosity; PIC = polymorphic information content; F_{IS} = inbreeding coefficient; B. Gumuz = Benshangul-Gumuz

3.3.5 Analysis of molecular variance (AMOVA)

Breeders commonly use breeding status and geographic origin to classify their breeding populations. In addition, the seed size is another important criterion used by breeders in common been. In this study, the three classification criteria were used to analyze genetic variation among and within the population, using AMOVA. Table 3.5 presents the analysis of molecular variance among common bean populations, based on their seed size, breeding

status and geographic origin. From the analysis, in all the classifications the highest variance component (71 - 73%) was observed among individuals within the population. The variance component computed among different populations, based on their seed size, breeding status and geographic origin were 12%, 10% and 7%, respectively. Wright's F-statistic was used to determine the deviation of the Hardy-Weinberg expectation within the population. Highly significant values were observed for all the population parameters, such as the fixation index or inbreeding coefficient (F_{IS}), the overall fixation index (F_{IT}) and the genetic differentiation (F_{ST}). The F_{IS} values for all the SNP loci were 0.805, 0.806 and 0.780, respectively, while the F_{IT} values were 0.828, 0.826 and 0.804, respectively, across the population, based on their seed size, breeding status and geographic origin (data not shown). The pairwise F_{ST} values showed a significant differentiation, ranging from 0.201 to 0.581, 0.178 to 0.578 and 0.117 to 0.602 among the sub-populations, based on their seed size, breeding status and geographic origin, respectively (data not shown).

Table 3.5 Analysis of molecular variance among and within common bean populations

Source of variation	df	SS	MS	Est. Var.	Perc. Var	F-Statistics
Seed Size						
Among populations	2	22284	11142	71.11	12	F_{ST} = 0.001
Among individuals	294	284491	968	431.62	71	F_{IS} = 0.001
Within individuals	297	31015	104	104.43	17	F_{IT} = 0.001
Total	593	337789	-	607.15	100	-
Breeding status						
Among populations	3	21412	7137	61.89	10	F_{ST} = 0.001
Among individuals	293	285373	974	434.77	72	F_{IS} = 0.001
Within individuals	297	31015	104	104.43	17	F_{IT} = 0.001
Total	593	337800	-	601.09	100	-
Geographic origin						
Among populations	4	15210	3802	38.48	7	F_{ST} = 0.001
Among individuals	197	179183	910	401.29	73	F_{IS} = 0.001
Within individuals	202	21609	107	106.97	20	F_{IT} = 0.001
Total	403	216002	-	546.75	100	-

df = degrees of freedom; SS = sum of squares; Est. var. = estimated variance, Perc. Var = percentage variance; SNNP = South Nation, Nationality and People; B. Gumuz = Benishangul Gumuz, F_{ST} = genetic differentiation, F_{IS} = fixation index or inbreeding coefficient and F_{IT} = Overall fixation index

3.3.6 Cluster analysis

The genetic distance measured among the genotypes, using phenotypic and SNP markers and, based on Jaccards genetic distance matrix revealed large genetic variations among the genotypes. Using SNP markers, the highest genetic distance between the genotypes was 0.82 and the lowest was 0.19, while the mean was 0.62 (data not shown). The highest genetic distance (0.82) was found between genotypes 215719, Beshbesh and Gofta, SCR-35. The

lowest genetic distance (0.19) was found between genotypes 228522, 211362 and 213198, 214662. The genetic distance values that were estimated, using phenotypic means ranged from 0.0 to 1.00, with a mean of 0.64. The highest phenotypic distance (1.00) was found among 3% of the genotypes, while the lowest distance (0.0) was found among 25% of the genotypes. This demonstrates that most of the genotypes were distantly related.

The discriminatory power of the SNP and phenotypic markers was also examined, using cluster analysis and by constructing phylogenetic trees on the basis of seed size and breeding status. The phylogenetic tree that revealed the genetic relationship among 297 common bean genotypes, based on 2554 SNP and 12 phenotypic markers is presented in Figure 3.1. The hierarchical cluster analysis conducted on the means of twelve phenotypic traits resulted in two major distinct clusters and three sub-clusters (Figure 3.1 A and B). The clustering of genotypes on the basis of seed size revealed a weak differentiation, while clustering based on breeding status displayed a relatively strong differentiation. In the latter case, the most susceptible genotypes were grouped in Cluster I, of which the majority were landraces, released genotypes and some breeding lines.

The phylogenetic tree that revealed the genetic relationship among the common bean genotypes, based on SNP markers showed a similar clustering pattern (Figure 3.1 C and D). The SNP markers were more effective in discriminating the genotypes into distinct clusters than the phenotypic markers. The cluster analyses revealed the presence of two distinct major clusters and three sub-clusters. The distinctiveness of the clusters was confirmed by the high cophenetic correlation coefficient for SNPs (r = 0.98) and for phenotypic markers (r = 092). In Cluster I, the first sub-cluster mainly consisted of small-seeded genotypes, while the other two sub-clusters contained both small and medium-seeded genotypes (Figure 3.1 C). In Cluster II, the large-seeded genotypes (red lines) uniquely separated from the medium and small-seeded genotypes. Based on their breeding status (Figure 3.1 D), the clustering patterns of the genotypes revealed that the breeding and resistant lines were clearly isolated. However, there was a moderate differentiation between varieties and landraces. Most of the landraces were small-seeded, suggesting that small-seeded genotypes are the most preferred ones in Ethiopia. The Mantel correlation test revealed a weak, but positive association between the Jaccard's genetic distance matrix of phenotypic and the SNP markers (r = 0.03, P = 0.07).

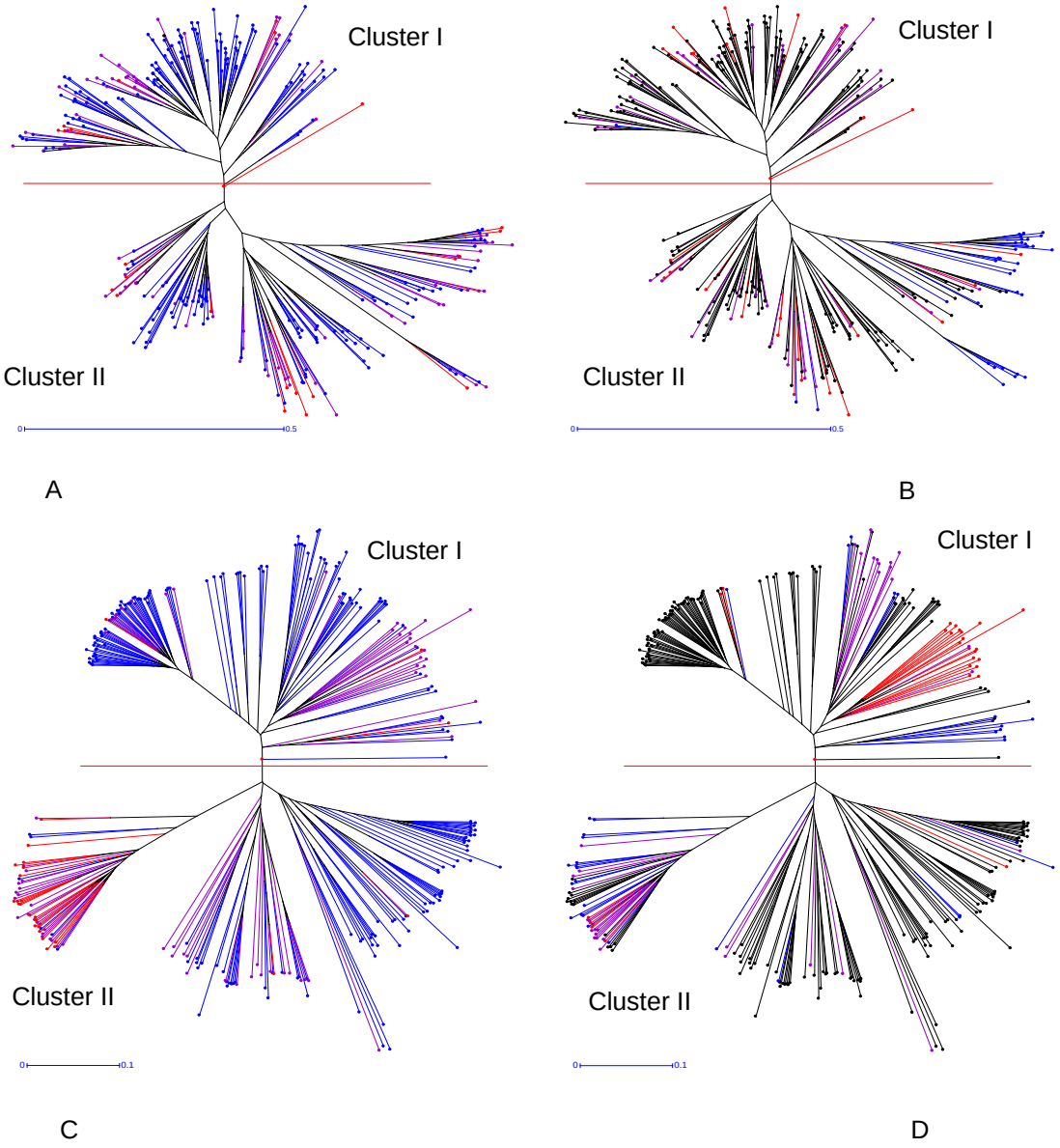

Figure 3.1 Neighbor-joining dendograms based on UPGMA genetic dissimilarity depicting genetic relationship between 297 common bean genotypes: A and B. Classification based on phenotypic traits; A = based on seed size and B = based on breeding status; C and D Classification based on SNP markers; C = based on seed size and D = based on breeding status. In A and C, Red lines large seeded, Purple – Medium seeded, Blue – small seeded genotypes. In C and D Red lines represent breeding line, Blue – resistant lines, Purple– variety; Black- landraces

3.4 Discussion

The present study assessed the genetic diversity of 297 common bean genotypes, using phenotypic and SNP markers. The Shannon-Weaver index (H') was measured for 12 phenotypic characters, related to their response to bruchid infestation and the genotypes displayed a wide range of variability for all the traits studied. In general, the mean diversity index (H') was 0.92 for all the phenotypic traits measured in all the genotypes, suggesting that all the traits were highly polymorphic. This was by far higher than the 0.56 mean diversity index measured by Okii et al. (2014b) on 284 Ugandan common bean accessions, using 22 morphological traits.

The diversity indices differed between their geographic origin and breeding status, and all the populations showed a high diversity for all the traits. The genotypes collected from the Oromiya and Benshangul-Gumuz regions revealed a relatively high level of variation for percentage adult emergence. Although the highest number of genotypes was represented by the Amhara (68) and Oromiya (54) regions, respectively, the genotypes displayed a relatively low level of diversity. The genotypes collected from the Benshangul-Gumuz (13) and those genotypes with unknown origin (18), on the other hand, revealed the highest diversity for the majority of insect related traits. As explained by Magurran (2004), the Shannon-Weaver index is highly correlated, with evenness (the number of genotypes per population) and/or number of unique genotypes represented within the population. All four population classifications, based on their breeding status, consistently showed a low level of diversity for the number of eggs, seed density and seed coat weight. The continued selection of these genotypes by breeders will result in a narrowing of genetic diversity and a reduced allelic richness when, compared to landraces and varieties.

The principal component analysis was performed to assess the relative importance of each trait for explaining the genetic diversity and the population structure present in the studied genotypes. The first four PCs, with eigenvalues greater than or equal to one were found to be significant in explaining the prevailing genetic diversity. The first PC, which explained about 50% of the total variation, was the cumulative effect of a number insect-related characters, such as the number of adults emerged, the index of susceptibility and the number of holes. The current results coincide with the results of Keneni et al. (2011), which demonstrated that the number of adults emerged, percentage adult emergence, number of holes, the index of susceptibility , the percentage seed weight loss and the percentage damage played a vital role in the delineation of the diversity into different groups. These critical traits were reported as an important parameter for bruchid resistance screening by several researchers (Reddenab and Mcguirea, 1983; Schoonhoven et al., 1983; Cardona, 2004). The contribution of each trait to

the principal components ranged from low to average, indicating that the grouping of the genotypes into different clusters was dictated by the additive effects of the number of characters. According to Chahal and Gosal. (2002), characters with larger absolute values closer to unity, within the first principal component analysis influence the clustering pattern more than those with lower absolute values, closer to zero. Similarly, the discriminant analysis revealed that about 90% of the total variation resulted from the variation in second generation percentage damage. This implies that this trait is vital in discriminating the genotypes, and it suggests that the selection efforts, based on this trait may be more effective in reducing bean bruchid infestation and the subsequent seed damage.

More than 76% of the genetic diversity values were between 0.40 and 0.50, indicating the presence of a high level of genetic diversity among the tested genotypes. Among the many phenotypic traits used for the classification of genotypes into Andean and Mesoamerican gene-pools, the seed shape and size are the most, commonly used traits (Singh et al., 1991a). In the current study, the 297 common bean genotypes were classified into three populations, based on the seed size. Large-seeded (Andean) genotypes were strongly differentiated from small (Mesoamerican) and medium-seeded genotypes. The genetic isolation or strong differentiation between the two gene-pools may be the result of reproductive barriers that cause difficulties in crossing between the two gene-pools (Acosta-Gallegos et al., 2007). These results also consistent with other genetic diversity studies (Asfaw et al., 2009; Blair et al., 2009; Kwak and Gepts, 2009; Corte´s et al., 2011; Fuente et al., 2013; Cichy et al., 2015). The weak differentiation between the small and medium-seed genotypes may be explained by the presence of significant inter gene-pool introgressions in the common bean, as reported by several other authors (Asfaw et al., 2009; Kwak and Gepts, 2009; Blair et al., 2010).

The high Shannon diversity index and gene diversity in medium and small-seeded genotypes was partly due to the presence of relatively equal allele frequencies and the alleles were evenly distributed. It may also be due to the large population size in Mesoamerican (200) genotypes, compared to the 26 Andean genotypes. This result is in line with several other studies (Beebe et al., 2000, 2001; Papa and Gepts, 2003; McClean et al., 2012; Fuente et al., 2013; Okii et al., 2014a; Cichy et al., 2015; Rodriguez et al., 2016). Unlike the diversity in phenotypic traits, the genotypes collected from Amhara and Oromiya revealed a high genetic diversity, compared to genotypes collected from other regions. Fisseha et al. (2016) also reported the presence of high genetic diversity in the Amhara and SSNP regions. This might be due to the high germplasm flow, as a result of importing seeds from abroad through common bean importers and/or the informal cross border seed exchange in Amhara, from Sudan, and in Oromiya, from Kenya.

Using both phenotypic and SNP markers, two distinct clusters of genotypes, with a cophenetic factor r > 0.90 were observed. These distinct clusters may have resulted from the breeders' and farmers' selection for adaptation to specific production areas, cooking values and market preference. The clustering of the genotypes, based on phenotypic traits and SNP markers showed some discrepancies in terms of the type and number of genotypes. SNP markers were better in clustering the genotypes according to seed size and breeding status. Between the two clusters, it is assumed that there is maximum homogeneity within clusters and maximum heterogeneity between clusters, in terms of the traits under consideration (Singh and Chaudhary, 1985; Hair et al., 1995). The clustering of most landraces, released genotypes and some breeding lines in the same cluster, may have resulted from their similar susceptible reaction to bean bruchid. Earlier studies reported that almost all the cultivated common bean varieties and landraces lack resistance to *Z. subfasciatus* (Schoonhoven and Cardona, 1982).

An analysis of molecular variance (AMOVA) among common bean populations, based on gene-pool (seed size), breeding status and geographic origin revealed that the highest variation (> 70%) was attributable to among individuals variation with populations, followed by the within individuals variation. The present result concurs with the reports of other scientists (Blair et al., 2009; Fisseha et al., 2016). The clustering of genotypes, based on both SNP and phenotypic markers did not show any association with geographic origin. Madakbaş and Ergin (2011) and Boros et al. (2014) also reported the lack of association between agro-morphological clustering and the geographic origin of the common bean in Turkey and Poland. This suggests that geographic origin may not always be a sufficient predictor for sub-dividing into to population into sub-populations (McClean et al., 2004; McClean and Lee, 2007; Keneni et al., 2011). Moreover, in countries like Ethiopia, where informal seed exchange is a vital part of the seed system, the geographic differentiation of genotypes could be very weak and the geographic association may be less clear (McClean et al., 2012). The examination of hierarchal partitioning showed that genetic variations, based on seed size demonstrated the highest genetic variation, followed by breeding status. The highly significant values observed among all the population parameters, such as F_{IS}, F_{IT} and $F_{ST,,}$ validate the fact that all the sub-population that were generated, based on the three classifications criteria were unique and the genotypes were isolated, to a certain extent.

Although there was a weak, but positive correlation (r = 0.03, P = 0.07) observed between the genetic and phenotypic distances, the phenotypic and genotypic data were effective in discriminating the genotypes. The week correlation may be due to the fact that phenotypic distances, based on few insect and seed-related traits due to the overall lack of genetic variability for these traits, compared to the genome-wide SNP markers. The positive

correlation, on the other hand, implies that phenotypic traits can be used as a useful tool in assessing genetic diversity. Generally, the use of two or more different methods helps to understand the genetic diversity, and the relationships within and among genotypes of crop species.

3.5 Conclusion

The present study demonstrated the presence of a broad genetic diversity in common bean genotypes in Ethiopia. The traits under consideration revealed a wide range of genetic diversity among common bean genotypes, thus these traits are recommended for use in the characterization and breeding of the common bean for bruchid resistance. In this study, the use of both SNP and phenotypic markers revealed the existence of a substantial genetic diversity in Ethiopian common bean germplasm. The cluster analyses, based on SNP markers confirmed the presence of genotypes from both the Mesoamerican and Andean gene pools. Based on the diversity analysis, 144 genotypes were selected as parental genotypes and further field performance evaluation was conducted. This information can be used in future common bean breeding and conservation activities in Ethiopia.

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CHAPTER 4

Population Structure and Genome-Wide Association Analysis of Bruchid Resistance in Ethiopian Common Bean (*Phaseolus Vulgaris* L.) Genotypes

Abstract

The common bean (*Phaseolus vulgaris*) is among the most important grain legume crops in Africa. However, the common bean grain is heavily damaged by the Mexican bean weevil (*Zabrotes subfasciatus* Boheman). This study was conducted to determine the population structure and genome-wide marker-trait association of bruchid resistance in the common bean. The phenotypic diversity and population structure of 297 genotypes were analyzed, using the Illumina BARCBean6K_3 SNP BeadChip. The genotypes consisted of landraces, released varieties, breeding lines and Mexican bean weevil resistant lines. A population structure analysis, based on Bayesian genotyping clustering approach classified the 297 genotypes into two subpopulations, namely, Middle American and Andean. Similar population patterns were also observed by using the principal coordinate analysis (PCoA). The genome-wide association study (GWAS) analysis identified 24 single- nucleotide polymorphism (SNPs) on nine chromosomes, with a significant ($P < 0.05$) association with the percentages of adult emergence and seed weight loss. The SNPs located on Pv4 and Pv7 were significantly ($P < 0.001$) associated with the two traits. Other significant SNPs were identified on other chromosomes of the common bean, but none of them were above the cutoff point (1.00×10^{-4}). Therefore, further verification of the SNPs that have a significant marker-trait association at $P < 0.05$ will be vital and accessions with these SNPs may be useful as parental materials.

Keywords: Genome wide association, Mexican bean weevil, *Phaseolus vulgaris*, population structure, single-nucleotide polymorphism

4.1 Introduction

The common bean (*Phaseolus vulgaris* L. 2n = 2x = 22) is a self-pollinating annual legume crop with a small diploid genome size of 521.1 Mb (Schmutz et al., 2014). It is among the most important food legumes for direct human consumption and a cheap source of protein, conferring nutritional security to poor people in eastern and southern Africa (Broughton et al., 2003; Katungi et al., 2009). However, in Africa the crop is grown under low-input agriculture systems and the productivity is hampered by different production constraints, the most important of which being their susceptibility to biotic and abiotic stresses (Miklas et al., 2006; Beebe, 2012).

Two major storage insect pests, namely, *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say) are the major common bean production constraints at a global level (Cardona and Kornegay, 1999; Cardona, 2004). The former is known to cause significant qualitative and quantitative losses to stored common bean seeds in Ethiopia (Tadesse et al., 2008) and elsewhere in Africa (Nchimbi and Misangu, 2002). Losses of 60%, after three to six months of grain storage, have been reported in Ethiopia (Getu et al., 2003), because proper control measures have not been put in place. Farmers have been using different management options to minimize the losses, including the use of multiple cultural practices and synthetic insecticides. However, the use of chemical insecticides has recently become more common in most parts of Ethiopia (Tadesse et al., 2008). The use of host plant resistance provides a better option for managing storage bruchids in the common bean, as chemicals have many negative side effects relating to sustainability, environmental pollution and food safety.

More effective breeding for bruchid resistance is possible with a better knowledge of the magnitude and pattern of genetic diversity in the source genetic material. With the current advances in molecular markers, a number of studies have been conducted to assess genetic diversity, population structure and genome wide association in the common bean. A wide range of molecular markers, including Restriction Fragment Length Polymorphism (RFLP) (Velasquez and Gepts, 1994; Freyre et al., 1998), Random Amplified Polymorphic DNA (RAPD) (Beebe et al., 2000; Dura´n et al., 2005), Inter Simple Sequence Repeat (ISSR) (González et al., 2005; Dagnew et al., 2014), Amplified Fragment Length Polymorphism (AFLP) (Beebe et al., 2001; Papa and Gepts, 2003), Simple Sequence Repeats (SSR) (Blair et al., 2007, 2009, 2010a; Asfaw et al., 2009; Kwak and Gepts, 2009; Burle et al., 2010; McClean et al., 2012; Fuente et al., 2013; Mercati et al., 2013; Okii et al., 2014) and Single-Nucleotide Polymorphism (SNP) (Corte´s et al., 2011; Blair et al., 2013; Goretti et al., 2013; Cichy et al., 2015a; Rodriguez et al., 2016), have been used in common bean.

As a high-throughput and cost-effective technology, SNP markers have become the marker of choice for map construction, genetic diversity analysis, genome wide marker–trait association and marker-assisted selection (Rafalski, 2010; Corte´s et al., 2011), because of their high level of polymorphism, wide genome coverage, locus specificity, better reproducibility and also have a fixed physical position on the chromosome (Yan et al., 2010). As a result, it has become increasingly possible to obtain genome-wide sequence data for any crop species, including the common bean (Schmutz et al., 2014). The sequence data are often used for the development of high-throughput and efficient genotyping platforms, such as BARCBean6K_3 BeadChip with a large number of SNP markers (about 6000) (Song et al., 2015). The availability and accessibility of this platform gives an opportunity to conduct a genome wide association study for different traits in common beans. Unlike QTL mapping, GWAS uses an association panel which exploits all of the recombination events and provides higher map resolution, because it has smaller linkage disequilibrium (LD) blocks than bi-parental population mapping (Nordborg and Weigel, 2008; Myles et al., 2009).

More recently, different genome wide association studies have been conducted for the identification of genomic regions associated with different traits, including agronomic performance (Kamfwa et al., 2015a; Moghaddam et al., 2016), cooking time (Cichy et al., 2015b), drought tolerance (Hoyos-villegas et al., 2017), symbiotic nitrogen fixation (Kamfwa et al., 2015b), anthracnose and angular leaf spot resistance (Morini et al., 2016; Zuiderveen et al., 2016) in the common bean, but limited attention has been given to bruchid resistance. Bean bruchid (*Zabrotes subfasciatus*) resistance was mapped on a gene cluster on linkage group B4 for APA (arcelin, phytohemagglutininand α-amylase inhibitor) locus, in bi-parental populations (Osborn et al., 1986; Blair et al., 2010 b; c). Arcelin is a lectin-like protein found in the cotyledons of the wild common bean and it confers antibiosis resistance to *Z. subfasciatusis* by delaying adult emergence and hampering insect growth and development (Osborn et al., 1988).

Nevertheless, beyond a few studies using ISSR (Dagnew et al., 2014) and SSR (Asfaw et al., 2009), SNP-based population structure and genome-wide association studies have not been conducted with the Ethiopian common bean collections. Currently, researchers from CIAT are developing new SNPs markers linked with the arcelin-based resistance genes to bean bruchid (*Zabrotes subfasciatus*). The objectives of this study were to examine the population structure of common bean genotypes collected from different breeding programs, bean types and geographic regions of Ethiopia and to identify genomic regions associated with bean bruchid resistance, using SNP markers distributed across the genome of the common bean.

4.2 Materials and methods

4.2.1 Plant material

A total of 297 common bean genotypes, consisting of 202 Ethiopian landrace collections and 95 released varieties and breeding lines, were used in this study. The landraces were collected from different eco-geographical regions, including Amhara (68), Oromiya (54), the South Nations, Nationalities and Peoples (SNNP) (49) and Benshangul-Gumuz (13), while 18 genotypes were from unknown origins. The remaining 95 genotypes consisted of 27 breeding lines, 33 released varieties and 35 lines with known resistance to the Mexican bean weevil. The released varieties and breeding lines were obtained from the Melkassa, Sirnka and Areka Agricultural Research Centers and the Haramaya University in Ethiopia. The Mexican bean weevil resistant genotypes were obtained from the International Center for Tropical Agriculture (CIAT) in Uganda, the Pannar Seed Company and the Malawi National Bean Improvement Program. Detailed descriptions of the genotypes are presented in Table 1 of Appendix A and Figure 1 of Appendix A.

4.2.2 Phenotyping and data analysis

The phenotyping of the 297 genotypes was was carried out in the Entomological Research Laboratory at the Melkassa Agricultural Research Center, Ethiopia. Twenty grams of seed was placed in transparent plastic jars (6 cm x 7 cm), with an opening at one end for free air circulation. The experiment was laid out in a randomized complete block design (RCBD), with three replications. Each jar was infested with five pairs (female and male) of newly-emerged bruchids. The insects were kept in the jars for 10 days to allow oviposition, after which the insects were removed and the number of laid eggs were counted. The jars were observed daily, to record bruchid emergence. The removal and counting of emerged adult bruchids were done every other day, starting from the first emergence till the last emergence. The temperatures and relative humidity for the 10 weeks' research period is given in Figure 2.1 of Chapter 2. Data on the number of adults that emerged were recorded and the percentage adult emergence (PAE) was calculated, based on the total number of adults emerged relative to the total number of eggs laid. The percentage seed weight loss (PSWL) was calculated by using the method suggested by Shaheen et al. (2006). Data on PAE and PSWL were used for the association analysis. The data were transformed by arcsine transformation, prior to the Analysis of Variance (ANOVA). The ANOVA was done with the SAS software Version 9.2 (SAS Institute, 2003) and the Tukey's honestly significant difference (HSD) test was used for mean comparison.

4.2.3 High-throughput genotyping

Total genomic DNA of 297 genotypes were extracted from young leaves of bean plants grown in the greenhouse at the Michigan State University (MSU), USA. Genomic DNA was extracted, using hexadecyl trimethyl ammonium bromide and following the method described by Rogers and Bendich (1985). The concentration of DNA was determined by using a Nano Drop Spectrophotometer (ND-8000; Nano Drop Products, Wilmington, DE) and the quality of the DNA was observed on a 1% agarose gel. The DNA samples of the genotypes were sent to the USDA/ARS Soybean Genomics and Improvement Laboratory (Beltsville MD, USA) for genotyping, using the Illumina BARCBean6K_3 SNP marker panel (Illumina Inc., San Diego, CA). A set of 5,398 SNP markers, distributed across the 11 pairs of common bean chromosomes were used. The Illumina BeadStation 500G was used to scan BACBean6K_3 BeadChips. Single-nucleotide polymorphism calling was conducted with the genotyping Module V2011.1 of the GenomeStudio software (Illumina Inc.), as described by Cichy et al. (2015a).

4.2.4 Population structure analysis

The Bayesian model-based clustering approach, using the STRUCTURE 2.3.4 program (Pritchard et al., 2000) was applied to determine the number of gene pools in the genotypes. An admixture model with independent allele frequencies, without prior population information, was used to simulate the population. The STRUCTURE program was set as follows: a burn-in period length of 10,000 and after burn-in 100,000 Markov Chain Monte Carlo (MCMC) repetitions were used. This model assumes that the genome of each individual is a mixture of genes originating from K unknown ancestral populations. For joint inference of the population substructure, K ranging from 1 to 10 was set up, with five independent runs for each K. The most probable value of K for each test was detected by ΔK (Evanno et al., 2005), using the Structure Harvester (Earl and VonHoldt, 2011). CLUMPP v.1.1.2 (Jakobsson and Rosenberg, 2007) was used to find the best alignments of replicate analyses from the structure. Bar plots were generated with average results of runs for the most probable K value, using DISTRUCT v.1.1(Rosenberg, 2003). A principal coordinate analysis (PCoA) was performed, using GenAlEx Version 6.5 (Peakall and Smouse, 2012).

4.2.5 Marker–trait association tests

Single-nucleotide polymorphism genotyping was conducted by using a set of 5398 SNP markers. After filtering monomorphic markers and SNPs with a minor allele frequency of less than 2%, only 2554 (47%) SNPs were retained for the population structure and association analysis. TASSEL Version 5.0 program was used to analyze the principal component analysis (PCA), based on the correlation matrix, kinship and mixed linear model (MLM) (Bradbury et al., 2007). Only four principal components, which together explained 56.5% of the total genotypic variance were used for the association analysis. To avoid cryptic errors, the kinship matrix (Φ) was calculated and included in the association analysis. To determine the marker-trait association, the MLM was conducted according to the methods described by Zhang et al. (2010), using the following formula:

$$y = X\alpha + P\beta + \Phi\mu + e$$

Where: *y* = the phenotype of a given genotype, *X* = the fixed effect of the SNP, *P* = the fixed effect of population structure (from the PCA matrix), *Φ* = the random effect of relative kinship from the kinship matrix and *e* = for residual effects.

The false discovery rate (FDR) (Storey and Tibshirani, 2003) was used for the cut-off of significant SNP markers for each trait, by using bioconductor in R (Gentleman et al., 2004) and markers above a 2.5 LOD score were considered as significant SNPs (Cichy et al., 2015b).

4.3 Results

4.3.1 Phenotypic diversity

Highly significant differences (*P*< 0.01) were recorded among the 297 genotypes for percentage adult emergence and seed weight loss (Table 4.1). The percentage adult emergence ranged from 0 to 100%, with a mean of 74.6%, while the seed weight loss (%) ranged from 0 to 50.3%, with a mean of 23.9%. Landraces exhibited a relatively high percentage of adult emergence and seed weight loss, compared to improved varieties and breeding lines (Table 4.1). Traditionally, researchers have used morphological markers, such as hundred seed weight (HSW) to group the gene pools. Genotypes with a hundred seed weight of <25 g are classified as Mesoamericans, while those with HSW >41 g are grouped as Andeans (Singh et al., 1991). In the present study, genotypes with an HSW of <25 g gave a significantly higher mean percentage of adult emergence and seed weight loss, compared to genotypes with an HSW of >41 g. The percentage of adult emergence and seed weight loss

in the genotypes under the Mesoamerican gene pool ranged from 0 to 100% and 0 to 50.3%, respectively. The range of the two traits in the Andean gene pool was from 0 to 100% and 0 to 47.2%, respectively.

Table 4.1 Means and ranges for percentage adult emergence and seed weight loss among 297 common bean genotypes that are grouped, based on breeding status and seed size after infestation by bean bruchid

Populations	NG	PAE			PSWL		
		Mean	Min	Max	Mean	Min	Max
Breeding status							
Landrace	202	82.8 ± 0.6	52.5	100	27.0 ± 0.8	0.7	50.3
Breeding line	27	77.0 ± 3.7	14.3	100	24.2 ± 2.2	3.3	47.1
Resistant line	35	20.2 ± 4.9	0.0	100	3.9 ± 1.3	0.0	31.9
Variety	33	78.5 ± 2.6	0.59	47.2	26.4 ± 1.8	45.9	100
Seed size							
<25 g (Mesoamerican)	200	77.4 ±1.5	0.0	100.0	24.7± 0.8	0.0	49.12
25 - 40	71	71.2 ±2.9	0.0	100	24.1 ±1.4	0.0	50.3
>40 g (Andean)	26	63.2±5.9	0.0	100.0	17.9 ± 2.6	0.0	47.2
All genotypes	297	74.6 ± 10.6	0.0	100.0	23.9 ± 6.5	0.0	50.3
CV%		17.5	-	-	20.9	-	-
P values		<0.01	-	-	<0.01	-	-

NG = number of genotypes; PAE = percentage adult emergence; PSWL = percentage seed weight loss; CV= Coefficient of variation

Mean ± standard error of the mean

Min and Max represent the minimum and maximum range of the trait

4.3.2 Population structure

The population structure revealed the genetic relationships and aided in genotype selection for breeding of bruchid resistance. The structure analysis, based on the Bayesian approach grouped the 297 genotypes of the common bean into two sub-populations, according to their gene pools as Middle American and Andean at K = 2 (Figure 4.1). The first subpopulation included a total of 243 genotypes from the Middle American gene pool. This subpopulation contained 185 landraces and 58 breeding and resistant lines. The second subpopulation contained only 44 (15%) genotypes from the Andean gene pool, of which 68% were improved varieties, as well as breeding and bruchid resistant lines. Ten genotypes (three landraces and seven breeding and resistant lines) were found to constitute the admixtures of the two gene pools. The further grouping of the population at K = 3 resulted in a separation of the Middle American gene pool into two sub-populations, while the Andean genotypes remained homogenous.

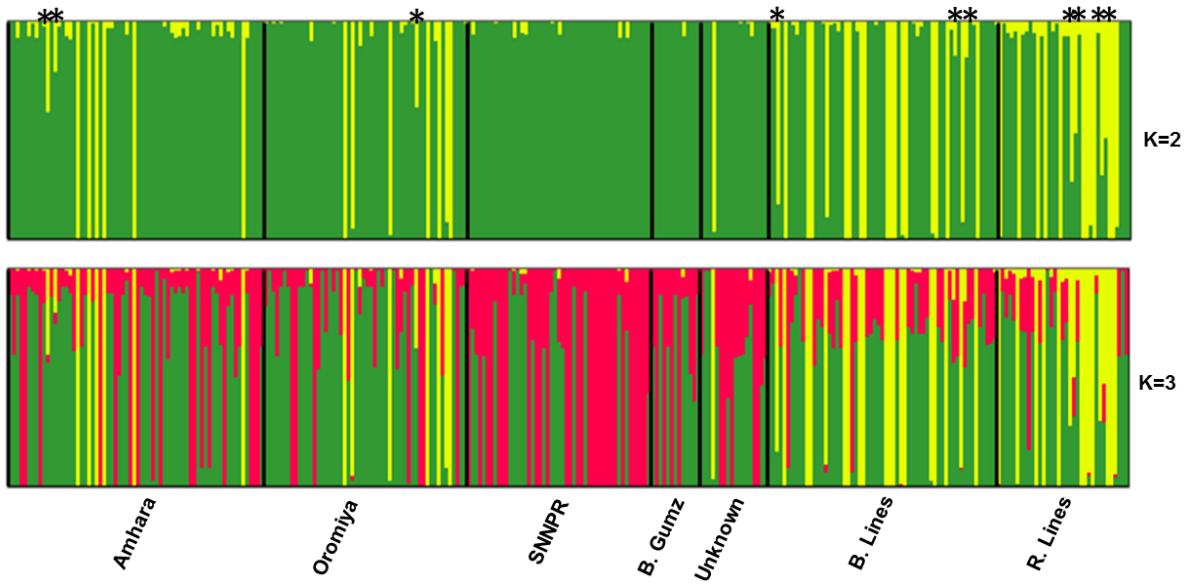

Figure 4.1 Population structure for 297 common bean genotypes. In K = 2 green represent Middle American and yellow represents Andean gene pool. In K = 3 green represents Middle American Group 1, red represents Middle American Group 2 and yellow represents Andean. Admixture genotypes are identified with an asterisk

Similar population structure patterns were also observed in the biplot of the principal component (Figure 4.2). The first two components of the principal coordinates accounted for 52.4% of the total genotypic variation. The pattern distribution of the genotypes over the scatter diagram of the planes of the first two principal components showed a clear correspondence with their classification, based on STRUCTURE output at K = 2, in that the admixture genotypes were positioned between the Andean and Middle American gene pools (Figure 4.2). Similarly, within the Middle American genotypes, the landraces were separated from the other groups i.e. the breeding lines, resistant lines and improved varieties, mainly on the second axis.

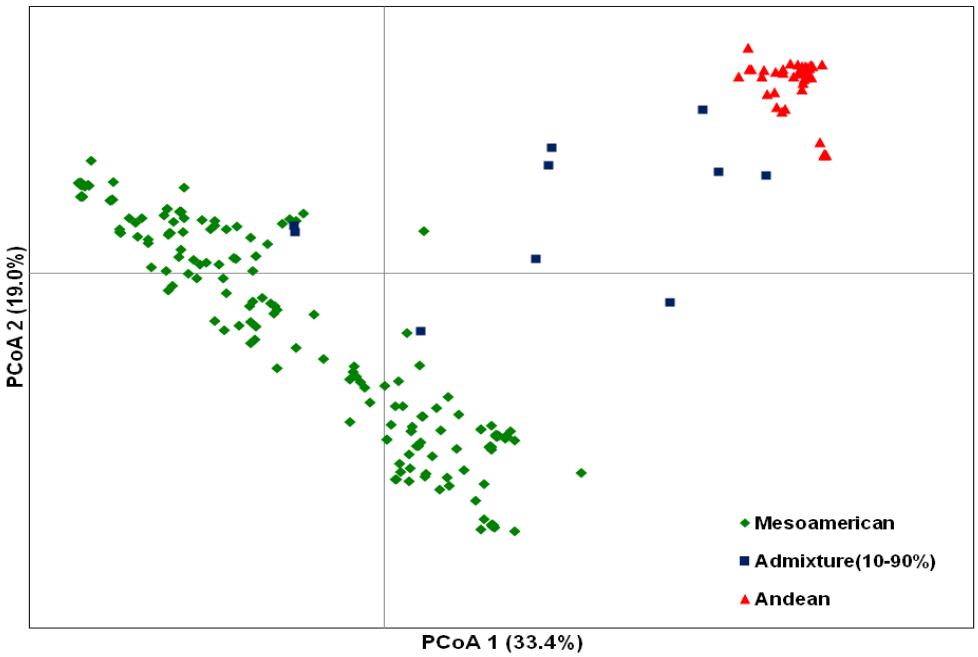

Figure 4.2 Principal coordinates analysis (PCoA) of 297 common bean genotypes, using 2554 SNP markers

4.3.3 Trait-single nucleotide polymorphism association

Percentage adult emergence

A total of 13 SNPs were detected, which were significantly (P < 0.05) associated with the PAE. Although individual SNPs explained less than 10% of the phenotypic variance observed in PAE, the 13 SNPs in total explained about 71% of the total phenotypic variation observed in PAE. The markers that were most significantly (P < 0.001) associated with PAE, were located on Pv04 and Pv07. The list of the most significantly associated SNPs, their chromosome positions, *P*-values and R^2 values, are presented in Table 4.2. SNP markers on Pv04 and Pv07, were significantly associated with the percentage of adult emergence (Figure 4.3A). These two most significantly associated SNPs, on Pv4 ($P = 2.55E^{-05}$) and Pv07 ($P = 5.61E^{-05}$) explained 7.5 and 6.9% of the variation in PAE, respectively. Other significantly associated SNPs for the PAE were detected on the same chromosomes and explained from 5.0 to 6.9% of the variation in PAE. Studying the marker-trait association on genotypes that do not have known resistance genes may lead to the identification of additional SNPs that are highly associated with the trait. Trait-marker association was also conducted, after excluding the resistant lines to see if there were significant marker trait associations in the 262 genotypes. Significant associations were detected between SNPs on Pv02, 09 and 10 and the PAE for all the genotypes, excluding the resistant lines. These significant SNPs explained 3.7 to 5.6% of the phenotypic variation observed in PAE (Table 4.2), but none of them were above the cutoff point (Figure 4.3B).

Percentage seed weight loss (PSWL)

A total of 11 SNPs were identified on Pv01, 04, 05, 06, 07 and 11, with a significant trait association with PSWL (Table 4.3 and Figure 4.4A). The 11 SNPs in total explained about 72% of the total phenotypic variation observed in the PSWL. The most significantly associated SNP (P = 1.75E^{-05}), which accounted for 7.8% of the variation observed in the PSWL, was located on Pv7. Two additional significantly associated SNPs, located on Pv04, explained about 11.3% of the phenotypic variation in the PSWL. Similarly, two more significantly associated SNPs, which explained 5.9 and 5.3% of the phenotypic variation were identified on Pv05. Another marker located on Pv01 explained 5.5% of the variation in the PSWL. The trait-marker association was also computed independently for the 262 genotypes, excluding the 35 introduced lines with pre-known resistance to the Mexican bean weevil. The result led to the identification of two additional significant SNPs that were located on Pv06 and Pv11. Except for the two significant SNPs found on Pv07 and Pv11, which explained 5.6 and 5.5% of the variation in PSWL, respectively, the other SNPs showed low R^2 values (<5%) and they were below the cut-off point (Figure 4.4B).

Table 4.2. Most significant markers, chromosome, position, *P*-value and for percentage adult emergence among common bean genotypes both with and without resistant lines, after infestation by bean bruchid

SNP	Chr.	SNP Position	*P* value	R^2 (%)
All genotypes including the resistant lines				
ss715647352	Pv04	47263067	2.55E-05	7.5
ss715646009	Pv07	37761323	5.61E-05	6.9
ss715646008	Pv07	37781465	5.96E-05	6.9
ss715649179	Pv04	45473345	2.03E-04	6.0
ss715646011	Pv07	37715936	2.27E-04	5.9
ss715646131	Pv04	44676327	3.32E-04	5.6
ss715645814	Pv04	46102860	7.73E-04	5.0
Genotypes excluding the resistant lines				
ss715642732	Pv10	2187490	8.46E-04	5.6
ss715639972	Pv02	28490308	0.00224	3.7
ss715647384	Pv10	44105878	0.00254	4.7
ss715639268	Pv09	35959997	0.00306	4.6
ss715646324	Pv10	40769883	0.00353	4.4
ss715645262	Pv01	49388242	0.00389	4.4

SNP = single nucleotide polymorphic code; Chr = chromosome; R^2 = percentage of phenotypic variation explained by the SNP marker

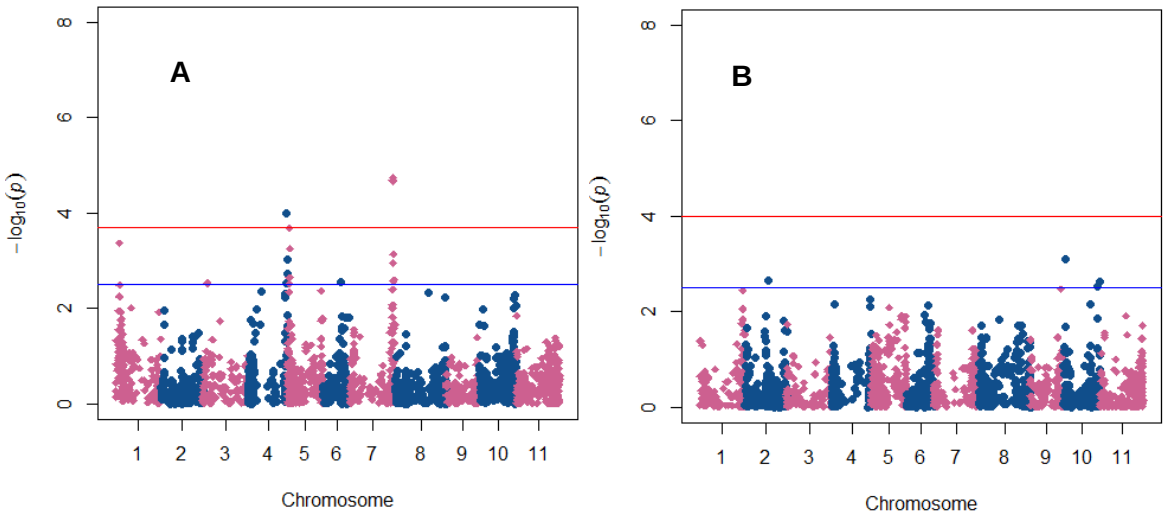

Figure 4.3 GWAS for PAE conducted on common bean genotypes and 2554 SNPs (> 0.02 minor allele frequency) using MLM. (A) Manhattan plots for 297 genotypes and (B) Manhattan plots for 262 genotypes (excluding the resistant lines), the red line is a false discovery threshold rate of 1.98×10^{-4} and 1.00×10^{-4}, respectively for significance and the blue line is p = 0.005 threshold for significanc

Table 4.3 Most significant markers, chromosome, position, *P*-value and R^2 for percent seed weight loss among common bean genotypes both with and without resistant lines, after infestation by bean bruchid

SNP	Chr.	SNP Position	*P* value	R^2 (%)
All genotypes including the resistant lines				
ss715646008	Pv07	37781465	1.75E-05	7.8
ss715646009	Pv07	37761323	2.06E-05	7.7
ss715646021	Pv07	37246486	2.10E-05	7.6
ss715645814	Pv04	46102860	1.13E-04	6.4
ss715646173	Pv05	1710978	2.22E-04	5.9
ss715645597	Pv01	3711904	4.25E-04	5.5
ss715650116	Pv05	2523099	5.48E-04	5.3
ss715645235	Pv07	37971629	7.84E-04	5.0
ss715647352	Pv04	47263067	8.70E-04	4.9
Genotypes excluding the resistant lines				
ss715639202	Pv06	29421468	6.95E-04	4.5
ss715646021	Pv07	37246486	7.88E-04	5.6
ss715645482	Pv11	385021	9.26E-04	5.5

SNP = single nucleotide polymorphic code; Chr = chromosome; R^2 = percentage of phenotypic variation explained by the SNP marker

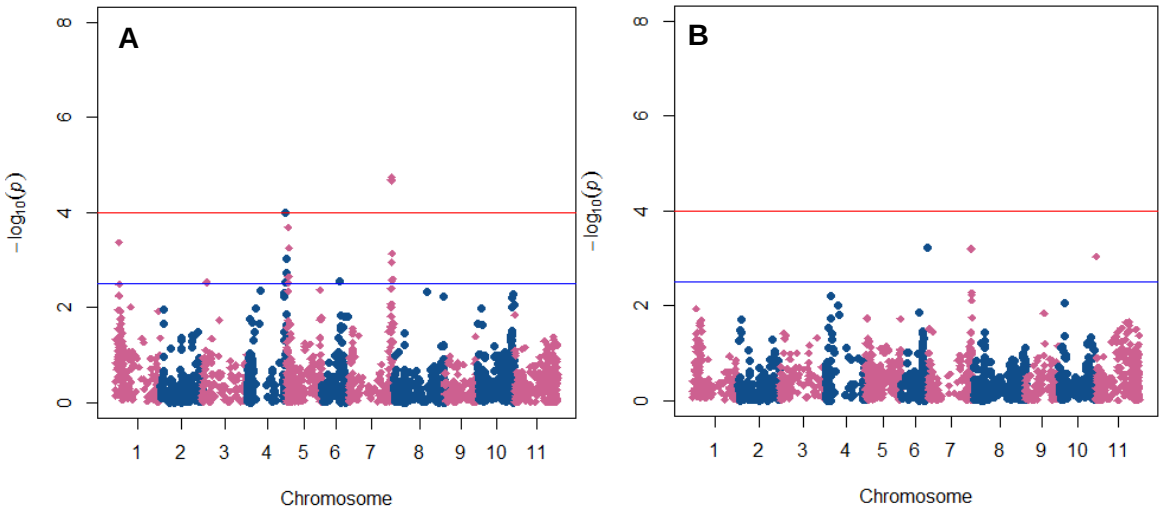

Figure 4.4 GWAS for PSWL conducted on common bean genotypes and 2554 SNPs (> 0.02 minor allele frequency) using MLM. (A) Manhattan plots for 297 genotypes and (B) Manhattan plots for 262 genotypes (excluding the resistant lines), the red line is a false discovery threshold rate of 1.02×10^{-4} and 1.00×10^{-4}, respectively for significance and the blue line is p = 0.005 threshold for significance

4.4 Discussion

4.4.1 Phenotypic diversity and population structure

The phenotypic diversity among 297 common bean genotypes for bruchid resistance was investigated and its genetic basis studied, using the genome-wide association approach. The percentage adult emergence and percentage seed weight loss among the tested genotypes ranged from 0 to 100% and 0 to 50.3%, respectively, suggesting the presence of significant variation among the genotypes for the two traits. Although there was no complete resistance observed in any of the landraces, breeding lines or released varieties, partial resistance was observed in a few genotypes, as reported by previous researchers (Schoonhoven et al., 1983; Acosta-Gallegos et al., 1998; Gallegos et al., 2008). A comparable result was observed in genotypes from Middle American and Andean origin for the two traits, signifying the existence of adequate genetic variability within the two gene pools to support genetic improvement in the common bean for bruchid resistance. The known bruchid resistant lines developed by CIAT, namely, Resistant to Zabrotes (RAZ) and Marker Assisted Zabrotes (MAZ) lines were genotypes from both gene pools.

Understanding the genetic basis and population structure of complex traits, such as insect resistance, assists in the selection of desirable genotypes (Hoyos-villegas et al., 2017). Various studies have been conducted to analyze the genetic diversity and population structure of *P. vulgaris*, using different types of markers (Freyre et al., 1998; Papa and Gepts, 2003; Dura´n et al., 2005; González et al., 2005; Blair et al., 2010a; McClean et al., 2012; Mercati et al., 2013; Cichy et al., 2015a; Rodriguez et al., 2016). The current study explored the population structure by using SNP markers and association of genomic regions with bruchid resistance, in a diverse population of common bean genotypes. Although several studies have been conducted, using SNP markers inthecommon bean, this is a first attempt to examine the population structure and marker-trait association on a large collection of Ethiopian common bean genotypes, using SNP markers.

The 297 genotypes evaluated in this study were grouped into two subpopulations at K = 2, based on the Bayesian genotyping clustering approach. This was expected, given that the common bean has evolved from the two gene pools described as Middle America and Andean (Koenig and Gepts, 1989; Gepts, 1998). The results obtained from the principal coordinate analysis revealed a similar clustering pattern to the Bayesian genotyping clustering analysis. The genetic structure identified in this study is generally consistent with the current hierarchical scheme of gene pools (Zhang et al., 2008; Blair et al., 2009; Kwak and Gepts, 2009; Corte´s et al., 2011; Fuente et al., 2013; Cichy et al., 2015a). At K = 3, the Middle American gene pool

was separated into two subpopulations, while genotypes with the Andean origin remained homogenous. This result was confirmed by other diversity studies (McClean et al., 2004; Benchimol et al., 2007; Asfaw et al., 2009; Rodriguez et al., 2016). More than 80% of the the Ethiopian common bean genotypes were observed to be from the Middle American gene pool. Asfaw et al. (2009) also confirmed the predominance of the Middle American gene pool within the Ethiopian common bean germplasm.

The structure analysis showed that most of the large-seeded bean genotypes were varieties, as well as breeding and resistant lines. However, a few landraces from the Amhara and Oromiya regions were observed in the Andean gene pool. This might be due to the importation of seeds from abroad, hybridization between the two major gene pools or the promotion of improved varieties as a research intervention in the regions. The majority of the admixture genotypes were from the breeding materials or resistant lines with a medium seed size. This may have resulted from artificial attempts to hybridize genotypes from different gene pools, in order to introgress different genes or through natural outcrossing among the genotypes.

4.4.2 Trait-single nucleotide polymorphism association

A genetically diverse, but not necessarily interrelated or highly structured population is required to perform an efficient GWAS (Flint-garcia et al., 2003). The population under study needs to exhibit a high degree of phenotypic diversity. In this study, 297 common bean genotypes collected from genetically diverse sources, such as landraces, released varieties and breeding lines were included. A set of 5,398 SNP markers was used and after removing nucleotide polymorphisms with missing rates ≥0.20 and minor allele frequency <0.05, a final set of 2554 SNPs was generated. The present study identified 24 SNPs that were significantly associated with PAE and PSWL. However, only those markers observed at P <0.001 were considered as important for marker-trait association in this study. The rest of the markers with a significant marker-trait association at P< 0.05 can also be useful for future bruchid resistance breeding programs. In the present study, the most significant SNPs were identified on Pv04 and Pv07 for both the PAE and PSWL. In addition, the proportion of the phenotypic variation (R^2 > 5%) observed for all significant markers suggests their possible influence on the respective traits. In addition to the Ethiopian collections, the 297 genotypes also comprised lines (RAZ and MAZ) with a known source of bruchid resistance, which is referred to as the APA (arcelin, phytohemagglutinin and α-amylase inhibitor) gene family. The members of the APA family provide resistance against bean bruchid and affect the survival and development of *Z. subfacsiatus* (Osborn et al., 1988; Cardona et al., 1989). The presence of significant markers on Pv04 and Pv07 could be associated with the APA gene family code for linked multi-gene family (Osborn et al., 1986). Previous studies reported that, with some exceptions,

the APA gene family is found in a locus on linkage group (LG) B4 of the common bean genetic map, and it has probably evolved from duplication and divergence and these (Suzuki et al., 1995; Freyre et al., 1998; Gepts, 1999; Lioi et al., 2003; Kami et al., 2006; Blair et al., 2010b). The other three-lectin genes [Lec-2, Lec-3 and FRIL (Flt3 receptor-interacting lectin)] that are loosely linked, are mapped to LG B7 (Nodari et al., 1993; Colucci et al., 1999) and now these LGs now refer to Pv04 for B4 and Pv07 for B7.

No SNPs were found above the cut-off point for all the traits in the 264 genotypes (genotypes excluding the resistant lines). For the 264 genotypes, the most significant SNPs for PAE were found on Pv02 and Pv10, which is different from the above result. For PSWL, three significant SNPs were identified on Pv06, 07 and 11. Kami et al. (2006) also reported that phaseolin, a seed protein, is located on linkage group B7. Previous studies on the genetic architecture of bruchid resistance in the common bean were related, with the APA gene family. Blair et al. (2010b) reported that different SSR markers around the arcelin bruchid resistance locus, for PAE and new SNP markers were developed for the same gene for both the Mesoamerican and Andean backgrounds (Bodo Raatz unpublished report). Both these SSR and SNP markers were located on Pv04. In this study, three significant SNPs, in addition to the one identified on Pv04 for PAE, were identified on three chromosomes and explained 5.9–6.9% of the variation observed in PAE. Accessions with these SNPs may be useful as parental materials, even though they lack the strong resistance genes like on Pv04 and Pv07.

4.5 Conclusion

In this study, SNP markers exhibited extensive genetic variability and two different allelic gene pools were distinguished. This suggests that SNP markers were highly valuable for distinguishing Andean and Middle American genotypes. Furthermore, the separation of the population to K = 3 resulted in a separation of the Middle American gene pool into two sub-populations. This indicated that the Middle American gene pool is more diverse than the Andean gene pool. The genetic variability information will be very critical for a robust common bean improvement program, to develop varieties suitable for the different production environments of Ethiopia.

In this study, a number of SNPs were identified that are associated with PAE and PSWL on Pv04 and Pv07, which is consistent with a previous study. In addition, other SNPs were identified on other chromosomes and the genomic region associated with these traits needs further investigation. The number of genotypes and the density of the markers were limited to identifying SNPs with smaller effects for bruchid resistance. The results of the present study can provide an important foundation for further studies to understanding the genetic

architecture of bean bruchid resistance, especially resistance other than from the APA gene family. The identified SNPs should be validated in different segregating populations for use in marker-assisted breeding, to accelerate the genetic improvement of bruchid resistance in the common bean.

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CHAPTER 5

Assessment of Diversity and Performance of Ethiopian Common Bean (*Phaseolus Vulgaris* L.) Genotypes for Yield and Yield Components

Abstract

The objectives of this study were to assess the agronomic performance of common bean genotypes, selected for their response to infestation, by Mexican bean weevil and to identify promising lines that can used as parents in breeding, for resistance to the Mexican bean weevil. Field experiments were conducted using 144 genotypes, under three different agro-ecologies in an unbalanced incomplete block design with three replications. The selected genotypes comprised of 109 landraces, 16 released varieties, 3 advanced breeding lines and 16 Mexican bean weevil resistant lines. Data on 15 important agro-morphological traits were collected and the mean yield performance of the genotypes was estimated. Multivariate methods, principal components and cluster analyses were used to examine the patterns of variation among the genotypes. The genotypes revealed a high level of phenotypic diversity for all agronomic traits recorded. The Ethiopian common bean landraces showed a wide genetic diversity for all the traits under consideration, suggesting that this germplasm could be a good source of valuable genes that could broaden the genetic base of the common bean breeding program in Ethiopia. Six principal components (PCs), which contributed 84% of the total variation among the genotypes, were identified. Thirty percent of the total variance was accounted for by PC1, which was highly correlated with the grain yield and aboveground biomass. The 15 agro-morphological traits classified the genotypes into three distinct major clusters and sub-clusters. The clustering patterns of the genotypes were according to the seed size, whereby the small and medium beans were distinctly separated from the large seeded beans. The study established the existence of considerable genetic variations among common bean genotypes. Unique genotypes, such as Nasir, Awash Melka and RAZ-36 from Cluster I, RAZ-2, RAZ-11 and RAZ-42 from Cluster II, and SER-125, SCR-15, MAZ-200, MAZ-203 and RAZ-120 from Cluster III, were selected based on their distinct agronomic performance and their response to Mexican bean weevil infestation. The selected genotypes could be useful for the common bean breeding program in general and breeding for bruchid resistance in particular.

Key word: Agro-morphological traits, cluster analysis, common bean, principal component analysis

5.1 Introduction

The common bean (*Phaseolus vulgaris* L.) is the third most important source of calories, after maize and cassava and the second most important source of dietary protein and minerals in the human diet (http://www.cgiar.org/our-research/crop-factsheets/beans/). In Africa, the major common bean-producing countries include Burundi, DR Congo, Ethiopia, Kenya, Rwanda, Tanzania, and Uganda, suggesting that East Africa is the most important region for the crop on the continent (Hillocks et al., 2006; Asfaw et al., 2009; Buruchara et al., 2011). In Ethiopia, most of the traditional foods, especially during the fasting seasons, are prepared from pulse crops, such as chickpeas, field pea, faba beans and lentils. However, recently there has been a growing interest in common beans, particularly among the low-income farmers, since the prices of other highland pulses are rising (Ferris and Kaganzi, 2008; Karanja et al., 2011). The common bean is widely grown in the country, in a range of agro-ecologies and production systems.

The major common bean production areas are Oromiya, the Southern Nations, Nationalities, and Peoples (SNNP) and the Amahara regions. These regions cover about 98% (51% Oromiya, 27% SNNPR and 20% Amhara) of the common bean production in the country (CSA, 2015). Although farmers from different parts of the country grow different types of beans, the most predominant types being white and red small beans (Asfaw et al., 2009). Both white and red small beans are produced in the Oromiya region and account for 61% and 44% of bean production in the country, respectively. The Amhara and SNNPR regions cover 35% and 43% of white and red bean production of the country, respectively. In the Oromiya region, only two zones (East Shewa and West Arsi) cover 76% of the total bean production of the region (CSA, 2015). In East Shewa, white beans (34%) are the most dominant types and are mainly grown for export, while red beans constitute only 10% of the bean produced and are mainly grown for household consumption. In West Arsi, on the other hand, farmers only produce red beans (Legesse et al., 2006; CSA, 2015). In the other bean-growing regions, especially in the SNNP, a range of cultivars, with different seed colours, sizes and shapes are grown, primarily for domestic consumption. Even though red, white and black bean varieties are produced in SNNP, the red bean is the most important and makes up 80-90% of the area allocated for bean (Ferris and Kaganzi, 2008).

The genetic improvement strategy of the National Common Bean Research Program in Ethiopia is focused mainly on consumer preferences and resistance to biotic and abiotic stresses. More than 55 improved common bean varieties have been released for different agro-ecologies and production systems (Assefa et al., 2006). These varieties have been adopted by farmers and have received high consumer acceptance, resulting in a high market

demand. Despite the success of developing acceptable common bean genotypes, harnessing the genetic potential of the common bean by delivering varieties, with high yield and related quality traits is still hindered by the narrow genetic base used in the breeding program (Fisseha, 2015). The National Bean Breeding Program relies mostly on exotic germplasm sources from the Center for Tropical Agriculture (CIAT) and very little improved germplasms from breeding programs in neigbhouring countries.

The common bean is believed to have been introduced to Ethiopia in the 16th century. Since then, farmers have been preserving and discovering important genotypes that are adapted to their local environments and needs, which has led to the evolution of morphologically-diverse landraces (Wortmann et al., 1998; Sperling, 2001). Landraces have been used as a source of desirable genes, in breeding for biotic and abiotic stresses (Shashidhar et al., 2013). A number of researchers have reported on the wide genetic diversity in the Ethiopian common bean genotypes for a number of important traits (Asfaw et al., 2009; Dagnew et al., 2014; Fisseha et al., 2016). However, the potential of the local landraces as sources of breeding material is not yet well-known and exploited. The objective of the present study, therefore, was to assess the performance of the yield and yield components of common bean landraces, varieties, breeding and resistant lines, which were selected for their response to bruchid infestation across different agro-ecologies and to select promising parents for breeding.

5.2 Materials and methods

5.2.1 Description of the study site

The study was conducted at three on-station trial sites in the Oromiya region of central Ethiopia. The sites were Melkassa (8^{0}24'52.04"N, 39^{0}19'41.22"E), Alem Tena (8^{0}17'32.29"N, 38^{0}56'48.77"E) and Arsi Negele (7^{0}22'30.29"N, 38^{0}40'17.78"E), which are located at an altitude of 1550, 1611 and 1960 metres above sea level (m.a.s.l.), respectively. The trials were conducted in 2014 during the main cropping season. The climatic data of Melkassa and Alem Tena were collected from Melkassa and Debrie Zeit Agricultural Research Centres, respectively. However, the weather station at Arsi Negele was not functional and the climatic data is not included in this study. The climatic data on rainfall and temperature for the two sites are presented in Figure 5.1 and 5.2. The soil types of Melkassa and Alem Tena are sandy loamy, while the soil is clay in Arsi Negele.

5.2.2 Experimental material

A total of 144 common bean genotypes were selected, on the basis of the prior screening of the genotypes for their response to bruchid infestation under laboratory conditions, a diversity

analysis and the population structure. The genotypes comprised of 109 landraces, 16 released varieties, and 19 pre-release breeding lines. The 109 common bean landraces were collected from different regions of Ethiopia, and of the 19 pre-released genotypes, 16 were resistant to the Mexican bean weevil. The list of the genotypes used in the study is presented the Table 5.1 and the seed colour, size and shape are shown in Figure 1 of Appendix A. The genotypes were grown during the off-season of 2014 (February-May) at Melkassa. They were under irrigation for seed increase and to offset any differences in seed age and the effects of the prior growing environments (Liao et al., 2008).

Table 5.1 List of 144 common bean genotypes used in the study

Breeding status	Number	Genotype name
Landrace	109	232196, 237079, 237080, 212860, 215391, 230044, 230661, 215048, 215049, 207934, 207935, 207938, 211346, 211347, 211348, 211349, 211356, 211361, 211362, 228522, NC-05, NC-07, NC-10, NC-16, NC-17, 207534, NC-25, NC-28, NC-29, NC-30, NC-34, 219231, 228812, 228813, 230526, 211315, 211320, 211323, 211333, 230525, 211331, 208367, 241736, 228077, 228082, 241734, 228085, 228086, 241748, 244805, 228911, 228913, 201066, 213197, 208995, 208699, 208702, 208703, 208705, 211340, 211546, 211552, 214663, 214664, 214675, 214676, 214678, 214665, 237993, 241739, 211279, 211280, 211284, 211286, 213046, 215051, 241752, 241756, 241757, NC-39, NC-44, NC-49, NC-50,NC-51, NC-52, NC-53, NC-54, NC-57, NC-61, 215719, 215720, 211266, 211267, 211269, NC-12, NC-13, NC-14, NC-15, NC-18, NC-20, 211302, 211304, 211313, 211311, 211314, 211325, 208638, 241134, 223329
Released varieties	16	Mexican-142, Kufanzik, Tinkie, Gofta, Red Wolayta, Awash-1, Awash Melka, Deme, DRK, Beshbesh, Roba, KAT-B1, Nasir, SER-125, Wedo, Ayenew
Resistant lines	16	KK25/MAIAWA/19, KK25/NAGAGA/184, MAZ 200, MAZ 203, MAZ 153, SMARC 4, RAZ-28-8, RAZ-9, RAZ-2, RAZ-40, RAZ-36, RAZ-44, RAZ-42, RAZ-11-1, RAZ-11, RAZ-120
Breeding lines	3	SCR-11, SCR-15, SCR-26

5.2.3 Experimental design and trial management

The 144 genotypes were planted in a 12 x 12 alpha lattice design, with three replications. The common bean genotypes were planted in one row of 3 m long, an inter-row spacing of 60 cm and an intra-row spacing of 40 cm. Weeds were controlled by frequent hand-weeding throughout the experimental period. Di-ammonium phosphate (DAP) fertilizer was applied during planting, at a rate of 100 kg/ha (Assefa et al., 2013) and other agronomic practices were carried out according to the cultivation practices recommended for each site.

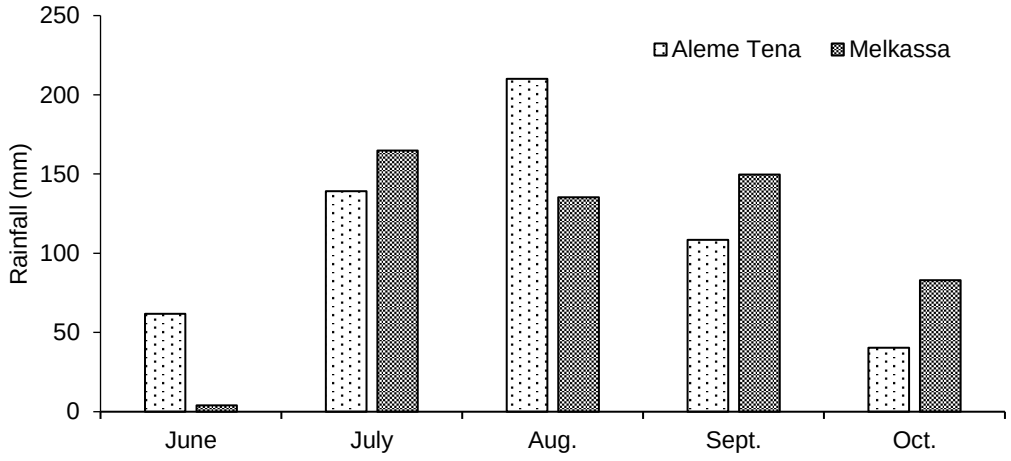

Figure 5.1. Rainfall (mm) of Melkassa and Alem Tena sites during the growing season

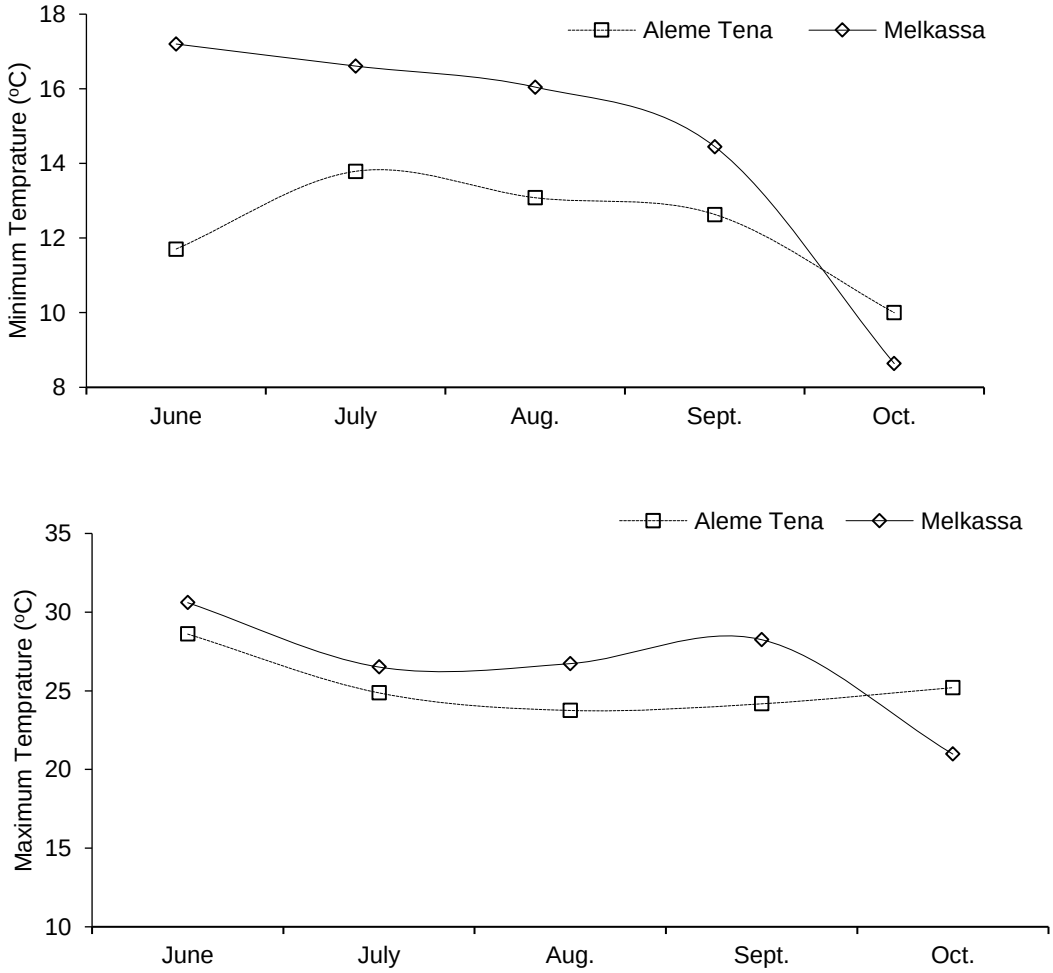

Figure 5.2 Minimum (A) and maximum (B) temperatures (^{0}C) of Melkassa and Alem Tena sites during the growing season

5.2.4 Data collection

In this study, a total of 15 phenological and agronomic traits were evaluated, based on the IBPGR (2002) common bean descriptors. For the agronomic traits, five randomly-selected plants were sampled for data collection, while the phenological traits were recorded on a whole plot basis. Data on phenological traits, such as days to 50% flowering (DTF) and days to 90% maturity (DTM) were recorded as the number of days from planting, to the date when 50% of the plants in the plot started flowering and when 90% of the plants reached physiological maturity, respectively. Data on the following agronomic traits were collected: plant height (PH) was measured (in cm) from the ground to the tip of the plant at 50% flowering, pods per plant (PPP) were recorded by counting the number of pods per plant at harvest, seeds per pod (SPP) were recorded by counting the number of seeds per pod at harvesting the hundred seed weight (HSW) was measured (in grams) as the weight of randomly-sampled 100 grains at 12.5% moisture content, the aboveground biomass (AGBM) was recorded (in grams) as the average fresh weight of five randomly-selected plants and converted to a per plant basis and the grain yield (GY) was measured (gm/plant) by weighing the grain yield of five plants and converting it to grain yield per plant.

In addition, the harvest index (HI) was measured as a proportion of the grain yield to the aboveground biomass, the grain filling period (GFP) was calculated by subtracting the number of days to 90% maturity from the days to 50% flowering, the grain production efficiency(GPE) was calculated as a proportion of the grain filling period to the duration of vegetative period, the biomass production rate (BPR) was estimated by dividing the above- ground biomass weight by the days to 90% physiological maturity and multiplied by 100 with and the economic growth rate (EGR) was calculated as a proportion of grain yield to the grain seed fill period. Other physiological parameters, such as leaf area and total chlorophyll content were also recorded. The leaf area was measured by a leaf area meter (LICOR model LI-3000) and the total chlorophyll content was measured by a non-destructive, hand-held chlorophyll meter (SPAD-502 Chlorophyll Meter) (Figure 5.3).

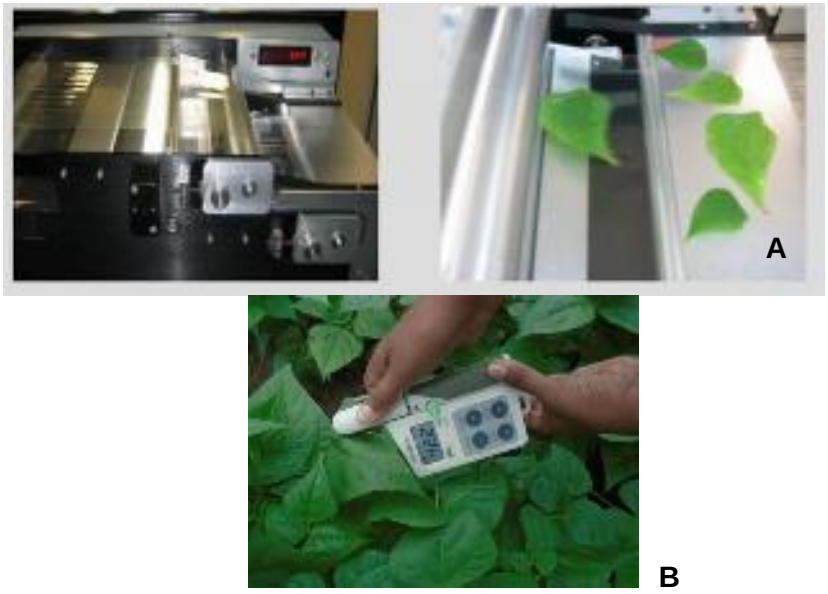

Figure 5.3 Leaf area meter (LICOR model LI-3000) (A) and SPAD-502 Chlorophyll Meter (B)

5.2.5 Data analysis

Data were subjected to the analysis of the unbalanced incomplete block design procedure, using GenStat Version 18 (Payne et al., 2017). Homogeneity of variances among the three locations was examined by using Bartlett's test for each of the studied agro-morphological traits. The Bartlett's test showed that all the traits had an equal error variance. All the agro-morphological traits were checked successively for normality, using GenStat, and all the traits showed a normal distribution. The three locations were treated as environments and a combined analysis of variance over the environments was done to estimate the variance component. Genotypes and environments were considered as fixed effects and replications, and blocks as random effects, and a combined analysis over environments was estimated from the linear additive model, which is expressed as:

$$Y_{ijklm} = \mu + r_i + b_j + \varphi_k + G_l + E_m + GE_{lm} + \varepsilon_{ijklm}$$

Where μ= the trait mean; r_i = the effect due to i^{th} replication; b_j = the effect due to the j^{th} block within the i^{th} replication; φ_k =the effect due to the k^{th} incomplete block within the j^{th} block; G_l = genotypic effect of the l^{th} genotype; E_m = environmental effect of the m^{th} environment; GE_{lm} = the interaction effect of the l^{th} genotype and the m^{th} environment.

Tukey's honestly significant difference test was used to comparing the genotypic means. The data were also subjected to the Principal Component Analysis (PCA) procedure, using Genstat Version 18. This method helps to classify the data set into components, which accounts for most of the variation in the recorded variables. This method is used to avoid multi-collinearity in the data set that might adversely affect the selection response in crop improvement programs. For multivariate analysis, the data were standardize to a mean of zero and a variance of unity was made to avoid the differences in scales used for recording data on the different characters (Manly, 1986). The top ten highest-yielding genotypes were selected based-on the traits that had the highest contribution to the first principal component i.e. grain yield. The genotypes were selected from small and medium market classes with red and white seed colours. Most of the bean-producing farmers in Ethiopia grow small and medium red beans and small white beans (Ferris and Kaganzi, 2008; Karanja et al., 2011). In the case of medium market class, some other seed colours were included, because of the outstanding performance of the genotypes.

The correlation coefficients between characters were estimated, based on the following formula:

$$r = {Cov_{xy}}\Big/{sqrt\left[\sigma_x^2 + \sigma_y^2\right]}$$

Where Cov_{xy} = co-variance of traits x and y, σ_x^2 = variance of x and σ_y^2 = variance of y.

A hierarchical cluster analysis was performed to examine the grouping patterns of the genotypes, based on their dissimilarity matrix with respect to the corresponding means of all the fifteen characters. The dissimilarity matrix was calculated, using the Dice similarity index and the cluster analysis was done by using the unweighted pair group method, the arithmetic mean (UPGMA) and the DARwin 6.0 software (Perrier and Jacquemoud- Collet, 2006). A dendrogram was then generated on the dissimilarity matrix and a bootstrap analysis was performed for node construction, using 10000 bootstrap values. The group means for all 15 agro-morphological traits were calculated and compared. Promising parental genotypes were selected.

5.3 Results

5.3.1 Agronomic performance

The analyses of variance for each location revealed a highly significant variability ($P < 0.001$) among the genotypes for all the traits studied. In addition, the performance of the genotypes was highly influenced by the prevailing environment. Thus, a combined analysis of variance

was conducted over three locations, which showed highly significant genotypes by the environment interactions for all the traits (Table 5.2). The mean squares partitioned, for genotype, environment and genotype by environment interaction indicated that environment (location) effects were more important for the variability recorded in all the traits, except for the pod per plant and the hundred seed weight. For the pod per plant, the genotype main effect and genotype by environment interaction had more influence than the environment main effect. However, for the grain yield per plant both the genotype and the environment effects were important for the expression of the traits (Tables 5.2). The coefficient of determination (R^2) estimated for all the traits ranged from 0.82 for the plant height to 0.99 for the hundred seed weight.

The minimum, maximum, mean and standard deviation as well as the coefficient of variation values of 15 agro-morphological traits recorded, at the three locations are presented in Table 5.3. The range for days to 50% flowering was recorded from 42 to 78 days, with a mean of 44 days. The plant height, leaf area and total chlorophyll content ranged from 20 - 90 cm, 0.80 - 5.80 m^2 and 30.8 - 62.4, respectively. The difference in days to maturity of late and early maturing genotypes was 44 days, with a mean value of 94 days, while the grain filling period ranged from 26 to 68 days. The number of pods per plant and seeds per pod ranged from 8.6 - 82.0 and 1.39 - 4.20, respectively. The genotypes revealed a high variation in seed size, ranging from small (10.1 g) to large (64.5 g). The minimum aboveground biomass was 21.25 gm and the maximum was 74.4 gm per plant, with the mean being 36.7 g/plant. The grain yield showed a wide variation, with the values ranging from 5.7 to 54.1 g/plant and the mean yield was 25.6 g/plant. In addition, the range of harvest index, the biomass production rate and economic growth rate were 19.4 - 99.1, 20.9 - 79.9 and 13.3 - 101.4, respectively. The coefficient of variation recorded in the traits studied ranged from 2.3% to 12.9%.

Table 5.2 Combined analysis of variance of 15 agro-morphological traits recorded on 144 common bean genotypes at three locations

Traits	Mean Square							
	Replication (DF= 2)	Block (DF = 11)	iblock (DF = 11)	Genotype (G) (DF = 121)	Environment (E) (DF = 2)	G x E interaction (GEI) (DF = 286)	Error (DF = 862)	R^2
Days to 50% flowering	1.87	71.38	44.09	49.00**	5174.08**	8.99**	2.41	0.93
Plant height	219.97	153.71	116.38	204.82**	19842.36**	197.00**	32.57	0.82
Total chlorophyll content	227.23	386.57	106.05	111.18**	2050.41**	65.64**	4.78	0.91
Leaf area (m^2/plant)	0.59	2.02	1.47	1.50**	224.60**	1.02**	0.04	0.97
Days to 90% maturity	50.43	539.39	124.70	182.58**	19915.01**	54.92**	4.55	0.96
Grain filling period	33.71	351.64	89.16	137.80**	6043.10**	51.46**	6.41	0.90
Pods per plant	55.55	971.81	682.06	540.76**	105.54**	148.81**	7.41	0.95
Seeds per pod	0.77	14.49	8.91	4.24**	9.17**	1.80**	0.21	0.88
Hundred seed weight (g)	12.83	2571.07	536.61	675.19**	589.14**	24.16**	2.10	0.99
Aboveground biomass (g/plant)	30.20	300.87	88.80	153.36**	4539.12**	82.36**	4.14	0.94
Grain yield (g/plants)	24.28	568.08	281.35	219.75**	240.55**	111.65**	4.30	0.95
Harvest index	15.62	1620.44	1042.57	783.12**	12005.66**	708.42**	23.46	0.95
Grain production efficiency (g/plant)	60.61	915.37	558.21	436.05**	1359.47**	184.89**	10.54	0.93
Biomass production rate (%)	17.38	652.56	75.51	164.86**	1352.50**	102.99**	5.15	0.93
Economic growth rate (%)	37.79	2672.08	832.04	851.69**	2826.06**	483.27**	24.76	0.93

DF = degree of freedom; R^2 = coefficient of determination; ** = significantly different at $p < 0.001$; * = significant ($P < 0.05$)

Table 5.3 Summary statistics on 15 agro-morphological traits evaluated on 144 common bean genotypes at three locations

Trait	Min	Max	Mean ± SE*	SD*	CV%*
Days to 50% flowering	37.00	51.90	44.17 ± 0.12	1.55	3.51
Plant height	31.70	56.20	44.20 ± 0.30	5.71	12.91
Total chlorophyll content	35.00	57.10	45.01 ± 0.17	2.19	4.86
Leaf area (m^2/plant)	0.80	5.80	3.00 ± 0.03	0.19	6.39
Days to 90% maturity	79.10	103.80	94.41 ± 0.23	2.13	2.26
Grain filling period	37.40	58.60	50.25 ± 0.18	2.53	5.04
Pods per plant	13.10	50.90	27.70 ± 0.28	2.72	9.83
Seeds per pod	2.10	6.10	4.21 ± 1.06	0.46	10.84
Hundred seed weight (g)	12.10	58.80	24.84 ± 0.27	1.45	5.84
Above ground biomass (g/plant)	27.70	53.20	36.74 ± 0.19	2.04	5.54
Grain yield (g/plant)	14.60	41.80	25.64 ± 0.21	2.07	8.09
Harvest index	39.76	92.10	69.99 ± 0.47	4.84	6.92
Grain production efficiency (g/plant)	14.00	54.50	29.52 ± 0.28	3.25	11.00
Biomass production rate (%)	30.30	56.20	39.18 ± 0.20	2.27	5.79
Economic growth rate (%)	28.50	82.00	51.48 ± 0.43	4.98	9.67

SE = standard error; CV = coefficient of variation; SD = standard deviation

5.3.2 Principal component analysis (PCA)

The PCA grouped the 15 phenotypic traits into 15 components, which accounted for the entire (100%) variability among the studied genotypes. However, the principal components, with an eigenvalue of less than 1 were eliminated. The first six principal components (PCs), accounting for 83.7% of the variability observed among the studied common bean genotypes, were maintained. Table 5.4 presents the eigenvectors and values, the percentage of total variance and total cumulative variance for the 15 phenotypic traits used in this study. The first principal component (PC1) explained that 29.7% of the total phenotypic variation among the 144 common bean genotypes was mainly due to the additive effects of the grain yield, grain production efficiency, economic growth rate, aboveground biomass and biomass production rate. The second PC, which accounted for 19.4% of the total variation was well associated with phenological traits, such as the days to 50% flowering, the days to 90% maturity and the grain filling period. Likewise, the third PC, which accounted for about 11% of the total variance of the genotypes, was due to the discriminatory effect of the hundred seed weight and the number of pods per plant. The variation in plant height, days to 50% flowering and the number of seed per pod constituted a large part of the total variation explained by the fourth PC. The fifth and sixth PCs accounted for 7.7% and 6.7% of the total variation, chiefly due to the contrast between the total chlorophyll content and the number of seeds per plant, and the harvest index and leaf area, respectively.

Table 5.4 Principal component (PC) analysis of various agro-morphological traits estimated at three locations

Trait	PC1	PC2	PC3	PC4	PC5	PC6
Aboveground biomass (g/plant)	0.41	0.00	-0.03	0.17	-0.22	0.19
Biomass production rate (%)	0.38	-0.23	0.06	0.15	-0.24	0.15
Days to 50% flowering	-0.12	0.31	0.02	0.51	-0.26	-0.30
Days to 90% maturity	0.02	0.55	-0.21	0.06	0.10	0.05
Economic growth rate (%)	0.42	-0.14	0.13	0.11	-0.04	-0.16
Grain filling period	0.10	0.46	-0.25	-0.23	0.26	0.23
Grain production efficiency (g/plant)	0.43	0.12	-0.08	-0.18	0.19	0.08
Grain yield (g/plants)	0.45	0.04	0.02	0.01	0.05	-0.06
Harvest index	0.19	0.11	0.05	-0.27	0.22	-0.64
Hundred seed weight (g)	0.10	-0.21	-0.63	-0.02	0.04	0.05
Leaf area (m^2/plant)	0.09	0.27	0.16	0.07	-0.22	0.44
Plant height	0.09	0.13	-0.38	0.50	-0.04	-0.27
Pods per plant	0.10	0.26	0.52	0.21	0.31	0.01
Total chlorophyll content	0.09	-0.27	0.08	0.22	0.48	-0.02
Seeds per pod	0.11	0.16	0.08	-0.42	-0.53	-0.28
Eigenvalue	4.45	2.91	1.68	1.36	1.15	1.00
% total variation	29.67	19.37	11.23	9.09	7.65	6.66
% cumulative variation	29.67	49.04	60.27	69.36	77.01	83.67

In order to select genotypes with the best performance, the contribution of each trait was determined by the PCA. It was found that the yield had a significant effect on the phenotypic variation among the 144 genotypes. Hence, the top ten best genotypes were selected from both small and medium market classes, based on grain yield performance. The mean performance of the top ten high- yielding genotypes from both small and medium-seeded genotypes is presented in Table 5.5. Genotypes, such as Nasir, SER-125, Awash Melka, RAZ-36, 241757, 230526, RAZ-44, 241734, 214665 and NC-51 were selected from the small-seed market class and they had a grain yield ranging from 29.2 to 41.8 g/plant. The top ten selected high-yielding genotypes from the medium market class included 207935, SCR-11, RAZ-40, NC-28, 211302, SCR-15, SCR-26, 228077, KK25/NAGAGA/19 and RAZ-120. These genotypes produced a grain yield ranging from 27.8 to 41.2 g/plant. There was no single genotype that showed consistent superiority for all the traits among the selected genotypes. However, the improved small- seeded variety Nasir exhibited the highest grain yield and grain production efficiency of all the tested genotypes. In addition, genotype 207935 showed the highest aboveground biomass, biomass production rate and economic growth rate of all the tested genotypes. Based on the field performance of the 144 genotypes, 45% of the selected

genotypes were landraces (241757, 230526, 241734, 214665, 207935, 211302, NC-51, NC-28 and 228077), 25% were resistant lines (RAZ-36, RAZ-44, RAZ-40, KK25/NAGAGA/19 and RAZ-120), 15% were released varieties (Nasir, SER-125 and Awash Melka) and 15% were advanced breeding lines (SCR-11, SCR-15 and SCR-26).

5.3.3 Correlations of yield and its components

The correlation among the 15 agro-morphological traits is presented in Table 5.6. Grain yield was highly significantly and positively ($P < 0.001$) correlated with the aboveground biomass, harvest index, grain production efficiency, biomass production rate and economic growth rate. Similarly, the grain filling duration and plant per pod was highly significant ($P < 0.01$), and the seeds per pod and hundred seed weight had a significant ($P< 0.05$) correlation with grain yield. The biomass production rate was found to be negatively and highly significantly ($P < 0.001$) correlated with days to 50% flowering, days to 90% maturity and the grain filling period, but highly ($P < 0.001$) positively correlated with the aboveground biomass and grain production efficiency. The total chlorophyll content, on the other hand, revealed a negative and significant association with the days to 50% flowering, the days to 90% maturity, the grain filling period and the seed per pod. Similarly, the hundred-seed weight had a negative and significant correlation with the leaf area, the days to 50% flowering, the pod per plant and seed per pod, and a positive and significant association with the plant height. The days to 50% flowering had a significant negative association with hundred-seed weight, grain production efficiency and biomass production rate. The relationship between the pods per plant and seeds per pod with the hundred seed weight was also significant, but negative.

Table 5.5 Mean performance of the top ten selected common bean genotypes for seed colour and yield and yield related traits

Genotype	SC	DTF	PH	TCC	LA	DTM	PPP	SPP	HSW	AGBM	GY	HI	GPE	BPR	EGR	GFP
Top ten small seeded genotypes																
Nasir	Red	41.6	53.3	48.0	2.5	95.4	36.0	5.1	24.6	46.1	41.8	63.5	54.5	48.3	77.5	53.9
SER-125	Red	41.8	41.7	47.1	2.6	90.7	26.0	3.5	25.6	39.7	36.5	77.3	42.6	44.3	75.9	48.9
Awash Melka	White	46.6	52.8	49.1	3.0	93.9	33.7	4.5	21.7	46.6	34.6	63.5	34.0	52.6	76.9	47.3
RAZ-36	White	42.7	45.0	53.4	3.2	96.2	46.3	3.1	18.1	45.5	33.1	66.9	41.3	47.1	63.3	53.6
241757	Red	47.0	47.2	41.5	2.9	95.7	29.8	4.4	22.7	43.7	32.9	76.3	34.0	45.6	68.7	48.7
230526	Red	42.9	41.1	40.4	3.4	96.6	27.0	5.0	23.6	37.0	32.2	86.9	40.3	38.3	59.8	53.7
RAZ-44	White	42.8	48.3	50.1	2.9	96.2	31.2	4.1	18.1	42.1	31.4	82.5	39.2	43.8	60.7	53.4
241734	Red	43.4	46.1	45.6	4.0	101.1	30.0	4.6	22.1	44.4	31.3	72.1	41.6	44.0	54.5	57.7
214665	Red	43.1	44.4	43.7	3.4	99.3	27.4	5.4	22.8	41.1	30.1	74.6	39.5	41.4	53.7	56.2
NC-51	Red	42.1	41.1	42.9	2.6	95.1	26.6	3.8	24.1	38.2	29.2	74.9	37.1	40.0	54.9	53.0
Top ten medium seeded genotypes																
207935	Carioca	44.9	51.1	49.6	3.2	95.6	24.2	5.7	29.4	53.2	41.2	80.7	46.8	56.2	82.0	50.7
SCR-11	Red	42.0	45.0	49.9	2.7	92.3	25.4	3.9	29.2	44.9	36.9	56.6	44.2	48.8	74.3	50.3
RAZ-40	White	41.4	37.8	49.5	3.1	89.6	20.3	3.7	36.7	35.8	32.6	62.4	32.2	40.6	60.7	48.1
NC-28	Cream	40.9	45.0	47.3	3.1	99.4	32.0	3.1	28.9	42.3	31.8	75.8	45.0	42.6	55.0	58.6
211302	Brown	39.8	38.3	47.8	2.8	89.0	21.6	4.2	36.5	42.3	31.7	77.7	39.0	47.2	66.1	49.2
SCR-15	Red	43.3	38.9	47.6	2.8	94.0	27.1	3.7	38.3	41.5	31.3	89.0	36.5	43.8	62.1	50.7
SCR-26	Red	43.6	49.4	47.2	3.0	92.6	23.9	4.2	27.7	42.9	29.2	67.5	31.8	46.1	57.8	49.0
228077	Red	42.9	43.3	37.5	3.4	100.7	26.3	5.7	25.9	38.4	28.4	75.8	39.3	38.1	48.8	57.8
KK25/MAIAWA/19	Red	43.6	47.2	42.0	2.8	95.4	20.8	5.6	36.9	33.1	28.2	77.3	33.7	34.9	54.7	51.9
RAZ-120	White	45.7	45.0	50.3	2.8	90.7	28.6	3.7	26.4	38.3	27.8	75.1	27.4	42.6	63.1	45.0

SC = seed colour; DTF = days to 50% flowering, PH = plant height (cm); TCC = total chlorophyll content; LA = leaf area (m2/plant); DTM = Days to 90% maturity; PPP = pods per plant; SPP = seeds per pod; HSW = hundred seed weight (g/100 seed); AGBM = aboveground biomass (g/plant); GY = grain yield (g/plant); HI = harvest index; GPE = grain production efficiency (g/plant); BPR = biomass production rate; EGR = economic growth rate, GFP = grain filling period

Table 5.6 Correlation analysis among 15 agro-morphological traits in 144 common bean genotypes recorded at three locations

Trait	DTF	PH	TCC	LA	DTM	GFP	PPP	SPP	HSW	AGBM	HI	GPE	BPR	EGR	GY
DTF	1.00														
PH	0.31***	1.00													
TCC	-0.26**	0.05	1.00												
LA	0.14	0.03	-0.12	1.00											
DTM	0.52***	0.29***	-0.33***	0.31***	1.00										
GFP	0.03	0.16	-0.23**	0.28***	0.87***	1.00									
PPP	0.21*	-0.04	0.04	0.24**	0.27***	0.20*	1.00								
SPP	-0.02	-0.04	-0.24**	0.14	0.14	0.17	-0.04	1.00							
HSW	-0.24**	0.25**	0.11	-0.22**	-0.10	0.01	-0.61***	-0.18*	1.00						
AGBM	-0.09	0.22**	0.15	0.24**	0.09	0.15	0.10	0.22**	0.18*	1.00					
HI	-0.07	0.06	0.08	0.07	0.12	0.18*	0.13	0.26**	-0.01	0.14	1.00				
GPE	-0.34***	0.12	0.05	0.15	0.26**	0.50***	0.23**	0.21*	0.20*	0.69***	0.40***	1.00			
BPR	-0.30***	0.11	0.27**	0.09	-0.34***	-0.22**	-0.01	0.12	0.19*	0.88***	0.08	0.55***	1.00		
EGR	-0.16	0.09	0.19*	0.01	-0.22**	-0.16	0.21*	0.12	0.15	0.70***	0.31***	0.72***	0.77***	1.00	
GY	-0.16	0.15	0.09	0.12	0.12	0.23**	0.26**	0.18*	0.18*	0.75***	0.39***	0.92***	0.68***	0.92***	1.00

PH = plant height (cm); LA = leaf area (m2/plant); TCC = total chlorophyll content; DTF = days to 50% flowering; DTM = Days to 90% maturity; PPP = pods per plant; SPP = seeds per pod; HSW = hundred seed weight (g/100 seed); AGBM = aboveground biomass (g/plant); GY = grain yield (g/plant); HI = harvest index; GPE = grain production efficiency (g/plant); BPR = biomass production rate; EGR = economic growth rate, GFP = grain filling period

*** = significant (P < 0.001); ** = significant (P < 0.01); * = significant (P < 0.05)

5.3.4 Cluster analysis

The relationship among the 144 common bean genotypes was revealed by using the neighbour-joining algorithm, using the unweighted pair group method (UPGMA). The cluster analysis on the mean of 15 phenotypic traits clearly classified the 144 genotypes into three major clusters and seven sub-clusters (Figure 5.4). The first cluster (Cluster I) was composed of 36 (25%) of the genotypes and was dominated by small-seeded beans. This cluster was further divided into two sub-clusters (sub-Cluster Ia and Ib), with 18 genotypes each. Cluster I consisted of 26 landraces, 2 resistant lines and 5 varieties. The second cluster (Cluster II) consisted of the largest number, mainly small-seeded genotypes (49%). This cluster was further sub-divided into three sub-clusters, with 26, 22 and 23 genotypes, respectively. Cluster III, consisted mainly of large and medium-seeded genotypes. This cluster was comprised of 37 genotypes, which were further sub-divided into two sub-Clusters, with 20 and 17 genotypes, respectively. Of the 16 resistant lines, 50% were in Cluster III, together with large-seeded released varieties.

5.3.5 Performances of genotypes in different clusters

Table 5.7 summarizes the cluster means of the 15 phenotypic traits for the three main clusters and seven sub-clusters. The mean performance of the clusters showed the presence of considerable phenotypic variation among genotypes within each cluster. Genotypes in Cluster I revealed the highest mean values for all the traits, except for plant height, hundred-seed weight, biomass production and total chlorophyll content. Genotypes in Cluster III had the highest mean values for plant height, hundred-seed weight, total chlorophyll content and biomass production rate.

Sub-cluster Ia contained accessions that had a large leaf area and a large number of seeds per pod. Genotypes grouped in sub-Cluster Ib were characterized by tall plants with a large number of pods per plant, as well as highest aboveground biomass, grain yield and economic growth rate. Although, sub-Clusters Ia and Ib consisted of genotypes with small-seed sizes, genotypes in sub-Cluster Ib were much smaller than those in sub-Cluster Ia. Genotypes in sub-Clusters IIa and IIb were relatively early maturing, with a short grain filling duration. However, sub-Cluster IIc consisted of genotypes that were late maturing and took long to fully fill the grain. In general, the genotypes clustered in sub-Clusters IIa and IIc were low performing genotypes that had an extended period of vegetative growth and the highest total chlorophyll content.

Out of the two sub-Clusters under Cluster III, sub-Cluster IIIb included the best performing genotypes in traits, such as grain yield, harvest index, grain production efficiency, biomass production rate and economic growth. These genotypes also had a high total chlorophyll content, a short flowering time and were of medium seed size. Sub-Cluster IIIa, on the other hand, consisted of tall genotypes with large seed sizes. The genetic distance averaged for all the genotypes in each cluster revealed that the genotypes in each respective cluster were diverse. The smallest mean genetic distance was observed among genotypes clustered in Cluster I sub-Cluster Ib, while the highest genetic distance was found among genotypes grouped in Cluster III sub-Cluster IIIa. Generally, a cluster analysis allows the selection of unique and genetically complementary genotypes for breeding and conservation. Genotypes Nasir, Awash Melka and RAZ-36 from Cluster I, RAZ-2, RAZ-11 and RAZ-42 from Cluster II, and SER125, SCR-15, MAZ-200, MAZ-203 and RAZ-120 from Cluster III were selected as potential parental genotypes. Although the agronomic performance of most of the resistant lines were relatively lower than the breeding lines and varieties, they can be used as a source of resistance. The selected genotypes have unique attributes, including grain yield, earliness and seed colour, shape and size.

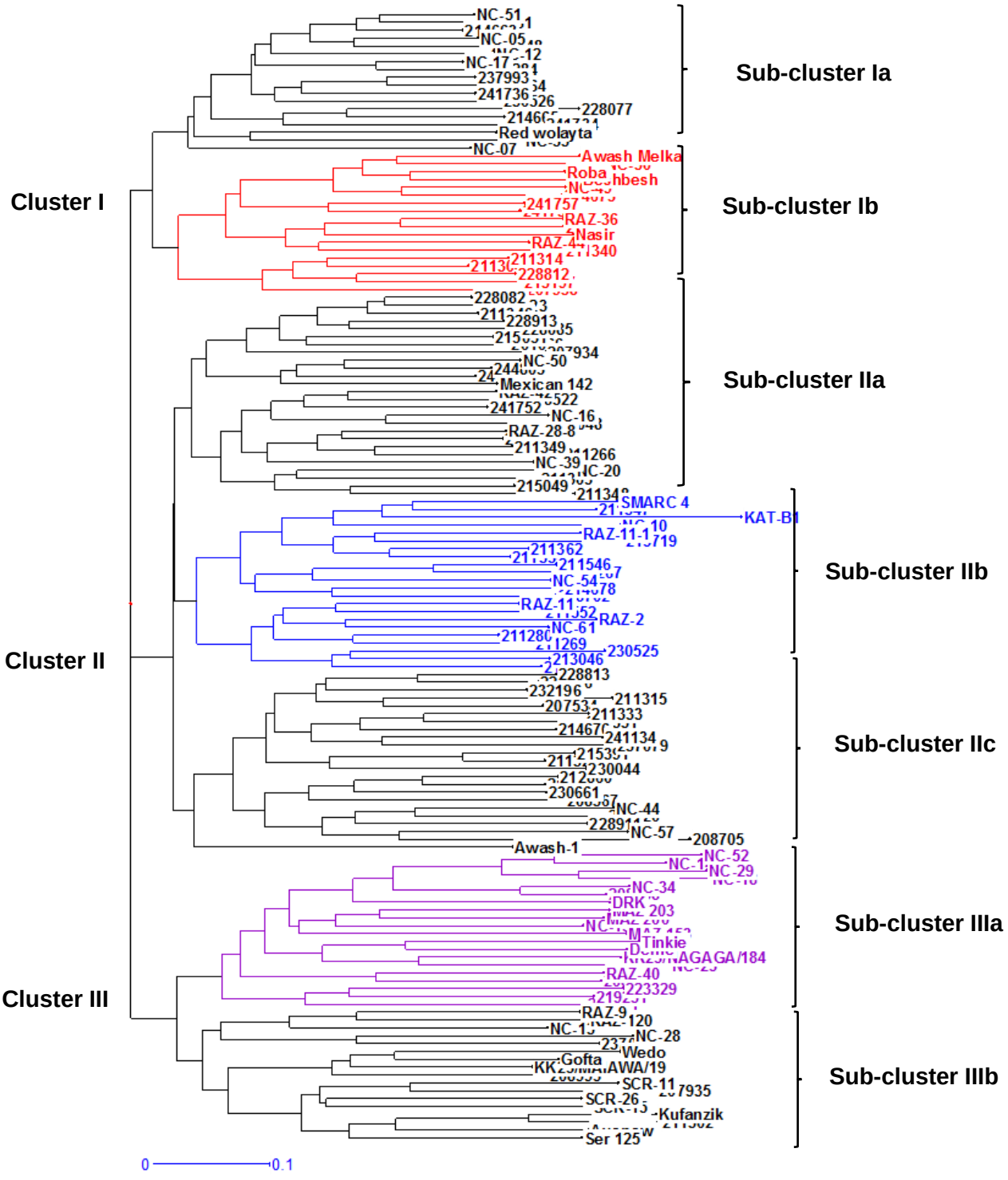

Figure 5.4 Dendrogram generated, based on hierarchical cluster analysis using UPGMA cluster algorithm, based on morphological data of 144 common bean genotypes

Table 5.7 The cluster means of 15 agro-morphological traits for the common bean genotypes, based on data recorded at three locations

Trait	Cluster means						
	C-I (n=36)		C-II (n=71)			C-III (n=37)	
	SC-Ia (n=18)	SC-Ib (n=18)	SC-IIa (n=26)	SC-IIb (n= 22)	SC-IIc (n=23)	SC-IIIa (n=20)	SC-IIIb (n=17)
Plant height (cm)	43.4	47.2	43.0	40.8	44.2	47.1	44.9
Leaf area (m^2/plant)	3.41	3.13	3.15	2.82	3.01	2.91	2.87
Total chlorophyll content	44.3	45.7	43.3	45.9	44.2	44.7	48.0
Days to 50% flowering	43.2	45.4	44.3	43.9	45.6	44.1	42.2
Days to 90% maturity	95.7	96.2	94.6	87.4	99.1	94.7	93.1
Grain filling period	52.4	50.9	50.4	43.6	53.6	50.6	50.9
Pods per plant	27.0	37.8	25.5	26.0	34.3	17.9	26.1
Seeds per pod	4.8	4.3	4.4	4.0	4.2	3.6	4.2
Hundred seed weight (g)	23.2	19.7	21.4	21.1	16.9	42.2	32.4
Aboveground biomass (g/plant)	38.8	41.3	36.2	33.6	32.8	36.1	40.8
Grain yield (g/plants)	28.2	32.2	22.8	21.9	22.0	25.3	30.7
Harvest index	75.8	75.6	64.6	65.9	68.7	67.2	76.6
Grain production efficiency (g/plant)	34.5	36.3	26.1	21.8	25.9	29.5	37.1
Biomass production rate (%)	40.5	43.2	38.7	38.7	33.3	38.2	44.0
Economic growth rate (%)	53.9	64.2	45.6	50.8	41.6	49.6	61.0
Genetic distance	0.45	0.53	0.51	0.59	0.56	0.60	0.58

C = cluster; SC = sub cluster; n = number

5.4 Discussion

The present study examined the genetic variability and agronomic performance of 144 selected common bean genotypes for 15 yield and yield-related traits in three locations. The highly significant genotype mean squares for all the characters demonstrated that the genotypes exhibited a wide genetic variability for yield and yield-related traits. The observed highly significant environmental main effects suggested that the three locations were diverse in terms of weather- and location-related factors, such as temperature, rainfall, relative humidity, wind, altitude, soil physical and chemical properties. The three test locations represented three different agro-ecologies, with Melkassa representing the dryland agro-ecology, Arsi Negele representing the highly productive highland agro-ecology and Alem Tena, representing the middling agro-ecology. Ceccarelli et al. (1991) indicated that the genotype and environment components are recognized as the primary sources of variability in agronomic and genetic studies. Similarly, the highly significant genotype by environmental-interaction indicated that genotypic performance is highly variable across the different environments. Ceccarelli (1994) also indicated that the expression of

morphological and physiological plant characteristics associated with yield, in optimal and stress conditions is different. Therefore, the discrimination and characterization of genotype adaptation across environments is crucial for optimizing the deployment of genetic resources.

In the present study, the means and ranges of phenological traits and yield-related traits, such as the number of pods per plant, the number of seeds per pod, the hundred seed weight and the weight of seeds per plant, revealed a wide range of genetic variation. A high phenotypic variation for these traits in the common bean were also reported by different authors (Stoilova et al., 2005, 2013; Negash, 2006; Burle et al., 2011; Boros et al., 2014; Scarano et al., 2014; Fisseha, 2015; Teame et al., 2017). The high phenotypic variation observed in this study may be attributed to the genetic variations among the genotypes and the environmental variations, in the tested locations. In this study, more than 75% of the genotypes were landraces, suggesting that there was ample genetic variability among the landraces that can be exploited in future common bean improvement program. Other researchers also reported on the presence of high phenotypic diversity in the Ethiopian common bean landraces (Negash, 2006; Asfaw et al., 2009; Fisseha, 2015). Similarly, several researchers from different parts of the world also reported the presence of a significant variation in the common bean in all the traits studied (Oscar et al., 2004; Duran et al., 2005; Negash, 2006; Lima et al., 2012; Awan et al., 2014; Boros et al., 2014; Fisseha, 2015; Prakash et al., 2015; Teame et al., 2017).

A Principal Component Analysis (PCA) was conducted to measure the relative contribution of each trait with regard to the total variation in the studied common bean genotypes. In this study, the first six components, with an eigenvalue of ≥1 explained 84% of the total variation; however, about 50% of the phenotypic variation was explained by the first two components. Similar results were recorded for agro-morphological traits in the common bean by Burle et al. (2011), Lima et al. (2012), Marzooghian et al. (2013), Okii et al. (2014) and Fisseha (2015). In the present study, about 30% of the phenotypic variations observed was due to the variation in grain yield and aboveground biomass. However, phenological traits also contributed significantly to discriminating the genotypes. The significant discriminatory effect of days to flowering was also reported by Burle et al. (2011) and Fisseha (2015). Likewise, about 11% of the variations detected among the tested genotypes was due to the variation in seed weight. In previous studies, this trait was reported as the most important trait used to differentiate the two common bean gene pools (Singh et al., 1991b). However, the contribution of the trait in this study was relatively low, compared to other previously-reported results (Burle et al., 2011; Fisseha, 2015). This could be

due to the fact that most of the genotypes were selected from small (74%) and medium (15%) seed sizes, as reported by Lima et al. (2012).

The top 20 common bean genotypes were selected as potential parents for breeding programs, based on their yield performance. The principal component analysis showed that grain yield had the most significant role in discriminating the 144 genotypes. The selection of the top genotypes was conducted according to the common bean market preferences in the major common bean-producing regions in Ethiopia, where the Mesoamerican beans (small and medium-seeded) have more market demand than the Andean (large-seeded) genotypes. Based on their agronomic performance, the selected genotypes were composed of 9 landraces, 5 resistant lines, 3 varieties and 3 advanced breeding lines. As can be expected, the released varieties in the selected small-seeded group topped the rank in grain yield. The majority (45%) of the selected genotypes were landraces, suggesting that landraces can be used as good source of valuable genes for future common bean breeding programs in Ethiopia (Mondini et al., 2009). Although, the local landraces were found to be better adapted, genetically diverse and agronomically suitable, the National Bean Breeding Program has been entirely, dependant on the exotic germplasm. The SCR lines (SCR-11 and SCR-15) were the two top selected genotypes from the medium-sized red bean group. These lines are red beans that were developed for drought-prone areas carrying drought tolerance, and with recessive genes for resistance to bean common mosaic virus (Darkwa et al., 2016). The SCR-15 line is one of the candidate varieties selected for release in 2017, after multi-environment variety trials (Negash et al., 2014). The lines with Zabrotes-resistance genes, such as RAZ-36, RAZ-40, RAZ-44 and RAZ-120, and the Malawian resistance variety (KK25/MAIAWA/19), were found to be agronomically suitable.

Yield is a complex trait and is the outcome of the interaction of a number of genes and traits. Moreover, the expression of the traits is highly influenced by the environmental factors, such as temperature, moisture and light. It is also, well known that the overall yield performance of genotypes is determined by the interaction of the traits, rather than the expression of individual traits (Ceccarelli et al., 1991). Blum (1988) also indicated that yield *per se* is not under direct genetic control, but under the control of the integrated effects of a multitude of physiological and biochemical processes. Hence, an understanding of the association between yield and yield-related traits is very crucial, in order to exploit the genetic variability through selection. In the present study, grain yield had a significant positive association with the grain filling period, the number of pods per plant, the hundred seed weight, aboveground biomass and harvest index. A selection, based on these traits can be used as an indirect selection criterion for the improvement

of grain yield in the common bean. Several researchers have also reported the positive significant correlation of grain yield with the, above mentioned traits (Duran et al., 2005; Negash, 2006; Mohammad et al., 2008; Karasu and Oz, 2010; Fisseha, 2015; Prakash et al., 2015). The strong positive correlation between hundred seed weight and grain yield has also been reported by different authors (Roy et al., 2006; Karasu and Oz, 2010; Negahi et al., 2014; Fisseha, 2015). Some reports, on the other hand, have indicated a strong negative correlation between grain yield and hundred seed weight (Duran et al., 2005; Negash, 2006; Kumar et al., 2009; Ahmed, 2013). Several authors also identified different sets of traits that had a significant association with yield. The variation in the sets of traits and the strength of the association might be a result of the variations in the environmental conditions and the genotypes used.

The hierarchical cluster analysis conducted on the means of 15 agro-morphological traits resulted in three distinct major clusters and seven sub-clusters. For the traits under consideration, the within-cluster variation was found to be the lowest, while the, between-cluster variation was the highest (Singh and Chaudhary, 1985; Hair et al., 1995). The mean performance of the genotypes grouped under the different clusters and sub-clusters showed considerable phenotypic variation. The clustering patterns were according to the seed size, where small and medium-seeded genotypes were clustered in Cluster I and II, while all the large-seeded genotypes were grouped in Cluster III. The present result is supported by several authors, such as Singh et al.(1991a; b), Burle et al. (2011), Madakbaş and Ergin (2011) Boros et al.(2014). Based on hundred-seed weight, genotypes with HSW <25 g are categorized as small-seeded, HSW $\geq$25-41g as medium-seeded and HSW > 41 g as large-seeded. The clustering of genotypes, based on their seed size (gene pools) was clearly observed in the molecular genetic diversity analysis, using SNP markers in the previous chapter. The clustering of landraces across all clusters indicated that Ethiopian landrace collections had a wide genetic variation for yield and yield-related traits. In addition, a large number (82%) of the genotypes was found to have a small to medium seed size, suggesting that the Ethiopian common bean genotypes are predominantly from the Mesoamerican gene pool, as reported in the previous chapter and supported by other authors (Asfaw et al., 2009).

5.5 Conclusion

The study identified a considerably wide genetic diversity among the 144 common bean genotypes for all the 15 phenotypic traits studied. Traits, such as the grain yield, the hundred seed weight and above ground biomass were found to be the most important traits in differentiating germplasm into different clusters. It was also found that the Ethiopian common bean landraces showed a wide range of variation for all 15 of the agro-morphological traits studied, which suggests these germplasms can be used as valuable sources of genes in the National Common Bean Improvement programs. Genetically unique genotypes, such as Nasir, Awash Melka and RAZ-36 from cluster I RAZ-2, RAZ-11 and RAZ-42 from Cluster II and SER-125, SCR-15, MAZ-200, MAZ-203 and RAZ-120 from Cluster III, were identified as suitable genotypes. Although the agronomic performance of the MAZ lines were far lower than the other genotypes, they can be selected as sources of the resistance genes. Released varieties, Nasir and Awash Melka are the top high yielding varieties that have been adopted in most of the bean growing areas. SER-125, on the other hand, is a recently released variety that possess most of the farmers' preferred traits. In addition, SCR-15 is one of the recent candidate variety submitted for verification for by the variety release committee in 2017. However, these varieties were more susceptible to Mexican bean weevil. These varieties will be selected as a female parent for the introgression of bruchid resistance genes in future breeding program.

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CHAPTER 6

Participatory Variety Selection of Common Bean Genotypes in the Oromiya Region of Central Ethiopia

Abstract

The study was conducted during the main cropping season of 2014 at three locations in the Oromiya region of central Ethiopia, to identify suitable parental genotypes for use in breeding for bruchid resistance and the farmers' preferred traits for choosing common bean varieties. One-hundred forty-four diverse common bean genotypes were planted in an alpha lattice design, with three replications at three research stations. Twenty common bean-producing farmers (10 males and 10 women) were involved in selecting the genotypes at each location. The participatory variety selection took place between late pod-filling to maturity. Participating farmers identified a number of criteria for selecting suitable varieties. However, the recognized selection criteria varied between location and gender groups. Yield and yield-related traits were ranked as the most important selection criteria by both gender groups at all locations. However, women ranked taste and cooking time as the top criteria for varietal choice, while men were more interested in marketability, seed size and seed colour. In all three locations, both farmer groups were able to select the 10 best genotypes, although varietal preferences across locations and gender groups were diverse. The majority of the genotypes selected at Melkassa and Alemetena were released varieties and breeding lines, which were dominated by white-seeded small beans. Awash-1 was the most preferred variety selected by both farmer groups. However, all the genotypes selected at Arsi Negele were red-seeded small beans, which were predominantly landraces. In addition, traits such as earliness and storage insect resistance, which were important in Melkassa and Alme tena received less attention in Arsi Negele. Culinary traits, which were a low priority for breeders, were also ignored by male farmers, while women found such traits important. The integration of the farmers' selection preferences with the breeders' criteria can improve the efficiency of plant breeding by developing crop varieties that better fit the specific needs of the farmers and that also increase the breeders' awareness of cultural and indigenous practices. The importance of addressing the missing link in the research-extension-farmer linkages in Ethiopia, for better diffusion and impact of improved varieties cannot be under-estimated.

Key words: *Phaseolus vulgaris*, farmers' selection criteria, participatory variety selection

6.1 Introduction

The common bean (*Phaseolus vulgaris* L.) is the most important food legume in Ethiopia. The crop is cultivated in several agro-ecological zones and farming systems. The common bean is mainly grown by small-scale farmers for household consumption, marketing and soil fertility improvement purposes (Wortmann et al., 1998; Asfaw et al., 2012; CSA, 2015). Ethiopian farmers have a higher preference to grow common beans, compared to other legumes, because they mature early, which helps them to obtain a cash income to buy food and other household needs. It also serves as an emergency crop in times of crop failure (Mekbib, 1997; Legesse et al., 2006).

The common bean was introduced to Ethiopia in the sixteenth century and farmers have been able to adapt, develop and maintain a large genetic diversity to suit their needs. A range of bean types are grown in the country, but small white and red beans are the most common and preferred types. The small white beans are mainly grown in the Oromiya (in the Central Rift Valley) and Amhara regions, for the export market. Ethiopia exports white beans to the canning industry in Europe (Ferris and Kaganzi, 2008). The small red beans, on the other hand, are grown mainly in the southern parts of the country and they are used for local and regional markets and for household consumption (Ferris and Kaganzi, 2008; Rubyogo et al., 2011; CSA, 2015). Recently, due to the rising demand in the international and domestic market, the common bean is being grown in almost all parts of the country, with varying intensity (Katungi et al., 2009; CSA, 2015). Common bean production in the Central Rift Valley (Oromiya region) comprises about 50% of the total bean production of the country. Ninety-five percent of common bean-growing farmers produce the small white beans (Alemu and Bekele, 2005; Legesse et al., 2006; CSA, 2015).

In Ethiopia, the National Common Bean Research Program plays an important role in meeting the increasing demand for the crop by releasing improved common bean varieties. Starting in the 1970s, the National Bean Program has developed and released more than 55 common bean varieties. Even though strong efforts have been made to disseminate these varieties, using different extension channels, the adoption rate has been slow, mainly due to the inaccessibility of improved seed (Pan Africa Bean Research Alliance, 2005; Dawit and Spielman, 2010; Buruchara et al., 2011). Over the pastfifteen years, the national bean research program, in collaboration with the International Center for Tropical Agriculture (CIAT) has been working on the decentralization of the seed systems. Consequently, a dramatic increase in the area of production and productivity of the common bean has been observed in the country. Between 2004 and 2012, the area for common bean production significantly increased from 181,600 to 330,000 ha and the total

production tripled to 387,000 tons per year. The average yield also increased from 0.62 to 1.50 t/ha (CIAT, 2013). Although considerable efforts have been made to improve the productivity of the crop in the country, there is still a huge gap between the potential and actual yield (Rubyogo et al., 2011; CIAT, 2013). Among the 55 improved varieties, only 18% were disseminated and adopted (Ferris and Kaganzi, 2008). The main reason for the poor adoption rate and low impact of the improved varieties are mainly due to the technological, socio-economical or agro-ecological constraints. Moreover, less coordinated efforts of the research and extension activities and poor integration of the farmers-consumers-traders value chain have by and large affected both the process and the outcome. To improve technology generation, dissemination and adoption, and to benefit from the available improved technologies, the different stakeholders (researchers, extension officers, farmers, consumers and traders) have to be part of the breeding process right from its inception. This can be done through participatory plant breeding in the identification of priority traits, on-farm demonstrations, popularization and re-evaluation of the technologies (Ceccarelli et al., 2000; Ceccarelli and Grando, 2007).

Participatory variety selection (PVS) is a powerful tool that involves farmers and other stakeholders to help orient breeding programs and to improve variety adoption (Sperling et al., 2001). It also assists plant breeders to develop technologies that fit into a specific production niche and the farmers' needs (Ceccarelli et al., 2000). The conventional plant breeding scheme uses a narrow range of selection criteria that addresses issues related to yield, uniformity and stability. Traditional farmers, however, employ more diverse and complex selection criteria, revolving around stable crop performance over seasons and they grow a range of genotypes that meet their needs in very complex and heterogeneous environments (Sperling and Loevinsohn, 1996; Ceccarelli and Grando, 2007). The farmers' preferences, as well as the socio-economic aspects, are often ignored by the conventional breeding programs. Farmer participation in setting breeding goals and varietal evaluation will remain critical for enhancing adoption and genetic diversity. A Participatory Variety Selection (PVS) can speed up the selection and fast-track the dissemination processes. In addition, it will eliminate a number of unacceptable varieties and save money and time (Mekbib, 1997; Assefa et al., 2006). The participatory evaluation of diverse common bean genotypes and the selection of parental material will be of paramount importance in designing possible improvement strategies, based on the farmers' priorities. Therefore, this study was carried out to evaluate diverse common bean genotypes, to identify suitable parental genotypes useful for breeding for bruchid resistance and to identify the farmers' selection criteria for choosing varieties.

6.2 Material and methods

6.2.1 Description of the study site

The study was conducted at three on-station trial sites in the Oromiya region of central Ethiopia (Figure 6.1). The three sites were Melkassa ($8^0$24'52.04"N, $39^0$19'41.22"E, 1550 m.a.s.l.), Alem Tena ($8^0$17'32.29"N, $38^0$56'48.77"E, 1611 m.a.s.l,) and Arsi Negele ($7^0$22'30.29"N, $38^0$40'17.78"E, 1960 m.a.s.l). This study was carried out in the main cropping season of 2014. The climatic data of Melkassa and Alem Tena were collected from the Melkassa and Debrie Zeit Agricultural research centers, respectively. The climatic data on rainfall and temperature for only the two sites is presented in Figure 5.1 and 5.2. The soil types of Melkassa and Alem Tena are sandy and loamy, while the soil in Arsi Negele is clay.

6.2.2 Experimental material

On the basis of their level of resistance, population structure and genetic distances, a total of 144 genotypes were selected. The selected common bean genotypes comprised of 109 landraces, 16 released varieties and 19 pre-release breeding lines. The 109 common bean landraces were collected from different regions of Ethiopia. Of the 18 pre-released genotypes, 15 genotypes were resistant to the Mexican bean weevil. The inclusion of landraces and pre-released varieties allows farmers to have more options and it allows them, to compare these genotypes with the released commercial varieties. This avoids the risk of the failure of adoption and allows the breeder to include the farmers' preferred traits in their breeding program. A list of the tested genotypes is given in Table 5.1. The 144 genotypes were planted in a 12 x 12 alpha lattice design, with three replications. The common bean genotypes were planted in one row of 3 m long, with an inter-row spacing of 60 cm and an intra-row spacing of 40 cm. Weeds were controlled with frequent hand-weeding throughout the experiment. Di-ammonium phosphate (DAP) fertilizer was applied during planting, at a rate of 100 kg/ha (Assefa et al., 2013a) and other agronomic practices were done as per recommendation for each site.

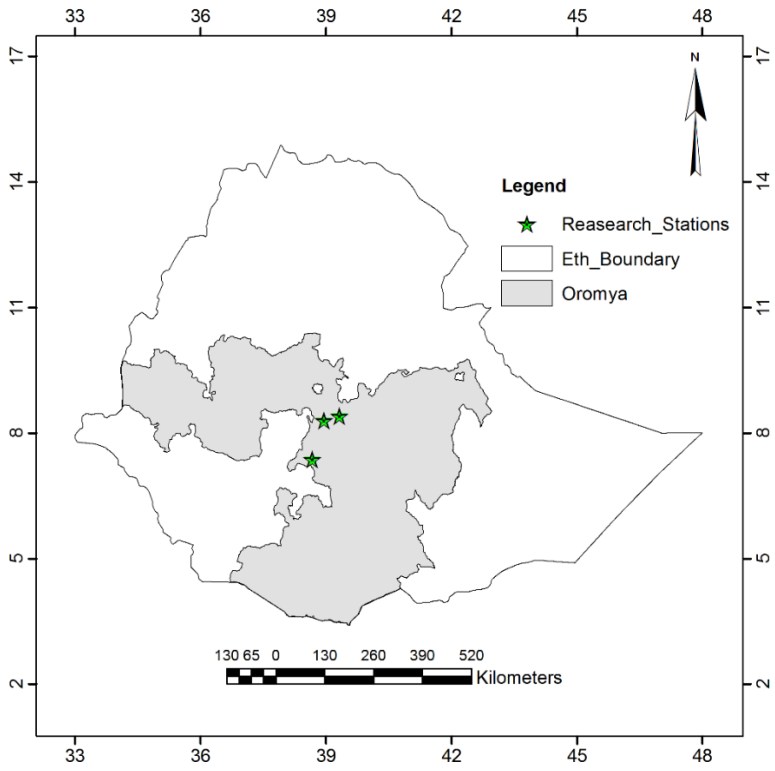

Figure 6.1 A map of Ethiopia showing the geographical positioning of the research stations used for participatory variety selection

6.2.3 Data collection and analysis

The participating farmers were selected, based on their indigenous knowledge, of bean production and their willingness to participate in the variety evaluation. The selection of participants was made with the help of development extension agents and technical assistants from each station. From each site, 20 (10 male and 10 female) common bean-producing farmers were selected. A visual evaluation of the genotypes was made when the crop was at the late pod filling and maturity stage. Focus group discussions were conducted to identify the common bean production constraints at each location. Local languages were used, to enable farmers to express their ideas easily during the discussion time.

The participating farmers were divided into male and female groups to explore the differences in the selection criteria between the two groups. A participatory variety selection (PVS) was applied to select common bean genotypes that possess the farmers' preferred traits and to facilitate the selection of parental genotypes for breeding for bruchid resistance. Initially, farmers were allowed to discuss and agree on criteria that they thought were important for selecting a given variety for

the different groups. Subsequently, the evaluation procedure was explained to the participating farmers and scoring was done individually. Four plastic tags, each with a different colour were given to the farmers, to facilitate the selection process (Figure 6.2 A). Plastic bags were put in each line in the field and farmers put the different coloured tags inside the plastic bags, based on their preferences (Figure 6.2 B). Seeds of each genotype were also displayed to the farmers, in order for the participants to observe the seed colours and sizes. The number of tags from each plastic bag from each genotype were counted. Immediately after the field evaluation, the best and the worst selected genotypes were identified and group discussions were held in the field, to rank the selection criteria of each group (Figure 6.3).

Figure 6.2 A. Farmers receive instruction on the participatory variety selection process at Melkassa. B. Farmers at Alem Tena select preferred varieties

Figure 6.3 A. Women farmers at Arsi Negele discussing the selection criteria, B. Group discussion at Melkassa after the variety selection

6.3 Results

6.3.1 Farmers' selection criteria

Farmers attending the participatory variety selection had different selection criteria for each gender group and location. In the focus group discussion, both men and women farmers were able to list 14 selection criteria. There were some traits listed by men and not by women. The ranking of the criteria was different for men and women, as well as for the different locations (Table 6.1). However, some similarities in the selection criteria were observed between Melkassa and Alem Tena. In all the locations, farmers used intricate combinations of traits for selecting common bean varieties. However, pod load and filling, as well as yield, were cited as the most important traits in both gender groups and in all locations. In addition, marketability, seed size and seed colour were perceived to be important selection criteria for men in all locations. Marketability was less important for women farmers in Melkassa and Arsi Negele, whereas taste was ranked fourth. In Melkassa and Alem Tena, earliness and drought tolerance were cited as important traits for both gender groups. The women farmers at Melkassa ranked earliness as the third most important selection criteria, while in Alem Tena and Arsi Negele, these traits were not important in the selection of common bean varieties (Figure 6.4). Resistance to insects and diseases was an important criterion in Arsi Negele, but not in other locations. In general, women ranked the taste and cooking time as the top criteria for varietal choice, while men did not consider these traits to be important. On the other hand, pod clearance and plant stand were ranked by the men, but they were not perceived as important by the women in all the locations. Stem strength ranked differently across locations, while the suitability of straw was ranked only at Melkassa and Alem Tena.

Table 6.1 Rank of selection criteria used by men and women farmers at Melkassa, Alem Tena and Arsi Negele

	Rank					
	Melkassa		Alem Tena		Arsi Negele	
Selection criteria	Male	Women	Male	Women	Male	Women
Pod load and filling	1	1	1	1	1	1
Yield	2	2	2	2	2	2
Drought tolerance	7	6	7	9	10	10
Marketability	3	9	3	3	5	7
Seed colour	4	7	4	4	3	3
Seed size and shape	5	8	5	5	4	5
Earliness	6	3	6	6	8	8
Insect and disease resistance	8	-	9	-	6	6
Taste	-	4	-	7	-	4
Cooking time	-	5	-	8	-	-
Plant stands	13	-	13	-	9	-
Stem strength	10	10	10	10	7	9
Pod clearance	12	-	11	-	8	-
Suitability of straw	9	11	8	11	-	-

- indicates that the criteria are not ranked for that location or farmers group

6.3.2 Farmers' variety selection

The results of the participatory variety selection of 144 genotypes at three research stations revealed that there was considerable variation among entries, based on the farmers' selection criteria. The ten best genotypes from each station were selected by farmers. At Melkassa, the ten genotypes selected by both men and women farmers were dominated by the white small-seeded beans. Genotypes, such as Awash-1, Awash Melka, 211333 and RAZ-42, were the top selected genotypes, followed by small and medium-sized red beans SCR-15, 211323 and SCR-11 (Table 6.2). In addition, yellow and speckled bean types were also selected by both groups. Red-and white-seeded beans (70% of selected genotypes) were most preferred by men, whereas yellow and white bean genotypes were selected by women. The commercial small white variety, Awash-1, was the most preferred variety and KAT-B1 was the earliest genotype in the trial selected at Melkassa.

In Alem Tena, the majority of the selected genotypes in both gender groups were small white beans, such as Awash-1, Awash Melka, 228812, 232196, 211347 and RAZ-40 and two yellow beans (NC-39, Wedo and Roba) (Table 6.2). The men selected six small white, two yellow (one small and one large), one speckled and one small red genotype. Genotypes selected by women farmers included six small white, four yellow (one small, one medium and two large) and one speckled coloured genotype. Awash-1, Deme, Awash Melka and NC-39 were the top selected, and the most preferred genotype in Alem Tena. In Arsi Negele, on the other hand, all the selected genotypes were small red-seeded beans, with the exception of SCR-15 and NC-16, which are large-seeded (Table 6.2). The landrace 214663 was the most selected genotype by both gender groups, while 214663, 241734, NC-07 and SER-125 were the most preferred genotypes of men. Similarly, 230526, 214663, NC-12 and SER-125 were the four top-ranked genotypes by women farmers in Arsi Negele. In Arsi Negele, 40% of the selected genotypes were landraces and only one released variety (Nasir) was selected. The two best genotypes selected in Aris Negele (214663 and 241734) were landraces collected from the southern part of Ethiopia. However, the majority of the selected genotypes in Alem Tena and Melkassa were released varieties and breeding lines, respectively.

A comparison of farmers' selection with the field performances of the selected genotypes revealed that farmers in Arsi Negele gave more attention to yield and yield-related traits than biotic and abiotic stress factors. However, in Melkassa and Alem Tena, farmers leaned more towards insect resistance and drought tolerance traits in their varietal selection practice (Table 6.1). In general, genotypes that were selected by Arsi Negele farmers were late maturing, with long grain filling duration, but relatively high grain and biomass yielders. At the other two stations, farmers' selected white small-seeded genotypes which are highly resistant to bruchid (RAZ lines), based on their agronomic performance. Similarly, the line RAZ 42 was one of the varieties selected by the national bean research program and submitted to the national variety release committee for verification and release. In Melkassa and Aleme tena, farmers selected genotypes that showed a wider range of variation for all the traits, except for grain yield. However, the genotypes selected in Arsi Negele had the widest range of variation for grain yield (Table 6.3).

Table 6.2 Lists of 10 best selected genotypes by farmers at Melkassa, Alem Tena and Arsi Negele

Rank	Melkassa			Alem Tena			Arsi Negele		
	Men	Women	All	Men	Women	All	Men	Women	All
1	Awash-1	NC-39	Awash-1	SCR-11	Awash-1	Awash-1	214663	230526	214663
2	Awash Melka	230525	NC-39	NC-39	Awash Melka	Deme	241734	214663	241734
3	SCR-15	Awash Melka	SCR-15	Awash-1	RAZ-42	Awash Melka	NC-07	NC-12	NC-07
4	NC-39	SCR-15	Awash Melka	211347	Deme	NC-39	201066	SER-125	SER-125
5	207934	Deme	KAT-B1	228812	Wedo	228812	SER-125	SCR-15	NC-12
6	KAT-B1	KAT-B1	211333	Deme	Roba	Wedo	214665	Nasir	SCR-15
7	211333	NC-15	211323	230661	NC-39	232196	NC-51	241734	Nasir
8	211323	NC-29	Deme	Awash Melka	RAZ-40	Roba	NC-12	NC-07	201066
9	NC-30	Awash-1	SCR-11	232196	NC-15	211347	SCR-15	NC-16	214665
10	SCR-11	RAZ-42	RAZ-42	Roba	228812	RAZ-40	Nasir	201066	NC-16

Table 6.3 Seed colour, size, breeding status and means of farmers selected common bean genotypes for bruchid resistant and other agro-morphological traits measured under laboratory and field conditions

Melkassa												
Genotype	SS	SC	Type	PAE	SWL	DTM	GFP	PPP	SPP	AGBM	GY	HSW
Awash-1	Small	White	VAR	86.5	46.0	84.7	45.3	27.0	3.8	28.9	24.6	18.7
NC-39	Medium	Yellow	LDR	84.5	29.0	86.0	46.7	15.8	3.4	35.9	22.8	26.3
SCR-15	Medium	Red	BRL	82.5	16.5	85.0	45.0	27.7	3.7	32.2	30.3	38.4
Awash Melka	Small	White	VAR	60.0	0.5	89.7	47.7	22.1	7.9	39.8	37.7	23.3
KAT-B1	Large	Yellow	VAR	80.0	26.0	76.0	40.3	11.9	3.4	23.6	12.9	42.6
211333	Small	White	LDR	86.0	22.0	88.0	47.0	26.0	5.4	32.9	17.5	14.0
211323	Small	Red	LDR	87.5	3.0	91.0	50.0	28.0	3.4	30.7	25.1	25.3
Deme	Large	Speckled	VAR	66.5	28.0	87.3	44.0	17.1	3.8	43.0	39.3	59.9
SCR-11	Medium	Red	BRL	14.5	6.0	84.7	45.3	27.4	4.5	36.5	34.7	29.4
RAZ-42	Small	White	RLN	2.0	0.0	85.0	46.3	31.2	4.8	43.8	29.6	22.0
Mean	-	-	-	65.0	18.0	85.7	45.8	23.4	4.4	34.7	27.5	30.0
SE	-	-	-	1.9	0.9	4.1	2.5	6.4	1.4	6.4	8.6	13.5
CV%				48.4	85.5	1.3	0.8	2.0	0.4	2.0	2.7	4.3
Aleme tena												
Genotype	SS	SC	Type	PAE	SWL	DTM	GFP	PPP	SPP	AGBM	GY	HSW
Awash-1	Small	White	VAR	86.5	46.0	86.7	43.7	28.2	4.1	29.7	25.0	18.0
Deme	Large	Speckled	VAR	66.5	28.0	97.0	50.7	15.0	3.1	37.1	30.1	57.1
Awash Melka	Small	White	VAR	60.0	0.5	90.0	44.3	39.3	5.4	50.2	37.0	21.5
NC-39	Medium	Yellow	LDR	84.5	29.0	86.7	44.0	20.2	6.1	37.8	25.7	25.4
228812	Small	White	LDR	77.5	40.5	94.0	48.7	36.4	3.7	36.7	22.9	16.3
Wedo	Medium	Yellow	VAR	89.5	18.0	81.0	40.3	17.0	4.1	34.1	24.3	32.8
232196	Small	White	LDR	74.5	14.5	91.7	46.3	47.0	3.8	36.6	29.5	15.7
Roba	Small	Yellow	VAR	49.5	22.0	93.7	46.7	35.8	5.0	44.1	29.9	18.1
211347	Small	White	LDR	79.0	6.5	80.1	37.3	32.3	3.8	31.5	18.9	18.4
RAZ-40	Medium	White	RLN	0.0	0.0	88.7	47.0	22.1	3.4	39.2	33.6	37.8
Mean	-	-	-	67.0	20.5	88.9	44.9	29.3	4.2	37.7	27.7	26.1
SE	-	-	-	1.7	1.0	5.5	3.9	10.6	0.9	5.9	5.3	13.2
CV%	-	-	-	39.8	76.8	1.7	1.2	3.3	0.3	1.9	1.7	4.2

				Arisnegele								
Genotype	SS	SC	Type	PAE	SWL	DTM	GFP	PPP	SPP	AGBM	GY	HSW
214663	Red	Small	LDR	90.0	23.0	101.3	55.7	28.4	4.9	41.2	34.7	24.0
241734	Red	Small	LDR	79.5	12.0	100.3	54.0	29.4	4.3	48.2	30.5	23.2
NC-07	Red	Small	LDR	70.5	34.0	102.0	46.7	34.2	3.8	38.9	25.4	25.0
SER-125	Red	Small	VAR	91.0	26.5	102.0	55.7	23.2	5.3	36.4	33.4	24.5
NC-12	Red	Small	LDR	84.0	34.0	103.0	55.0	21.8	5.5	36.9	29.8	23.8
SCR-15	Red	Medium	BRL	82.5	16.5	103.0	56.3	28.7	3.6	46.3	32.6	38.5
Nasir	Red	Small	VAR	83.5	43.0	105.7	60.7	42.6	3.8	50.0	45.4	23.5
201066	Red	Small	LDR	84.5	9.5	99.0	53.3	21.9	6.3	44.6	30.9	23.5
214665	Red	Small	LDR	93.5	29	102.7	57.0	31.5	5.0	39.2	30.7	21.6
NC-16	Red	Small	LDR	62.5	17.5	99.3	53.3	20.1	5.5	44.0	24.4	23.1
Mean	-	-	-	82.0	24.5	101.8	54.8	28.2	4.8	42.6	31.8	25.1
SE	-	-	-	0.6	0.7	2.0	3.6	6.9	0.9	4.8	5.8	4.8
CV%	-	-	-	11.6	43.8	0.6	1.1	2.2	0.3	1.5	1.8	1.5

SS = seed size; SC = seed colour; PAE = percentage adult emergence; SWL = seed weight loss; DTM = days to maturity; GFP = grain filling period; PPP = pods per plant; SPP = seeds per pod; HSW = hundred seed weight; AGBM = aboveground biomass; GY = grain yield

Figure 6.4 Women farmers select for earliness at Melkassa (A) and red bean selection by farmers at Arsi Negele (B)

The grain yield and days to maturity of the farmers' selected genotypes at all locations are presented in Figures 6.5. A significant range of the variations for grain yield and maturity was recorded among the selected genotypes. Among the selected genotypes, KAT-B1 and Nasir were the earliest (76) and latest (106) genotypes to mature, respectively. In addition, the mean grain yield for selected genotypes ranged from 12.9 g/plants (KAT-B1) to 45.4 g/plants (Nasir).

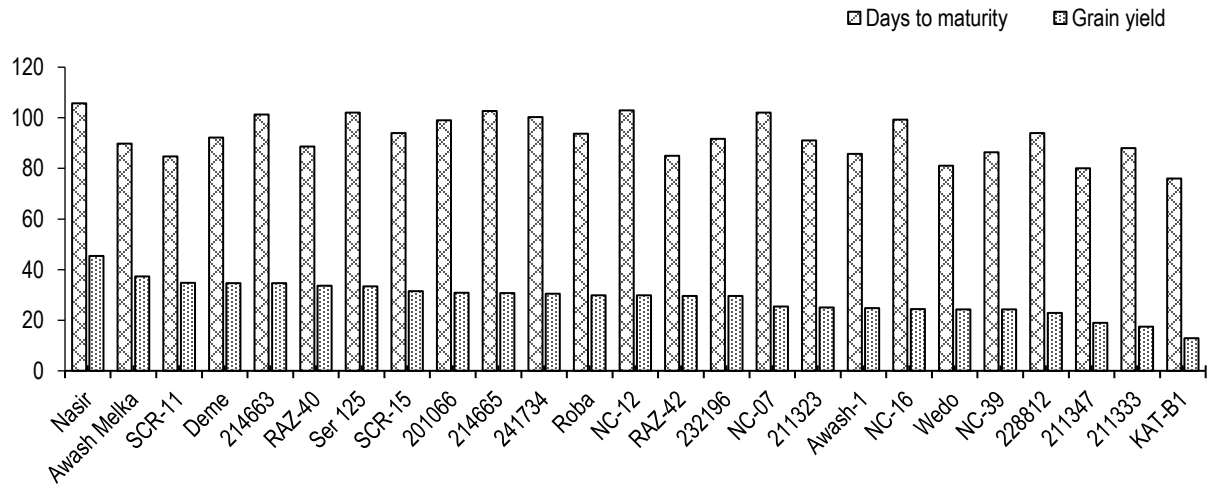

Figure 6.5 Genotypes selected by farmers and their average yield (g/plant) and days to maturity in 2014 at Melkassa, Alem Tena and Arsi Negele

6.3.3 Post-harvest usage and problems

Farmers at Melksassa and Alem Tena generally use beans as boiled grain (*Nifro*) and for stew (*Wot*). Red and yellow beans are primarily used for boiled grain and the white beans are used for stew. Yellow beans are considered very tasty and have a short cooking time. In Alem Tena, farmers also use speckled beans for consumption. In addition to boiled grains and stews, farmers in Arsi Negele use beans for making soup. In this region, yellow and speckled bean types are more preferred than the white ones. The red bean variety (Nasir) is the most marketable bean in the area. However, speckled and yellow beans have also recently been attracting the consumers' attention in the market.

Farmers in Melkassa and Alem Tena recognized bruchids as the most important storage pest, while farmers in Arsi Negele perceived bruchid as a less important problem. Farmers used several insect management practices, such as chemicals (Phostoxin and Malathion) and different cultural practices, such as mixing beans with ash and hot pepper powder to reduce the grain loss caused by the insect. Storing beans with ash and pepper powder hinders the mobility and oviposition of the insect. According to the farmers in Melkassa, Awash Melka had some tolerance, compared to other released varieties, but the variety has a low marketability in the area. Alem Tena farmers indicated that yellow beans are more susceptible to bruchid than red and white genotypes. Farmers at both locations sold their beans soon after harvest, to avoid losses due to bruchids.

6.4 Discussion

The common bean, because of its short life-cycle compared to cereal crops, is perceived by farmers as a food security crop. In the present study, male and female farmers were invited to assess and select the genotypes, based on their preferences in on-station trials at three sites. The farmers were well aware of the selection criteria and that the different areas had different selection criteria. The selection criteria were associated with the socio-cultural and agro-ecological conditions of the areas. McGuire (2007) also reported that, in a highly heterogeneous farming environment, the farmers' preference and varietal choice is a result of the interaction of the social, economic and environmental factors. Furthermore, in such diverse farming systems, farmers employ a wide range of criteria for selecting their preferred genotypes. In order to understand the farmers' preferences and to closely work with farmers, participatory studies are an essential component of a plant breeding program. Ceccarelli and Grando (2006) also reported that participatory research is important, in order to understand the traits or combinations of traits that are of interest to farmers.

The common bean genotypes selected by farmers varied amongst the three sites and the gender groups. The farmers' preference and selected genotypes at Melkassa and Alem Tena showed some level of similarity. This is mainly due to the fact that these two environments are both drought-prone areas. In these areas, farmers traditionally prefer small white-seeded bean varieties, such as Awash-1 and Awash Melka which were ranked as the best genotypes. These two varieties were released for the Central Rift Valley areas. Moreover, farmers in the Central Rift Valley produce white beans for export and Awash-1 is the dominant genotype for this purpose (Alemu and Bekele, 2005; Legesse et al., 2006; Assefa et al., 2013b). In addition to the white beans, farmers at Melkassa and Alem Tena selected yellow, red and speckled beans of various seed sizes. The most recently-released yellow seeded variety, which was introduced from Kenya, gained popularity in the Melkassa area due to its extreme earliness.

Arsi Negele, on the other hand, is situated in the mid-altitude area of the southern Rift Valley region and has a relatively high rainfall climate. In this area, all the selected genotypes were small to medium-sized red cooking bean types. Farmers in this area produce the red beans for household consumption and the local markets (Ferris and Kaganzi, 2008; Asfaw et al., 2009, 2012; Rubyogo et al., 2010), although some red beans are informally exported to the regional markets of northern Kenya (Ferris and Kaganzi, 2008; Rubyogo et al., 2010). Similarly, Asfaw et al. (2012) also reported that the small red and black beans were the most preferred varieties in the southern part of Ethiopia, while the small white beans were rated poorly. Of the top ten red beans selected in Arsi Negele, the first three genotypes were landraces. Acc.no 214663 was the most preferred landrace, which was originally collected from the southern part of the country. At Arsi Negele, some farmers have started growing the speckled beans for both local consumption and the market. Although farmers have a strong preference towards red and white bean types in the study area, farmers grow several bean genotypes for multiple household uses. This suggests that farmers are flexible and willing to produce a range of common bean types, in addition to the well-acquainted white and red small- seeded bean varieties.

Varietal choice and related selection criteria may vary for different groups of farmers (de Boef and Thijssen, 2006). Gender is one major social category in which variation can be expected, in this regard. Women and men have different gender roles and responsibilities in their society, on the farm and in the household. Consequently, these roles affect their decision to choose a variety or sets of varieties. Both gender groups had a similar preference for yield and yield- related traits and a variety with high-grain yield is obviously preferred by both men and women. In the present study, all farmers who participated in the PVS ranked yield and pod load and filling as their top

selection criteria in all the locations. A similar result from a PVS was reported for bean genotypes by other researchers in the Central Rift Valley and eastern parts of Ethiopia (Mekbib, 1997; Assefa et al., 2005, 2013b).

However, differences were observed between men and women for other selection criteria. Men tended to focus more on seed size, seed colour and market-related traits. The tendency towards seed colour and size is highly influenced by market preferences in the different locations. However, women are generally more interested in post-harvest processing and food preparation aspects. Women have an important role in the assessment of postharvest qualities, such as taste, cooking quality and time. On the other hand, men totally ignored the culinary quality of beans. Assefa et al. (2013b) also reported on the variation between the gender groups with regard to market and use-related traits. This signifies the importance of involving participants from different farming systems and farmers' groups in the participatory studies. In Arsi Negele, diseases and insects are the most prevalent production constraints. The pressure of disease is generally high, compared to the other locations, due to the high rainfall in the area. Arsi Negele farmers totally ignored the cooking time and the suitability of straw, as selection criteria because fuel wood and other forage crops are available in abundance.

The choice of selection criteria was significantly associated with the prevalent environmental conditions. In areas where drought and disease problems are prevalent, men and women farmers tend to have similar preferences. In Melkassa and Alem Tena, drought-related traits, such as earliness and drought tolerance were among the top listed traits, whereas in Arsi Negele, disease and insect resistance was more vital. Melkassa and Alem Tena are characterized by low rainfall of a short duration. In these areas, farmers consider earliness and drought tolerance as important traits for selecting bean varieties (Asfaw et al., 2012). Although the yield potential of KAT-B1 is low, farmers selected it because of its earliness, as the common bean is the first food available for the household in drought-prone areas in central Rift Valley. Women farmers prefer early genotypes to the high yielding late maturing genotypes, in order to fulfill the food needs of the household (Assefa et al., 2013b).

Although the storage insect pests were found to be the major problem in all locations, the problem is more severe in Melkassa and Alem Tena than in Arsi Negele. This may be related to the favorable environmental conditions for the growth and the development of the insect at the two sites, compared to the cool and humid Arsi Negele. Farmers were able to recognize the good

tolerance level of the released variety Awash Melka, to the insect. This fact was also confirmed in our laboratory analysis (Chapter 2).

6.5 Conclusion

In the present study, the farmers' most preferred traits and genotypes were identified. The selection criteria included yield, pod load and filling, drought tolerant, seed colour, size and shape, earliness, drought tolerance, insect and disease resistance, taste and cooking time. The relative importance of the selection criteria varies from location to location, and among farmer groups. The variation in the selection criteria is highly influenced by socio-cultural, economic and agro-ecological factors. The study confirmed that both men and women need to be involved in identifying farmers' preferences, setting priorities and re-orienting research directions. Understanding of varietal trait preferences across farming systems and farmer groups, will provide new insights for breeders to anticipate which traits and trait combinations can benefit the target farming system or farmer group. This can be done through a participatory variety selection that enhances the development of demand-driven, client- oriented crop technologies, dissemination and adoption. The information generated from this study can be utilized by plant breeders for the incorporation of farmers preferred traits into the beans breeding program.

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CHAPTER 7

Introgression of Arcelin Gene into Commercial Varieties and Advanced Lines of the Common Bean

Abstract

The Mexican bean bruchid (*Zabrotes subfasciatus* Boheman) is the most destructive storage pest of beans in the Central Rift Valley regions of Ethiopia and can cause 38% to 60% grain loss within six months of storage. Breeding for resistance against the bean bruchid is a major component of an integrated pest management strategy in bean production. The objective of the study was to introgress arcelin-based resistance into selected commercial cultivars and advanced breeding lines. In this study, the phenotyping results of the crosses derived from SCR-15 (highly susceptible parent) and MAZ-200 (highly resistance parent) are presented. For the specific cross above, a total of 208 progeny families were developed from pedigree breeding. Phenotyping was conducted at the F_4 generation, using five response characters to bruchid infestation, under laboratory conditions and using a randomized complete block design, with three replications. Highly significant differences ($P < 0.001$) among the entries, parents and offspring were recorded for almost all of the parameters, in response to bruchid infestation. However, there was no significant difference between the two parents in the number of eggs laid. Based on the percentage adult emergence, 34.6% of the progeny genotypes were categorized as highly resistant, 12.0% were resistant, 21.6% were moderately resistant and 32.7% were susceptible. The study observed considerable phenotypic variations among the offspring and parental lines for the susceptibility parameters. The levels of broad sense heritability ranged from 68.5%–93.9% for all the traits, suggesting that selection may be useful in improving bruchid resistance. The results obtained in the present study can be utilized to develop new bruchid resistance varieties of common bean.

Key words: Arcelin gene, common bean, Mexican bean weevil

7.1 Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most important legumes worldwide and serves as the daily diet for more than 300 million people (Lioi and Piergiovanni, 2013). In eastern and southern Africa, it is the second most important dietary protein source (Cardona and Kornegay, 1999). Despite its importance, the crop is affected by various biotic and abiotic stress factors. Of the biotic factors, the storage insect pests *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say) are the most destructive grain pests (Cardona et al., 1992; Cardona, 2004). The grain damage caused by the insects reduces the quality, marketability, germination and seedling vigour (Blair et al., 2010a). In developing countries, the two bean bruchid species cause up to a 13% loss in stored bean grain (Cardona and Kornegay, 1999). In the warmer regions of eastern and central Africa, *Z. subfasciatus* is the most common (Nchimbi and Misangu, 2002; Tadesse et al., 2008) storage pest. In the Central Rift Valley of Ethiopia, where the common bean is one of the major crops, *Z. subfasciatus* is considered to be a serious storage pest that causes immense damage on stored beans (Negasi, 1994; Wortmann et al., 1998; Getu et al., 2003).

The Mexican bean weevil (*Z. subfasciatus*) is more prevalent in the lower altitudes (< 1000 meters above sea level) and warmer areas. Hence, *Z. subfasciatus* is more important in the tropical and subtropical regions (Hill, 1983). The infestation and damage by *Z. subfasciatus* occurs only in storage (Schoonhoven, 1976) and most of the cultivars and landraces are susceptible to the pest (Schoonhoven and Cardona, 1982). Very few wild common bean genotypes, which originate from the central highlands of Mexico, were found to be resistant (Schoonhoven et al., 1983; Acosta-Gallegos et al., 1998). One mechanism of resistance is believed to be antibiosis, which is conferred by the seed storage proteins produced by the APA (arcelin, phytohemagglutinin and α-amylase inhibitor) gene family (Schoonhoven et al., 1983; Acosta-Gallegos et al., 1998). The antibiosis exhibited through insecticidal activities affects the emergence of adult bruchids and hampers insect growth and development (Osborn et al., 1988). Although the APA proteins differ in their biochemical and physiological properties, their expression shows similar patterns (Moreno et al., 1990). Based on protein size and electrophoresis patterns, arcelin is different from all the other APA proteins (Romero-Andreas et al., 1986). Several variants of arcelin were identified, each with a different effect on *Z. subfasciatus*. Currently, eight variants of arcelin (Arc-1 to Arc-8), with different levels of resistance have been identified from wild accessions of the common bean (Osborn et al., 1986; Lioi and Bollini, 1989; Santino et al., 1991; Acosta-Gallegos et al., 1998; Zaugg et al., 2012). Arc-5 and Arc-1 showed the highest level of resistance to *Z. subfasciatus*,

followed by Arc-4, Arc-2 and Arc-3, in their order of importance (Cardona et al., 1990). The Resistance to Zabrotes (RAZ) lines have been developed by CIAT, using Arc-1 variant lines through backcrossing (Cardona et al., 1990), while the Marker Assisted Zabrotes (MAZ) lines were developed by crossing RAZ lines and different genotypes. Several RAZ and MAZ resistant lines were identified in this study, while all of the commercial varieties, landraces, and advanced breeding lines were susceptible to the insect. Therefore, the objective of this study was to interogress arcelin genes from the resistant lines into a highly-productive and susceptible commercial variety and advanced breeding lines.

7.2 Materials and methods

7.2.1 Plant material and crosses

Based on the level of resistance to bruchid infestation, agronomic suitability and participatory variety selection, 15 parental materials were selected from different market classes. Two separate crossing blocks were established, based on the market class of the genotype. The first crossing block was composed of eight small white bean genotypes, of which three were commercial varieties (Awash-1, Awash-2 and Awash Melka) and five were donor resistant to Zabrotes (RAZ) lines (RAZ-2, RAZ-11, RAZ-36, RAZ-42 and RAZ-120). The second crossing block consisted of five small to medium red beans, namely, two commercial varieties (Nasir, SER-125), an advanced breeding lines (SCR-15) and two donor Marker Assisted Zabrotes (MAZ) lines (MAZ-200 and MAZ-203).

The females were selected, based on their agronomic performance and the participatory variety selection. SCR-15 is an advanced breeding line that has been evaluated under 18 different bean-growing environments and submitted to the variety release committee for release in 2017 (Negash et al., 2014). However, this line is highly susceptible to bean bruchid (Figure 7.1). Figure 7.1 presents the selected parental genotypes and their level of resistance to bean bruchid. The crosses were made at the Melkassa Agricultural Research Center. Although most of the bean crossing activities of the center have been conducted under field conditions, the crosses of the present study were conducted in the screen house, to avoid natural insect and disease infestation. The F_1 plants were selfed, in order to generate enough seed for the subsequent experiments (Figure 7.2).

Figure 7.1 Selected susceptible (SCR-15) and resistant (MAZ-200) red bean parental genotypes

Figure 7.2 Screen house crossing block at Melkassa during 2015 offseason

The F_1 seeds of the cross between Nasir and MAZ-200 either failed to germinate or the F_1 plants were dwarfed and failed to grow beyond the two-leaf stage (Figure 7.3 B). This may be related to the incompatibility of the inter-gene pool cross (Singh, 1989), as the two parents were from different gene pools i.e. Nasir was from the Mesoamerican gene pool and MAZ-200 was form the Andean gene pool.

Figure 7.3 Parental and offspring genotype performance under field condition: A: parental genotype Nasir and B: F1 plants derived from the cross between Nasir/MAZ-200

7.2.2 Phenotyping

The screening of a very large number of F_4 genotypes for bruchid resistance, under laboratory conditions requires a large quantity of seeds and a large laboratory space. Although two sets of crosses were made, using the MAZ and RAZ lines, the author gave priority to phenotype the MAZ lines. In this chapter, out of the 19 successful crosses, only F4 plants derived from the SCR-15 and MAZ-200 crosses were considered.

The crosses were advanced to the F_4 generation to get sufficient seed for the laboratory experiments. For the present study, a total of 208 F_4 families derived from the cross SCR-15 and MAZ-200, were phenotyped in the Entomological Research Laboratory at the Melkassa Agricultural Research Center, Ethiopia, under ambient temperature and relative humidity conditions. The insect-rearing and bruchid- screening protocols were described in detail in Chapter 2. Fifteen seeds were placed in a transparent plastic jar of 6 cm x 7 cm size. To provide for proper ventilation, the lids of the plastic jars were perforated and covered with mesh that had a small pore size, to prevent the bruchids from escaping. Each jar was infested with three pairs of (female and male) newly- emerged bruchids. The jars that contained the bean seeds and bruchids were laid out in the laboratory, in a randomized complete block design (RCBD), with three replications. The jars were incubated for oviposition for 10 days, under controlled laboratory conditions. After 10 days, the parental bruchids were removed and the number of eggs laid was counted. The jars were monitored on a daily basis, to observe for any progeny bruchid

emergence. From the first appearance of bruchid progeny, the jars were monitored every two days, for recording purposes, and for the removal of newly-emerged bruchids.

Data were recorded on the number of eggs per adult female, the number of adult bruchids emerged, the percentage of adult bruchids emerged, seed damaged (%) and the seed weight loss (%). The detailed procedure for data collection and analysis are presented in Chapter 2. The percentage adult weevil emergence was calculated, based on the total number of adults emerged compared to the number of eggs laid. The genotypes were classified, based on the percentage adult emergence as described by Blair et al. (2010b). The genotypes with adult emergence from 0 to 15% were classified as highly resistant (HR), those from 15 to 30% as resistant (R), those from 30 to 50% as intermediate resistance (IR) and those from 50 to 100% as susceptible (S).

To ensure the homogeneity of variance, the data, based on count and percentage values were transformed, using natural log and arcsine transformation, respectively. The phenotypic data were subjected to statistical analysis, using GenStat Version 18 (Payne et al., 2017). Tukey's honestly significant difference test was used for comparisons of the mean values. The variance components were estimated, using the REML tool in GenStat Version 18. During the analysis, both the genotypes and replications were considered as random effects. Heritability, in a broad sense was estimated as:

$$h^2 = \frac{\sigma^2 G}{\sigma^2 P}$$

Where:

$\sigma^2 G$ = genotypic variance and $\sigma^2 P$ = total phenotypic variance

7.3 Results

7.3.1 Analysis of variance (ANOVA) and heritability estimates

The mean squares for five response characters to bruchid infestation are shown in Table 7.1. The analysis of variance revealed highly significant differences (P<0.001) among the genotypes for all the traits. Traits, such as the number of adults emerged and the percentage adult emergence were significantly different (P<0.001), and the percentage seed damage and the percentage seed weight loss were significantly different (P<0.05), while they were not significant for the number of eggs (P>0.05) between the two parents. However, the 208 offspring revealed highly significant differences (P<0.001) for all the traits.

Table 7.1 Analysis of variance (ANOVA) for five response characters studied on 208 F_4 families' and parental genotypes after infestation by bean bruchid

Source of variation	DF	Mean Squares				
		NE	NAE	PAE	PD	SWL (%)
Rep	2	139.80	157.38	526.70	552.20	11.10
Genotypes	209	622.10**	402.41**	2342.60**	2335.10**	174.71**
Parents	1	860.70^{ns}	2742.00**	7351.30**	2722.90*	310.11*
Offspring	207	624.80**	384.09**	2288.40**	2320.40**	172.62**
Residual	418	195.90	36.63	130.60	140.20	18.98

DF = degrees of freedom; NE = number of eggs; NAE = number of adult emerged; PAE = percentage adult emergence; PD = percent seed damage; SWL (%) = seed weight loss
* significant at P = 0.01; ** significant at P < 0.001; ns= non-significant

The mean, minimum, maximum, heritability and coefficient of variation values, for five susceptibility parameters of bean bruchid infestation are presented in Table 7.2. The number of eggs laid ranged from 3.0 to 114.0, with a mean of 35.7 eggs per genotype. However, the number of adults emerged ranged from 0.0 for the resistance genotypes, to 58.0 for the highly susceptible genotypes, with a mean of 12.0 adults per genotype. Similarly, out of the total eggs laid on each genotype, none of the eggs (0.0%) hatched on the resistant genotypes, while all the eggs (100.0%) hatched into adults on the highly-susceptible genotypes, with a mean percentage adult emergence of 34.3 per genotype. The range for the percentage seed damage and seed weight loss was from 0.0 to 93.3% and 0.0 to 33.8, respectively. The coefficient of variation calculated for the five parameters among the 210 genotypes ranged from 12.2% for number of eggs to 42.7% for percentage seed damage. The minimum (68.54%) and the maximum (93.93%) broad sense heritability were recorded on the number of eggs and percentage adult emergence, respectively.

Table 7.2 Summary statistics on five response characters evaluated on 208 F4 families' and parental genotypes under bean bruchid infestation

Trait	Min	Max	Mean	H^2 (%)	CV (%)
Number of eggs	3.00	114.00	35.67	68.54	12.2
Number of adult emerged	0.00	58.00	12.02	89.96	24.6
Percentage adult emergence	0.00	100	34.29	93.93	42.4
Percentage seed damage	0.00	93.30	32.75	93.68	42.7
Percentage seed weight loss	0.00	33.80	11.33	88.72	38.7

H^2 = broad sense heritability; CV = coefficient of variation

7.3.2 Phenotypic performance of parents and offspring

On the basis of percentage adult emergence, the progenies were classified into four classes. Genotypes with a PAE value of between 0.0 to 15.0% were categorized as highly resistant, 15. 0% to 30.0% as resistant, 30.0% to 50.0% moderately resistant, and greater than 50.0% as susceptible. Based on the above classifications, 34.6% of the progenies were found to be highly resistant, 12.0% resistant, 21.6% moderately resistant and 32.7% susceptible (Table 7.3). Although the maximum number of eggs were laid in the highly-resistant genotype category, less than 15% of the eggs emerged into adults and the percentage seed damage and percentage seed weight loss ranged from 0.0 to 26.7% and 0.0 to 16.9%, respectively. In the susceptible genotypes, on the other hand, the least number of eggs were laid, but about 95% of the laid eggs hatched into adults and caused significant seed weight loss. As the number of emerged adults increased, the percentage seed damage and seed weight loss increased. In the present study, the resistant parent MAZ-200 was grouped in the highly- resistant genotype category and 40 of its offspring revealed a similar resistant response, with a 0% percentage adult emergence (Table 7.4). Figure 7.4 presents some of the highly resistant offspring and the resistant parent MAZ-200. The eggs laid were clearly observed and pictures were taken at the end of the experiment (60 days after the first infestation).

Table 7.3 Classification of progeny genotypes, based on bruchid susceptibility classes using percentage adult emergence

Class	Offspring		Traits				
	No	%	NE	NAE	PAE	PD	PSWL
Highly Resistant (0 - 15%)	72	34.6	7.3 - 74.7	0.0 - 0.7	0.0 - 14.6	0.0 - 26.7	0.0 - 16.9
Resistant (15 - 30%)	25	12.0	9.0 - 62.7	2.3 - 14.0	15.7 - 29.4	8.9 - 40.0	7.2 - 18.2
Intermediate (30 - 50%)	45	21.6	5.7 - 66.7	2.7 - 29.0	31.8 - 47.1	15.6 - 64.4	11.0 - 22.0
Susceptible (50 - 100%)	68	32.7	8.0 - 62.0	5.7 - 53.3	50.4 - 95.3	20.0 - 88.9	5.4 - 24.7

NE = number of eggs; NAE = number of adult emerged; PAE = percentage adult emergence; PD = percentage seed damage; PSWL = percentage seed weight loss

Figure 7.4 Parental line MAZ-200 and some of the highly resistant progeny genotypes

Twenty-six (12.5%) progenies showed >70% percentage adult emergence (Table 7.5), while 68 (33%) progenies had >50% adult emergence. The highly susceptible parent SCR-15 revealed that a maximum percentage adults emerged (95.3%) and that there was an above average percentage seed damage and seed weight loss (Table 7.5). Figure 7.5 shows some of the susceptible progenies, and the highly susceptible parent SCR-15 60 days after the first infestation, with the damaged seed clearly visible. In the present study, the majority of the progenies (47%) were found to be resistant.

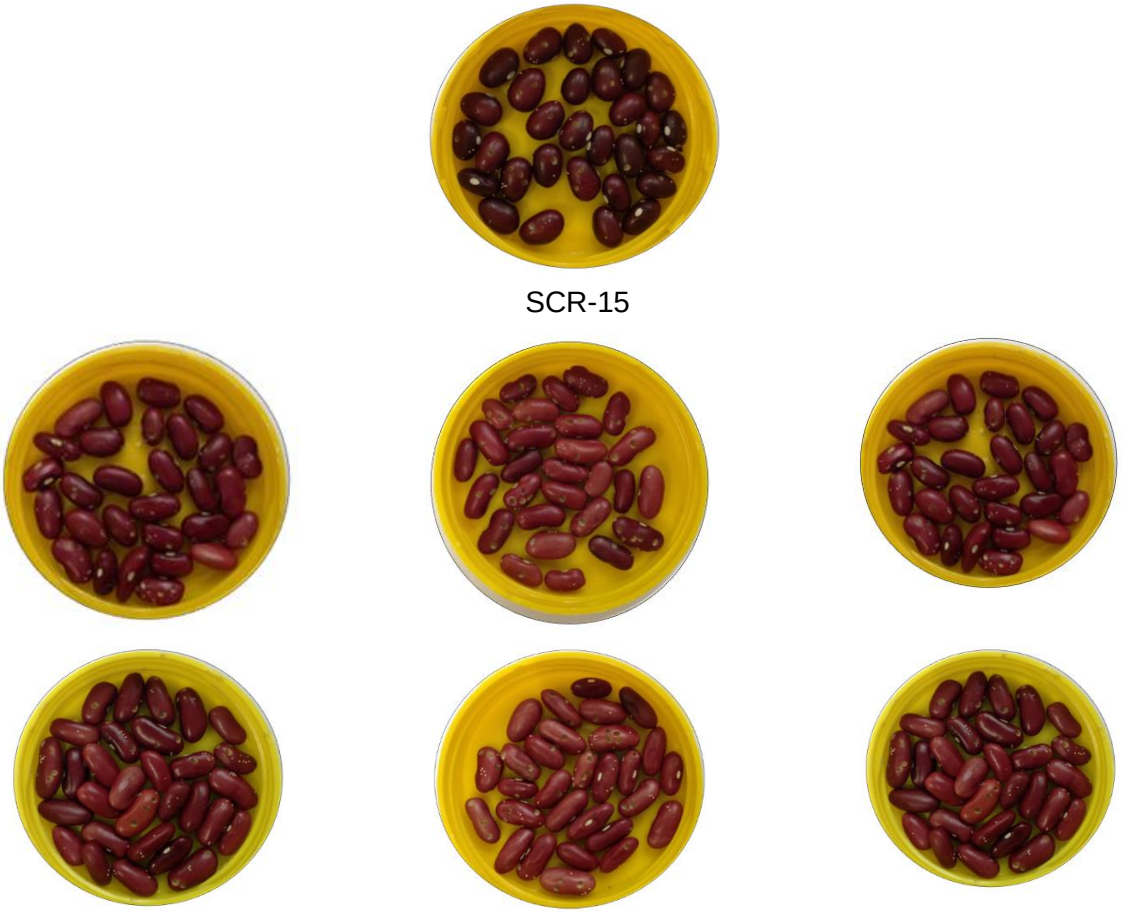

Figure 7.5 Parental line SCR-15 and some of the susceptible progeny genotypes

Table 7.4 Mean responses to bruchid infestation among the highly resistant F_4 families

Offspring	NE	NAE	PAE	PD	PSWL
SCR15/MAZ-200-010	41.7	0.0*	0.0*	0.0*	0.0*
SCR15/MAZ-200-018	18.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-020	21.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-022	27.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-028	17.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-030	45.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-031	41.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-032	44.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-034	7.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-038	34.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-054	43.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-056	34.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-060	26.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-068	7.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-084	43.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-089	24.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-103	25.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-104	72.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-106	29.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-107	46.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-115	74.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-121	46.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-129	52.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-131	53.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-135	51.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-146	45.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-149	35.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-153	41.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-158	24.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-160	29.3	0.0	0.0	0.0	0.0
SCR15/MAZ-200-161	19.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-167	14.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-168	51.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-180	36.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-187	27.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-188	48.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-191	65.7	0.0	0.0	0.0	0.0
SCR15/MAZ-200-194	45.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-198	40.0	0.0	0.0	0.0	0.0
SCR15/MAZ-200-207	18.7	0.0	0.0	0.0	0.0
MAZ-200 (Resistant parent)	**48.7**	**0.0**	**0.0**	**0.0**	**0.0**

NE = number of eggs; NAE = number of adult emerged; PAE = percentage adult emergence; PD = percentage damage; PSWL = percentage seed weight loss; * 0 value indicated that there is no adult emerged and concequently no percentage adult emergence, percentage damage and seed weight loss

Table 7.5 Mean responses for bean bruchid infestation characters among the highly susceptible F_4 families with percent adult emergence >70% after infestation

Entry	NE	NAE	PAE	PD	PSWL
SCR15/MAZ-200-009	46.3	35.7	77.0	66.7	14.8
SCR15/MAZ-200-011	48.7	33.7	71.2	80.0	7.0
SCR15/MAZ-200-014	45.0	33.3	74.4	62.2	10.7
SCR15/MAZ-200-015	25.7	19.7	75.6	60.0	21.6
SCR15/MAZ-200-051	34.3	30.3	88.3	68.9	10.1
SCR15/MAZ-200-065	42.7	33.7	78.6	84.4	5.4
SCR15/MAZ-200-066	35.3	25.3	71.7	73.3	12.6
SCR15/MAZ-200-071	24.7	19.7	79.4	68.9	11.6
SCR15/MAZ-200-074	43.3	31.0	71.6	80.0	7.6
SCR15/MAZ-200-079	8.0	5.7	70.6	20.0	11.1
SCR15/MAZ-200-080	32.7	29.7	91.0	80.0	15.5
SCR15/MAZ-200-097	62.0	50.7	82.4	84.4	9.8
SCR15/MAZ-200-099	33.3	23.3	74.3	80.0	14.0
SCR15/MAZ-200-101	49.0	38.7	78.4	77.8	11.7
SCR15/MAZ-200-109	24.7	20.7	83.8	77.8	18.0
SCR15/MAZ-200-113	32.7	28.7	88.3	73.3	13.6
SCR15/MAZ-200-114	34.3	24.3	76.9	68.9	13.9
SCR15/MAZ-200-117	25.7	18.7	74.3	57.8	21.5
SCR15/MAZ-200-119	21.7	17.3	80.0	48.9	24.7
SCR15/MAZ-200-141	45.0	35.7	78.5	88.9	6.3
SCR15/MAZ-200-154	20.7	16.3	79.3	42.2	23.3
SCR15/MAZ-200-175	37.7	29.3	83.6	71.1	20.4
SCR15/MAZ-200-177	30.0	23.0	76.6	60.0	24.1
SCR15/MAZ-200-182	41.0	29.0	75.0	66.7	14.4
SCR15/MAZ-200-186	9.7	6.7	72.2	24.4	12.0
SCR15/MAZ-200-199	27.7	20.0	73.3	57.8	13.1
SCR-15 (Susceptible parent)	**55.7**	**53.0**	**95.3**	**60.0**	**20.2**

NE = number of eggs; NAE = number of adult emerged; PAE = percentage adult emergence; PD = percentage damage; PSWL = percentage seed weight loss

7.4 Discussion

In this chapter, out of the two crossing blocks, only F_4 plants derived from SCR-15 and MAZ-200 crosses were considered. The F_1 seeds derived from the cross between Nasir and MAZ-200 were dwarfed and did not grow beyond the two leafs stage. The occurrence of the dwarf-lethal incompatibility in some inter-gene pool crosses was also reported by Singh and Gutiérrez (1984). In the past, breeders attempted to combine the large-seed size traits of the Andean and the high-yield potential trait of the Mesoamerican gene pools. However, these attempts were not successful (Singh, 1989) and Welsh et al. (1995) also reported that the average performance of the progeny derived from the inter-gene pool crosses was below the performance of the parents. This may have been resulted from their geographical isolation, which may have led to the development of a reproductive barrier between the two gene pools. Singh (2001) also suggested that the divergence between the two gene pools may have resulted from the independent genes that control the overall performance in Mesoamerican and Andean gene pools.

The highly significant ($P<0.001$) mean squares for all the susceptibility traits recorded in this study suggest the presence of a wide range of genetic variation among the offspring for bruchid resistance. The parents also revealed significant differences ($P<0.01$) for all the traits, except for the number of eggs indicating that the parents were very diverse in relation to their level of resistance to bean bruchid. The present results are also consistent with the results from Chapter 2. The non-significant difference in the number of eggs between the two parents confirmed that the mechanism of resistance of the arcelin gene is antibiosis, which is characterized by delaying adult emergence and hampering insect growth and development (Osborn et al., 1988). In the present study, it was recorded that, out of the 56 eggs laid, on average 53 hatched into adults (95%) in the susceptible parent (SCR-15). However, in the resistant parent (MAZ-200), out of the 49 eggs laid, none of the eggs hatched into adults. This indicates that the parents differentiated well in their response to bruchid infestation. Similar results were also reported by Blair et al. (2010b) for the arcelin gene. In addition, the highest number of eggs laid (91) was on SCR15/MAZ-200-178 and the lowest (6) was on SCR15/MAZ-200-127, with a percentage adult emergence of 35% and 50%, respectively. The results indicated that the highest and lowest number of eggs laid were not proportional to the number of adults that emerged. In other words, the high number of eggs may not necessarily correspond with the high number of progeny, and vice versa. This shows that the number of eggs laid per female may not be a good indicator for resistance. The lack of proportionality in number of eggs laid and the number of adults emerged was supported by other similar studies (Negasi and Abate., 1992; Shiferaw, 2004). The 21.6% of

the offspring grouped under intermediate resistant in the present study, may be due to the heterozygous (Arc^+/Arc^-) state of the arcelin gene. Kornegay et al. (1993) reported that arcelin is inherited as a monogenic dominant trait, which provides a higher level of resistance to bruchids when it is in the homozygous (Arc^+/Arc^+) state than when it is in its heterozygous (Arc^+/Arc^-) state.

For an efficient and effective breeding program, it is important to know the genetic variability, by using appropriate parameters, such as heritability estimates (Atta et al., 2008). In the present study, moderate to high levels (69 to 94%) of heritability values were recorded, indicating that a greater proportion of the phenotypic variation observed in this study was explained by the genotypic variations among genotypes. These values were highly inflated by the non-heritable dominant and epistasis effects (Holland et al., 2003). The high heritability values reported by Kasozi (2013) for the susceptibility parameters, such as F_1 weevil progeny emergence, percent grain damage and Dobie's index of susceptibility on the maize weevil (*Sitophilus zeamais*) were consistent with the present results. However, Keneni (2012) in his study on adzuki bean beetle (*Callosobruchus chinensis* L.) on chickpea, reported low heritability values (0.20 -11%) for all insect-related traits, such as the number of eggs, the number of adult emerged and the percentage adult emergence) and high heritability (76%) for seed weight loss. The high heritability values recorded in this study indicated the extent to which the phenotype is determined by the genotype. This suggests that selection may be useful for the enhancement of bruchid resistance in reducing the grain loss caused by bean bruchid (Singh, 2002). The genotyping and phenotyping of the all the crosses are still in progress, with the support of projects from Bill and Melinda Gates Foundation and the National Bean Improvement Program.

7.5 Conclusions

The introgression of the arcelin locus into commercial and highly-productive varieties was effective in the case of SCR-15/MAZ-200 crosses. Within the 208 segregating progeny population, half of the progenies were found to be resistant, to highly resistant using phenotypic screening. Natural infestation and damage of *Z. subfasciatus* only occurs in storage starting from pre-existing bruchid populations. All genotypes were equally exposed to the same age and number of insects and the laboratory conditions were conducive to insect growth and development. This type of screening is standard procedure for storage pests and the outcome is conclusive, since the test is a non-choice test. In addition, the parameters used to screen the population revealed moderate to high levels of heritability, indicating that the considerable variations among the progeny population may be due to the genes. This, in turn, suggests that

selection may be effective for developing new varieties with high levels of bruchid resistance. In order to increase the efficiency of breeding for bruchid resistance, MAS can be applied, as linked markers are available and MAS has been proven to be effective for traits with high heritability, such as selection for resistance to diseases and insect pests.

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CHAPTER 8

An Overview of the Research Findings

8.1 Introduction and objectives of the study

The common bean is the second most widely-grown grain legume crop. It contributes to the growth of the national economy in Ethiopia and is a source of income for millions of smallholder farmers. The Ethiopian common bean germplasm revealed a wide range of genetic variation for different agro-morphological traits. Landraces are a source of many desirable genes in most of the national crop breeding programs in a number of countries. Assessing the available genetic diversity through a well-designed and systematic genetic diversity analysis is of paramount importance for developing high-yielding, bruchid-resistant common bean varieties. Moreover, participatory variety selection will improve and facilitate the development of new varieties with all the desirable traits. A summary of the major findings of this thesis, and their implications for the development of high-yielding and bruchid resistant common bean varieties is presented in this chapter. This information may be utilized for the future improvement and genetic conservation efforts of the common bean in Ethiopia.

This study was undertaken with the following specific objectives:

(g) to evaluate the Ethiopian common bean landrace collections, commercial varieties, advanced breeding lines and elite resistant lines for resistance to the Mexican bean weevil;

(h) to assess the magnitude and pattern of genetic diversity in Ethiopian common bean landraces, commercial varieties, advance breeding and exotic resistant lines for the response to infestation by bean bruchid, using phenotypic and SNP markers;

(i) to examine the population structure among common bean genotypes collected from different breeding status, seed colours and sizes, and to identify genomic regions that are associated with bean bruchid resistance, using SNP markers distributed across common bean genome;

(j) to assess the agronomic performance of common bean genotypes selected for their response to bruchid infestation, using yield and yield components under different agro-ecologies;

(k) to identify suitable parental genotypes that are useful for breeding for bruchid resistance and to identify the farmers' selection criteria for choosing varieties; and
(l) to interogress arcelin genes into commercial varieties and an advanced breeding line.

8.2 Research findings in brief

Evaluation of Ethiopian common bean (*Phaseolus vulgaris* L.) genotypes for resistance to the Mexican bean weevil (*Zabrotes subfasciatus* Boheman)

In this study, a total of 300 common bean genotypes, including 204 landraces, 34 commercial varieties, 34 Mexican bean weevil resistant lines, 27 advance breeding lines and 35 resistant lines were evaluated under laboratory conditions. Data on fifteen insect and seed traits were collected. A comparison of the analysis of variance means was made. The main findings were as follows:

- A wide range of phenotypic variation was observed among the landraces, commercial varieties, advanced breeding and resistant lines for all the traits under bruchid infestation.
- Except for the CIAT resistant lines, with known arcelin-based resistance, almost all the genotypes were susceptible to bean bruchid. However, SCR-11 and NC-16 were found to be partially resistant to bean bruchids, compared to other genotypes.
- The Resistance to Zabrotes (RAZ) and Marker-Assisted Zabrotes (MAZ) lines revealed a wide level of resistance to the insect. The resistant lines were classified as resistant (73%) and moderately resistant (27%), and some RAZ and MAZ lines showed complete resistance.
- Eleven genotypes, RAZ-11, RAZ-36, RAZ-2, RAZ-42, RAZ-44, RAZ-120, RAZ-40, MAZ-200, and MAZ-203, were identified as highly resistant lines for bean bruchid. Similarly, advanced breeding line (SCR-11) and landrace (NC-16) were found to be moderately resistant.

Genetic diversity analysis of common bean (*Phaseolus vulgaris* L.) genotypes for resistance to the Mexican bean weevil (*Zabrotes subfasciatus*), using single nucleotide polymorphism (SNP) and phenotypic markers

In this study, three out of the 300 genotypes, were omitted because of the complete damage of the seed by bruchids. A total of 297 common bean genotypes, of different breeding status, seed size class and geographic origin were analyzed with single nucleotide polymorphism (SNP) markers and phenotypic traits. Both genotypic and phenotypic data was subjected to different genetic diversity analysis software. The Shannon diversity index, within and among breeding status and collection sites was calculated by using phenotypic traits. The loci-and population-

specific genetic diversity parameters were estimated for the genotypic data. The cluster analysis was made, using both markers systems. The main outcomes were as follows:

- The genotypes revealed very high polymorphism for traits, such as the number of holes per seed, percentage adult emergence, second progeny percentage damage and susceptibility index.
- Traits, such as the number of adults emerged, susceptibility index, percentage adult emergence, percentage damage, percentage seed weight loss and the number of holes were proved to be important traits in discriminating genotypes into different susceptibility classes. These traits are important traits, useful for selection for bean bruchid resistance, and selection efforts, based on these traits may be most effective in reducing bean bruchid infestation and the subsequent seed damage.
- Population classification, based on the seed size and breeding status of the genotypes provided more meaningful classification than geographic origin.
- Resistant lines and commercial varieties were less diverse than landraces and breeding lines.
- About 89% of the SNP markers used in this study had PIC values exceeding 0.30, demonstrating the high discriminatory power of the markers.
- The genetic diversity estimates, using SNP markers revealed that 76% of the gene diversity values was found between 0.40 and 0.50, indicating the presence of a high level of genetic diversity among the genotypes.
- Two distinct clusters were found, using both phenotypic and SNP markers, but SNP markers were better in clustering the genotypes according to their seed size and breeding status.
- The clustering of genotypes, based on both SNP and phenotypic markers did not show any association with geographic origin, indicating the existence of a high level of gene flow among regions.
- The analysis of molecular variance (AMOVA) among populations, which was classified based on their seed size, breeding status and geographic origin, revealed that out of the total genetic variation, more than 70% was attributable to individuals within the population, and the remaining 30% was due to variation amongst populations and with a genotype variation.
- Based on phenotypic and genotypic distances, 144 diverse genotypes were selected for further agronomic performance study.

Population structure and genome-wide association analysis of bruchid resistance in Ethiopian common bean (*Phaseolus vulgaris* L.) genotypes

A population of 297 common bean genotypes, which were collected from different seed size classes, breeding status and geographic origins were genotyped with high throughput SNP markers. Population structure analysis and genome-wide association mapping were undertaken, based on 2554 SNP markers with < 2% missing data. The main outcomes were as follows:

- The tested genotypes were grouped into two sub-populations at K = 2, based on the Bayesian genotyping clustering approach, as Middle American and Andean gene pools. In addition, at K = 3 the Mesoamerican gene pool separated into two subpopulations. Most (>80%) of the genotypes were grouped under the Middle American gene pool, suggesting that genotypes from the Mesoamerican gene pool are predominant in Ethiopia.
- Most of the landraces were found to have a Middle American gene pool background; however, some landraces from the Amhara and Oromiya regions were from the Andean gene pool. This may have resulted from seed importation from abroad, since there are number of seed importers in these regions.
- About seven highly significant (P<0.001) and six significant (P<0.01) marker-trait associations (MTAs) were detected explaining about 71% of the phenotypic variation observed for percentage adult emergence. Similarly, six highly significant (P<0.001) and six significant at (P<0.01) marker-trait associations, that explained 72% of the phenotypic variation observed for seed weight loss, were discovered.
- Most of the markers associated with percentage adult emergence and seed weight loss are located on Pv4 and Pv7 of the common bean genome.
- No SNPs were found above the cut-off point for all the traits in the 264 Ethiopian genotypes, excluding the resistant lines.

Assessment of diversity and performance of Ethiopian common bean (*Phaseolus vulgaris* L.) genotypes for yield and yield components

The above selected 144 genotypes were evaluated, using a 12 x 12 alpha lattice design with three replications under three different agro-ecological conditions. The performance of the genotypes was assessed, using 15 yield and agronomic traits. An analysis of variance, correlation, principal component and cluster analyses were made. The core findings of this study were as follows:

- The genotypes exhibited a wide range of variations for 15 yield and yield-related traits. In addition, highly significant genotypes by environmental interactions were observed, which is an indication that the yield performances of the genotypes were highly influenced by the environment and that the selected environments were variable, in terms of temperature, moisture, relative humidity, altitude, as well as soil physical and chemical properties.
- Out of the tested genotypes, 75% were landraces and they showed a wide range of variation for all the tested traits, and out of the top 20 selected high-yielding genotypes, 45% genotypes were landraces. This suggests that landraces can be used as sources of valuable genes and can be exploited in future common bean improvement programs.
- Grain yield, aboveground biomass, days to 50% flowering and days to 90% maturity were found to be important traits in discriminating the genotypes.
- Significant and positive correlations were observed between the grain yield and grain filling period, the number of pods per plant, hundred seed weight and aboveground biomass
- In the small bean category, genotypes Nasir, SER-125, Awash Melka, RAZ-36, 241757, 230526, RAZ-44, 241734, 214665 and NC-51 were selected, based on their high yield and other yield-related traits. Similarly, genotypes SCR-11, SCR-15, SCR-26, 228077 KK25/MAIAWA/19, RAZ-40, RAZ-120, NC-28, 207935 and 2113002 were selected from the medium-seed size category.
- The lines with Zabrotes resistant genes, such as RAZ-36, RAZ-40, RAZ-44 and RAZ-120 and KK25/MAIAWA/19 (Malawian resistance variety) were found to be agronomically suitable.
- A hierarchical cluster analysis, based on the agro-morphological traits, classified genotypes into three distinct major cluster and seven sub-clusters. The clustering patterns of the genotypes were highly correlated with the seed size category of the genotypes.

Participatory variety selection of common bean genotypes in Oromiya region of central Ethiopia

A total of 60 farmers were invited to evaluate the genotypes in all three agro-ecologies. Farmers were requested to identify their preferred traits and rank them. Based on the selected traits, farmers were able to evaluate the 144 common bean genotypes. The main outcomes were as follows:

- Farmers identified 14 preferred traits that should use as selection criteria, of which grain yield, pod load and filling were the most important traits across agro-ecologies and gender groups.

- Farmers used a combinations of traits for selecting common bean varieties. Women were more interested in post-harvest traits, such as earliness, taste and cooking time, while men were more focused on marketability, seed colour, size and shape.
- At Melkassa and Alem Tena, farmers were more concerned to moisture stress and storage insect pests, while at Arsi Negele farmers gave more weight to grain yield and other yield-related traits.
- Farmers at Melkassa preferred small white bean types whereas, in Arsi Negele, small red beans are more preferred.
- Based on the diversity analysis and the participatory selection, six female parental genotypes (Nasir, SER-125, SCR-15, Awash-1, Awash-2 and Awash Melka) and seven donor resistant lines (RAZ- 2, RAZ-11, RAZ-36, RAZ-42, RAZ-120, MAZ-200 and MAZ-203) were selected from each seed colour category.

Introgression of the Mexican bean weevil resistance (arcelin) gene into commercial varieties and advanced lines of the common bean

The above selected genotypes from the red and white bean category were crossed in the North Carolina II factorial mating design. A total of 21 crosses were made, with eight and five parental genotypes of white and red beans, respectively. Only 208 offspring were derived from a cross between SCR-15 and MAZ-200 and the parents were phenotyped

- A highly significant variation was observed within and among the offspring and parental genotypes.
- Moderate to high levels of heritability (69 to 94%) were observed for all the susceptibility parameters used, suggesting selection can be a useful breeding method for the enhancement of bruchid resistance in reducing the grain loss.
- About 45% of the offspring were found to be resistant, of which 40 (19%) of the genotypes revealed complete resistance, with zero adult emergence.
- The phenotyping and genotyping of the other crosses is still under progress.

8.3 Implications of the research findings

- The success of any breeding program depends on the availability of diverse genetic resources for the target traits. The wide genetic diversity detected amongst common bean genotypes, collected from different seed size classes, breeding status and geographic origins, indicated that there is potential for selection of source germplasm for breeding for yield and yield-related traits. The low level of bruchid resistance observed among the Ethiopian common bean genotypes can be improved through the introduction of resistant lines and hybridization with the more adapted and high-yielding commercial cultivars.
- The two distinct sub-groups (gene pools) observed from the structure analysis, will enable breeders to select distinctive alleles and exploit the potential of transgressive segregation between the two sub-groups, as well as and the broadening of the genetic base of the common bean with regard to some traits. The co-occurrence of the two gene pools in the Ethiopian common bean germplasm, the very low (<4%) inter-gene pool hybrids and the significant population differentiation indicated that the cultivation of a pure gene pool has been geographically isolated, with regard to market demand and household consumption needs.
- Bruchid resistance breeding efforts has been underway for long period of time; however, these efforts have not been actively deployed in many local breeding programs. This may be related to the tedious, more technical and high resource requirements. To improve the efficiency and effectiveness of resistance breeding, conventional breeding methods should be complemented with the application of molecular markers. Very few efforts have been made in the identification of molecular markers linked to insect resistance genes in the common bean. The 13 highly significant marker-trait associations detected in this study could be useful for initiating marker-assisted selection and the introgression of bruichid resistance in the common bean. This will avoid the tedious phenotypic screening, using controlled insect infestation on seeds and insect raring. However, the validation of these markers needs to be carried out before any large-scale application can be made.
- For the better diffusion and impact of improved technologies, farmers have to be part of the breeding process right from the very beginning. Farmers were able to identify and rank the most preferred traits and were able to select potential parents for crossing. The pure lines that will be identified from the crosses generated from farmers-selected parental genotypes at a later stage of breeding, and may have farmer-preferred traits across different agro-ecologies, which will make the diffusion of these varieties easier.

Appendix A

Table 1. Description of the genotypes used in this study

No	Genotype	Seed colour	Seed size*	Geenpool	Origin/Source	Breeding status
1	207934	Red	Small	Mesoamerican	B.Gumz	Landrace
2	207935	Carioca	Medium	Mesoamerican	B.Gumz	Landrace
3	207938	Black	Small	Mesoamerican	B.Gumz	Landrace
4	211346	Red	Small	Mesoamerican	B.Gumz	Landrace
5	211347	Black	Small	Mesoamerican	B.Gumz	Landrace
6	211348	Black	Small	Mesoamerican	B.Gumz	Landrace
7	211349	Yellow	Small	Mesoamerican	B.Gumz	Landrace
8	211356	White	Small	Mesoamerican	B.Gumz	Landrace
9	211361	Red	Small	Mesoamerican	B.Gumz	Landrace
10	211362	White	Small	Mesoamerican	B.Gumz	Landrace
11	228522	Red	Small	Mesoamerican	Amhara	Landrace
12	NC-02	Brown	Small	Mesoamerican	Amhara	Landrace
13	NC-03	Carioca	Small	Mesoamerican	Amhara	Landrace
14	NC-05	Red	Small	Mesoamerican	Amhara	Landrace
15	NC-07	Red	Small	Mesoamerican	Amhara	Landrace
16	NC-10	Brown	Small	Mesoamerican	Amhara	Landrace
17	NC-16	Red	Small	Mesoamerican	Amhara	Landrace
18	NC-17	Red	Small	Mesoamerican	Amhara	Landrace
19	211266	Cream	Small	Admixture	Amhara	Landrace
20	211267	White	Small	Mesoamerican	Amhara	Landrace
21	211269	Black	Small	Admixture	Amhara	Landrace
22	NC-08	White	Small	Mesoamerican	Amhara	Landrace
23	NC-09	Carioca	Medium	Mesoamerican	Amhara	Landrace
24	NC-12	Red	Small	Mesoamerican	Amhara	Landrace
25	NC-13	Cream	Small	Mesoamerican	Amhara	Landrace
26	NC-14	Speckled	Large	Andean	Amhara	Landrace
27	NC-15	Cream	Large	Mesoamerican	Amhara	Landrace
28	NC-18	Speckled	Large	Andean	Amhara	Landrace
29	NC-20	Black	Small	Mesoamerican	Amhara	Landrace
30	NC-39	Cream	Small	Mesoamerican	Amhara	Landrace
31	NC-44	Brown	Small	Mesoamerican	Amhara	Landrace
32	NC-48	Red	Small	Mesoamerican	Amhara	Landrace
33	NC-49	Black	Small	Mesoamerican	Amhara	Landrace
34	NC-50	Carioca	Small	Mesoamerican	Amhara	Landrace
35	NC-51	Red	Small	Mesoamerican	Amhara	Landrace
36	NC-52	Speckled	Large	Andean	Amhara	Landrace
37	NC-53	Yellow	Small	Mesoamerican	Amhara	Landrace
38	NC-54	Carioca	Small	Mesoamerican	Amhara	Landrace

No	Genotype	Seed colour	Seed size*	Geenpool	Origin/Source	Breeding status
39	NC-57	Carioca	Small	Mesoamerican	Amhara	Landrace
40	NC-61	Black	Small	Mesoamerican	Amhara	Landrace
41	215719	Pinto	Small	Mesoamerican	Amhara	Landrace
42	215720	White	Small	Mesoamerican	Amhara	Landrace
43	207534	White	Small	Mesoamerican	Amhara	Landrace
44	NC-25	Brown	Medium	Mesoamerican	Amhara	Landrace
45	NC-28	Cream	Medium	Mesoamerican	Amhara	Landrace
46	NC-29	Speckled	Large	Andean	Amhara	Landrace
47	NC-30	Carioca	Small	Mesoamerican	Amhara	Landrace
48	NC-34	Speckled	Large	Andean	Amhara	Landrace
49	232196	White	Small	Mesoamerican	Oromiya	Landrace
50	237079	White	Small	Mesoamerican	Oromiya	Landrace
51	237080	Yellow	Medium	Mesoamerican	Oromiya	Landrace
52	212860	White	Small	Mesoamerican	Oromiya	Landrace
53	215391	White	Small	Mesoamerican	Oromiya	Landrace
54	230044	White	Small	Mesoamerican	Oromiya	Landrace
55	230661	White	Small	Mesoamerican	Oromiya	Landrace
56	215048	White	Small	Mesoamerican	Oromiya	Landrace
57	215049	Black	Small	Mesoamerican	Oromiya	Landrace
58	228911	White	Small	Mesoamerican	Oromiya	Landrace
59	228913	Red	Small	Mesoamerican	Oromiya	Landrace
60	201066	Red	Small	Mesoamerican	Oromiya	Landrace
61	213197	White	Small	Mesoamerican	Oromiya	Landrace
62	208995	Red	Large	Andean	Oromiya	Landrace
63	208699	Yellow	Medium	Andean	Oromiya	Landrace
64	208702	Black	Small	Mesoamerican	Oromiya	Landrace
65	208703	Black	Small	Mesoamerican	Oromiya	Landrace
66	208705	Black	Medium	Mesoamerican	Oromiya	Landrace
67	211340	Black	Small	Mesoamerican	Oromiya	Landrace
68	211302	Brown	Medium	Andean	Oromiya	Landrace
69	211304	White	Small	Mesoamerican	Oromiya	Landrace
70	211305	Yellow	Small	Admixture	Oromiya	Landrace
71	NC-22	Cream	Medium	Not known	Amhara	Landrace
72	211311	Yellow	Medium	Andean	Oromiya	Landrace
73	211314	White	Small	Mesoamerican	Oromiya	Landrace
74	211325	Red	Small	Mesoamerican	Oromiya	Landrace
75	208638	Speckled	Large	Andean	Oromiya	Landrace
76	241134	White	Small	Mesoamerican	Oromiya	Landrace
77	223329	Mottled	Medium	Andean	Oromiya	Landrace
78	219231	Speckled	Medium	Andean	Oromiya	Landrace
79	228812	White	Small	Mesoamerican	Oromiya	Landrace
80	228813	White	Small	Mesoamerican	Oromiya	Landrace

No	Genotype	Seed colour	Seed size*	Geenpool	Origin/Source	Breeding status
81	230526	Red	Small	Mesoamerican	Oromiya	Landrace
82	211315	White	Small	Mesoamerican	Oromiya	Landrace
83	211320	White	Small	Mesoamerican	Oromiya	Landrace
84	211323	Red	Small	Mesoamerican	Oromiya	Landrace
85	211333	White	Small	Mesoamerican	Oromiya	Landrace
86	230525	Cream	Small	Mesoamerican	Unknown	Landrace
87	211331	White	Small	Mesoamerican	Unknown	Landrace
88	211546	Carioca	Small	Mesoamerican	SNNPR	Landrace
89	211552	Black	Small	Mesoamerican	SNNPR	Landrace
90	214663	Red	Small	Mesoamerican	SNNPR	Landrace
91	214664	Red	Small	Mesoamerican	SNNPR	Landrace
92	214675	Black	Small	Mesoamerican	SNNPR	Landrace
93	214676	White	Small	Mesoamerican	SNNPR	Landrace
94	214678	Pinto	Small	Mesoamerican	SNNPR	Landrace
95	228084	Red	Small	Not known	SNNPR	Landrace
96	214665	Red	Small	Mesoamerican	SNNPR	Landrace
97	237993	Red	Small	Mesoamerican	SNNPR	Landrace
98	241739	Red	Small	Mesoamerican	SNNPR	Landrace
99	211279	Black	Small	Mesoamerican	SNNPR	Landrace
100	211280	Cream	Small	Mesoamerican	SNNPR	Landrace
101	211284	Red	Small	Mesoamerican	SNNPR	Landrace
102	211286	Carioca	Small	Mesoamerican	SNNPR	Landrace
103	213046	Black	Small	Mesoamerican	SNNPR	Landrace
104	215051	White	Small	Mesoamerican	SNNPR	Landrace
105	241752	Red	Small	Mesoamerican	SNNPR	Landrace
106	241756	Brown	Small	Mesoamerican	SNNPR	Landrace
107	241757	Red	Small	Mesoamerican	SNNPR	Landrace
108	208367	White	Small	Mesoamerican	SNNPR	Landrace
109	241736	Red	Small	Mesoamerican	SNNPR	Landrace
110	228077	Red	Medium	Mesoamerican	SNNPR	Landrace
111	228082	Red	Small	Mesoamerican	SNNPR	Landrace
112	241734	Red	Small	Mesoamerican	SNNPR	Landrace
113	228085	Red	Small	Mesoamerican	SNNPR	Landrace
114	228086	White	Small	Mesoamerican	SNNPR	Landrace
115	241748	Red	Small	Mesoamerican	SNNPR	Landrace
116	244805	White	Small	Mesoamerican	SNNPR	Landrace
117	KK25/MAIAWA/19	Red	Medium	Mesoamerican	Malawi	Resistant line
118	KK25/NAGAGA/184	Red	Large	Andean	Malawi	Resistant line
119	MAZ 200	Red	Large	Andean	CIAT	Resistant line
120	MAZ 203	Red	Large	Andean	CIAT	Resistant line
121	MAZ 153	Mottled	Large	Mesoamerican	CIAT	Resistant line
122	SMARC 4	White	Small	Mesoamerican	Pannar seed company	Resistant line

No	Genotype	Seed colour	Seed size*	Geenpool	Origin/Source	Breeding status
123	RAZ-28-8	Mottled	Small	Mesoamerican	Pannar seed company	Resistant line
124	RAZ-9	Yellow	Medium	Mesoamerican	Pannar seed company	Resistant line
125	RAZ-2	White	Small	Mesoamerican	Pannar seed company	Resistant line
126	SCR-11	Red	Medium	Mesoamerican	CIAT	Breeding line
127	SCR-15	Red	Medium	Andean	CIAT	Breeding line
128	SCR-26	Red	Medium	Mesoamerican	CIAT	Breeding line
129	RAZ-40	White	Medium	Andean	CIAT	Resistant line
130	RAZ-36	White	Small	Mesoamerican	CIAT	Resistant line
131	RAZ-44	White	Small	Mesoamerican	CIAT	Resistant line
132	RAZ-42	White	Small	Mesoamerican	CIAT	Resistant line
133	RAZ-11-1	White	Small	Mesoamerican	CIAT	Resistant line
134	RAZ-11	White	Small	Mesoamerican	CIAT	Resistant line
135	RAZ-120	White	Medium	Mesoamerican	CIAT	Resistant line
136	Mexican 142	White	Small	Mesoamerican	EIAR	Variety
137	Kufanzik	Pinto	Medium	Mesoamerican	HU	Variety
138	Tinkie	Red	Large	Andean	HU	Variety
139	Gofta	Cream	Medium	Mesoamerican	HU	Variety
140	Red Wolayta	Red	Small	Mesoamerican	EIAR	Variety
141	Awash-1	White	Small	Mesoamerican	EIAR	Variety
142	Awash Melka	White	Small	Mesoamerican	EIAR	Variety
143	Deme	Speckled	Large	Andean	EIAR	Variety
144	DRK	Red	Large	Admixture	EIAR/AQOS	Variety
145	Beshbesh	Cream	Small	Mesoamerican	EIAR	Variety
146	Roba	Cream	Small	Mesoamerican	EIAR	Variety
147	KAT-B1	Yellow	Medium	Andean	EIAR	Variety
148	Nasir	Red	Small	Mesoamerican	EIAR	Variety
149	SER-125	Red	Small	Mesoamerican	EIAR	Variety
150	Wedo	Cream	Medium	Mesoamerican	ARARI/SARC	Variety
151	Ayenew	Pinto	Small	Mesoamerican	HU	Variety
152	207942	Black	Small	Mesoamerican	B.Gumz	Landrace
153	207943	Black	Small	Mesoamerican	Oromiya	Landrace
154	207949	White	Small	Mesoamerican	Oromiya	Landrace
155	211274	Brown	Small	Mesoamerican	Unknown	Landrace
156	211278	Carioca	Small	Mesoamerican	SNNPR	Landrace
157	211282	Red	Small	Mesoamerican	SNNPR	Landrace
158	211293	Red	Medium	Mesoamerican	SNNPR	Landrace
159	211294	Red	Small	Mesoamerican	SNNPR	Landrace
160	211313	White	Small	Mesoamerican	Oromiya	Landrace
161	211321	White	Small	Mesoamerican	Oromiya	Landrace
162	211322	White	Small	Mesoamerican	Oromiya	Landrace
163	211324	White	Small	Mesoamerican	Oromiya	Landrace
164	211327	White	Small	Mesoamerican	Oromiya	Landrace

No	Genotype	Seed colour	Seed size*	Geenpool	Origin/Source	Breeding status
165	211329	White	Small	Mesoamerican	Oromiya	Landrace
166	211332	Red	Small	Mesoamerican	Oromiya	Landrace
167	211339	White	Small	Mesoamerican	Oromiya	Landrace
168	211341	Black	Small	Mesoamerican	Oromiya	Landrace
169	211344	Red	Small	Mesoamerican	B.Gumz	Landrace
170	211350	White	Small	Mesoamerican	B.Gumz	Landrace
171	211365	White	Small	Mesoamerican	Unknown	Landrace
172	211366	Red	Small	Mesoamerican	Unknown	Landrace
173	211367	Yellow	Small	Mesoamerican	Unknown	Landrace
174	211382	Black	Small	Mesoamerican	Amhara	Landrace
175	211483	Black	Small	Mesoamerican	Amhara	Landrace
176	211553	Black	Small	Mesoamerican	Unknown	Landrace
177	213198	Red	Small	Mesoamerican	Oromiya	Landrace
178	214662	Red	Small	Mesoamerican	SNNPR	Landrace
179	214667	Red	Small	Mesoamerican	SNNPR	Landrace
180	214671	Red	Small	Mesoamerican	SNNPR	Landrace
181	214677	Black	Small	Mesoamerican	SNNPR	Landrace
182	215050	White	Small	Mesoamerican	Oromiya	Landrace
183	215542	Yellow	Small	Andean	Unknown	Landrace
184	215545	Red	Small	Mesoamerican	Unknown	Landrace
185	216820	White	Small	Mesoamerican	Oromiya	Landrace
186	219233	Red	Small	Mesoamerican	Unknown	Landrace
187	219337	Red	Small	Mesoamerican	SNNPR	Landrace
188	219338	Red	Small	Mesoamerican	SNNPR	Landrace
189	219389	Red	Small	Mesoamerican	SNNPR	Landrace
190	219340	Red	Small	Mesoamerican	SNNPR	Landrace
191	228079	Red	Small	Mesoamerican	SNNPR	Landrace
192	228080	Red	Medium	Mesoamerican	SNNPR	Landrace
193	228081	Red	Small	Mesoamerican	SNNPR	Landrace
194	228083	Red	Medium	Mesoamerican	SNNPR	Landrace
195	228088	Red	Small	Mesoamerican	SNNPR	Landrace
196	228089	Red	Small	Mesoamerican	SNNPR	Landrace
197	228090	Red	Small	Mesoamerican	SNNPR	Landrace
198	228246	White	Small	Mesoamerican	Oromiya	Landrace
199	228247	Red	Small	Mesoamerican	Oromiya	Landrace
200	228811	White	Small	Mesoamerican	Oromiya	Landrace
201	228912	White	Small	Mesoamerican	Oromiya	Landrace
202	229814	Red	Small	Mesoamerican	Amhara	Landrace
203	235502	White	Small	Mesoamerican	Unknown	Landrace
204	235503	Black	Small	Mesoamerican	Unknown	Landrace
205	235506	Red	Small	Mesoamerican	Unknown	Landrace
206	235507	Carioca	Small	Mesoamerican	Unknown	Landrace

No	Genotype	Seed colour	Seed size*	Geenpool	Origin/Source	Breeding status
207	235508	Yellow	Medium	Mesoamerican	Unknown	Landrace
208	RAZ-19	White	Small	Mesoamerican	CIAT	Resistant line
209	241729	Red	Medium	Mesoamerican	Unknown	Landrace
210	241730	Red	Small	Mesoamerican	Unknown	Landrace
211	241737	Red	Small	Mesoamerican	SNNPR	Landrace
212	241750	Red	Small	Mesoamerican	SNNPR	Landrace
213	RAZ-138	White	Small	Admixture	CIAT	Resistant line
214	241807	White	Small	Mesoamerican	Amhara	Landrace
215	241813	Red	Small	Mesoamerican	Amhara	Landrace
216	241814	White	Small	Mesoamerican	Amhara	Landrace
217	241809	Black	Small	Mesoamerican	Unknown	Landrace
218	MAZ 112	Mottled	Medium	Mesoamerican	CIAT	Resistant line
219	MAZ 151	Mottled	Medium	Admixture	CIAT	Resistant line
220	MAZ 172	Mottled	Medium	Andean	CIAT	Resistant line
221	MAZ 174	Mottled	Large	Andean	CIAT	Resistant line
222	MAZ 179	Mottled	Medium	Andean	CIAT	Resistant line
223	MAZ 180	Mottled	Medium	Andean	CIAT	Resistant line
224	MAZ 198	Pinto	Medium	Mesoamerican	CIAT	Resistant line
225	MAZ 202	Red	Large	Admixture	CIAT	Resistant line
226	MAZ 204	Red	Large	Andean	CIAT	Resistant line
227	MAZ 205	Red	Medium	Andean	CIAT	Resistant line
228	MAZ 215	Mottled	Medium	Andean	CIAT	Resistant line
229	MAZ 217	Mottled	Large	Andean	CIAT	Resistant line
230	RAZ-28	Pinto	Medium	Mesoamerican	CIAT	Resistant line
231	SAB 14	White	Medium	Andean	CIAT	Breeding line
232	SCR-28	Red	Medium	Mesoamerican	CIAT	Breeding line
233	SAB 17	White	Medium	Andean	CIAT	Breeding line
234	SCR 24	Red	Medium	Mesoamerican	CIAT	Breeding line
235	SCR 14	Red	Medium	Mesoamerican	CIAT	Breeding line
236	SCR 19	Red	Medium	Mesoamerican	CIAT	Breeding line
237	Line smc-21	White	Medium	Admixture	CIAT	Breeding line
238	Line SEC-8	White	Large	Admixture	CIAT	Breeding line
239	SAB 9	White	Large	Andean	CIAT	Breeding line
240	SCR-6	Red	Medium	Mesoamerican	CIAT	Breeding line
241	SCR-37	Red	Medium	Mesoamerican	CIAT	Breeding line
242	SCR-36	Red	Medium	Mesoamerican	CIAT	Breeding line
243	SCR-9	Red	Medium	Mesoamerican	CIAT	Breeding line
244	SCR-25	Red	Medium	Mesoamerican	CIAT	Breeding line
245	SCR-35	Red	Medium	Mesoamerican	CIAT	Breeding line
246	SCR-34	Red	Medium	Mesoamerican	CIAT	Breeding line
247	SCR-7	Red	Medium	Mesoamerican	CIAT	Breeding line
248	SCR-1	Red	Medium	Mesoamerican	CIAT	Breeding line

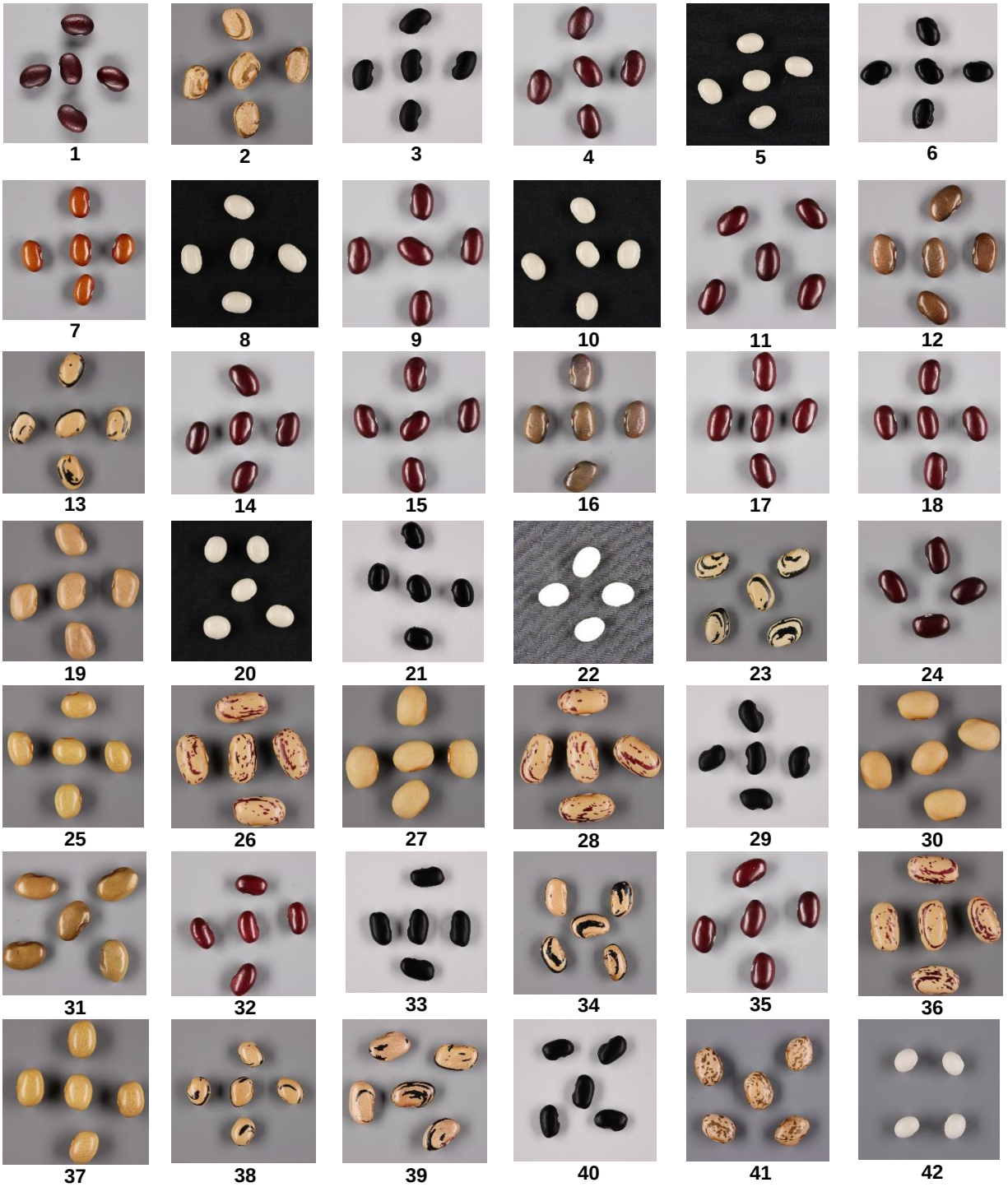
No	Genotype	Seed colour	Seed size*	Geenpool	Origin/Source	Breeding status
249	SCR-22	Red	Medium	Mesoamerican	CIAT	Breeding line
250	SCR-20	Red	Medium	Mesoamerican	CIAT	Breeding line
251	SCR-3	Red	Medium	Mesoamerican	CIAT	Breeding line
252	SCR-13	Red	Medium	Mesoamerican	CIAT	Breeding line
253	Hirna	Red	Large	Andean	HU	Variety
254	Chercher	White	Small	Mesoamerican	EIAR	Variety
255	Dursitu	Red	Small	Mesoamerican	EIAR	Variety
256	Babile	Red	Medium	Andean	HU	Variety
257	Hundene	Mottled	Medium	Andean	HU	Variety
258	Haramaya	Cream	Medium	Mesoamerican	HU	Variety
259	Fidise	Mottled	Medium	Andean	HU	Variety
260	AFR 702	Red	Large	Andean	SARI	Variety
261	Ibado	Mottled	Medium	Andean	SARI	Variety
262	OMO-95	Red	Small	Mesoamerican	SARI	Variety
263	ETAW- 01-L-7A	White	Small	Mesoamerican	CIAT	Breeding line
264	SARI-1	Red	Small	Mesoamerican	SARI	Variety
265	ETAW-01-L-7-6K	Mottled	Medium	Andean	SARI	Variety
266	ETAW- 01-L-7-20A	Mottled	Large	Andean	CIAT	Breeding line
267	Dinknesh	Red	Small	Mesoamerican	EIAR	Variety
268	Batu	White	Large	Andean	EIAR	Variety
269	KAT-B9	Red	Medium	Andean	EIAR	Variety
270	Nazareth-2	White	Small	Mesoamerican	EIAR	Variety
271	Cranscope	Speckled	Medium	Andean	EIAR	Variety
272	Lehode	Cream	Medium	Not known	ARARI/SARC	Variety
273	NC-01	Cream	Small	Mesoamerican	Amhara	Landrace
274	NC-06	Red	Small	Mesoamerican	Amhara	Landrace
275	NC-11	Cream	Medium	Mesoamerican	Amhara	Landrace
276	NC-19	Red	Small	Mesoamerican	Amhara	Landrace
277	NC-24	Red	Small	Mesoamerican	Amhara	Landrace
278	RAZ-111	White	Small	Admixture	CIAT	Resistant line
279	NC-32	Yellow	Medium	Mesoamerican	Amhara	Landrace
280	NC-33	Yellow	Medium	Mesoamerican	Amhara	Landrace
281	NC-35	Carioca	Small	Mesoamerican	Amhara	Landrace
282	NC-36	Black	Small	Mesoamerican	Amhara	Landrace
283	NC-37	Cream	Small	Mesoamerican	Amhara	Landrace
284	NC-38	Cream	Medium	Mesoamerican	Amhara	Landrace
285	NC-40	Red	Small	Mesoamerican	Amhara	Landrace
286	NC-41	Black	Small	Mesoamerican	Amhara	Landrace
287	NC-43	Brown	Small	Mesoamerican	Amhara	Landrace
288	NC-45	Brown	Medium	Mesoamerican	Amhara	Landrace
289	NC-46	Black	Small	Mesoamerican	Amhara	Landrace
290	NC-47	Red	Small	Mesoamerican	Amhara	Landrace

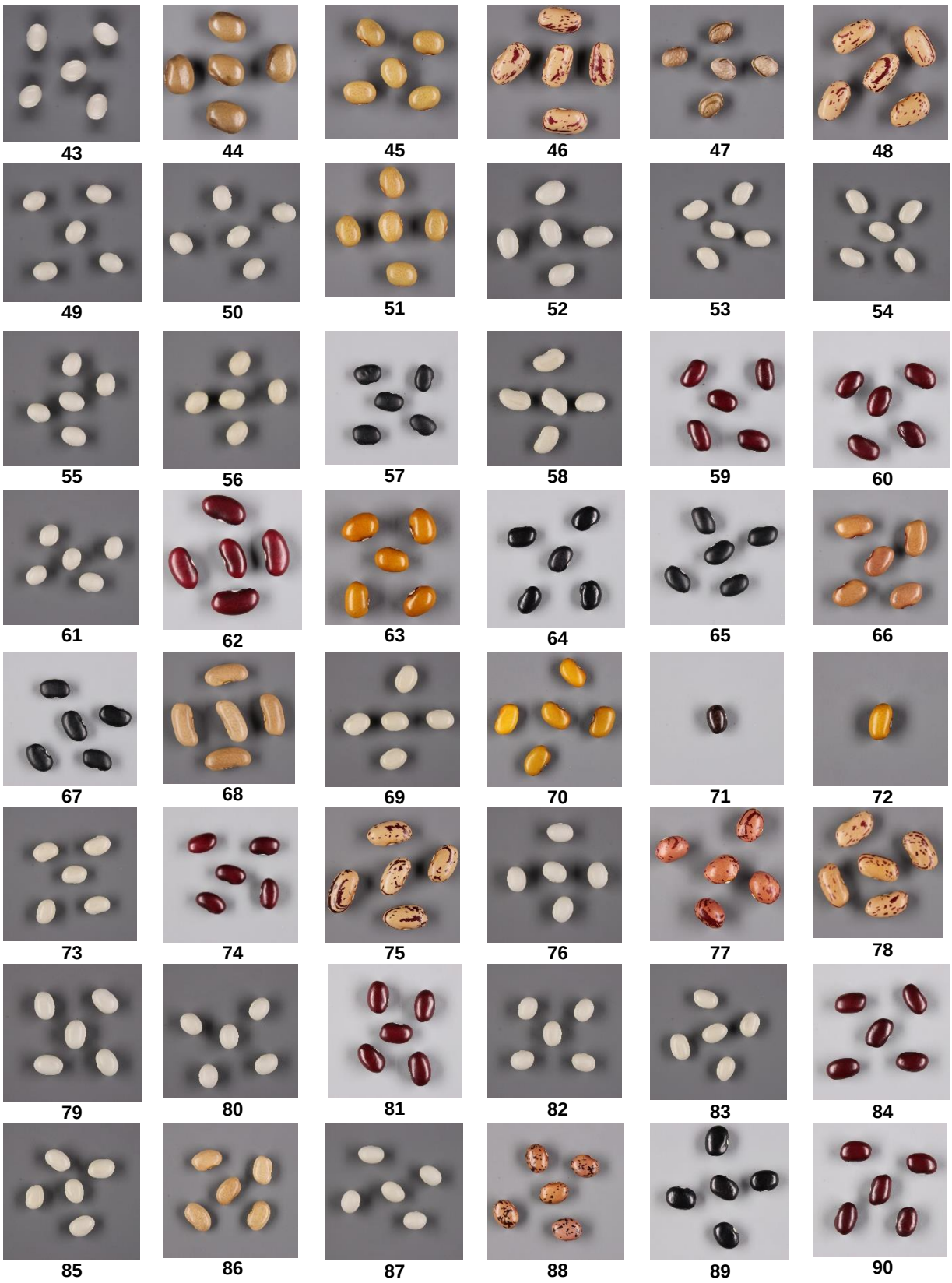
No	Genotype	Seed colour	Seed size*	Geenpool	Origin/Source	Breeding status
291	NC-55	Black	Small	Mesoamerican	Amhara	Landrace
292	NC-56	Speckled	Medium	Andean	Amhara	Landrace
293	NC-58	Black	Small	Mesoamerican	Amhara	Landrace
294	NC-59	Red	Small	Mesoamerican	Amhara	Landrace
295	NC-60	Red	Small	Mesoamerican	Amhara	Landrace
296	NC-62	Red	Small	Mesoamerican	Amhara	Landrace
297	NC-63	Black	Small	Mesoamerican	Amhara	Landrace
298	RAZ-34	White	Small	Mesoamerican	CIAT	Resistant line
299	RAZ-114	White	Small	Mesoamerican	CIAT	Resistant line
300	RAZ-119	White	Small	Mesoamerican	CIAT	Resistant line

* Seed size, based on hundred seed weight (g/100 seed) (small <25 g, medium = 26-40 g and large >40 g)

Appendix A

Figure 1. Seed colour and size description of the genotypes used in this study

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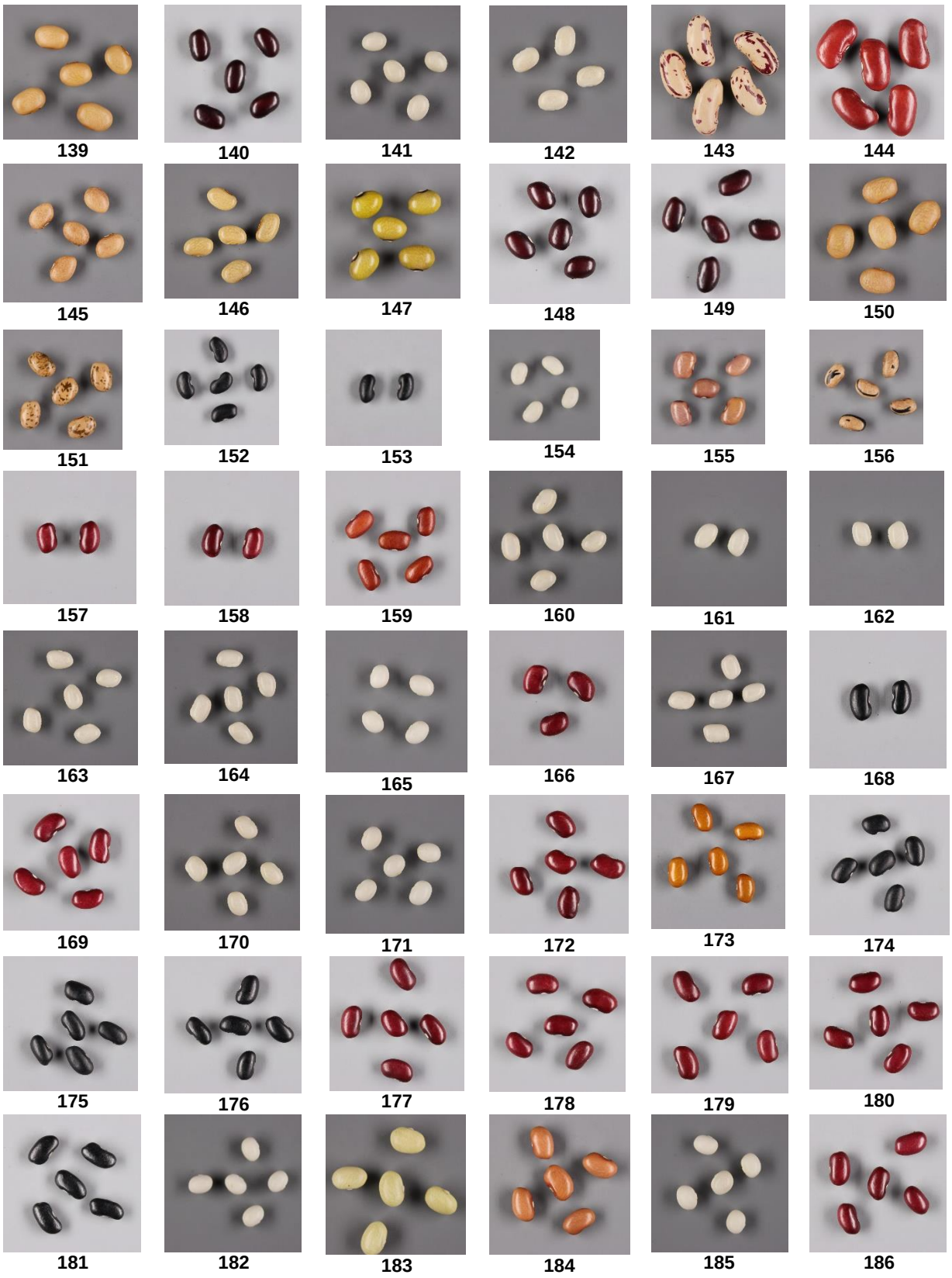
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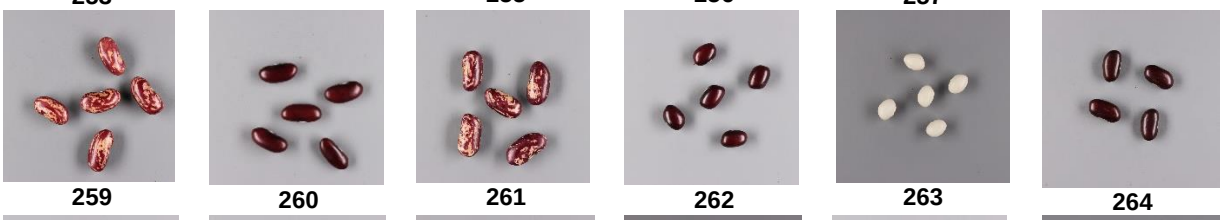
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