Genetic analysis and characterization of faba bean (*Vicia faba*) for resistance to chocolate spot (*Botrytis fabae*) disease and yield in the Ethiopian highlands

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THESIS ABSTRACT

Faba bean (*Vicia faba* L.) is one of the most important food legumes cultivated in Ethiopia. However, its production has been limited by several factors, among which include chocolate spot (CS) disease caused by the fungus *Botrytis fabae*. This is an important disease reducing faba bean yields and hence food security in the Ethiopian smallholder sector and other countries where the crop is grown. This study, conducted from 2011 to 2013 in Ethiopia, was designed to assess (i) farmers' awareness of chocolate spot and their influence on faba bean varieties of their preference; (ii) the genetic diversity, potential of several landracees for CS resistance; (iii) the mode of inheritance to CS and (iv) breeding potential of CS resistant lines in hybrid combinations. Simple sequence repeat (SSR) markers were used for genetic diversity study, while the full diallel mating design was used for genetic analysis of CS resistant moderately resistant and susceptible faba bean lines from Ethiopia breeding programme and International Center for Agriculture Research in the Dry Area (ICARDA). This study revealed that CS is a major production constraint in Ethiopia with high incidence and severity levels in the farmers' fields. The level of CS resistance in locally grown landraces thus needs to be improved. Farmers preferred new varieties that combined early maturing, high yield potential and CS resistance. The study also showed that faba bean genotypes with a wide genetic variation to CS existed in the Ethiopian germplasm, with nine of the local landraces being potential sources of resistance. Additive gene effects were more important than non-additive gene effects for CS resistance, suggesting that selection would be useful for improving the resistance of chocolate spot in faba bean. Lines ILB-4726, ILB-938 and BPL-710 had good resistance to CS and were good combiners for yield. Non-additive gene action was important for grain yield, implying that hybridization would be effective as a strategy for faba bean yield improvement. No maternal effects were recorded in the inheritance of CS resistance. Further genotypes x environment interactions were significant for CS resistance in faba bean, suggesting breeding for specific adaptation. These strategies will be recommended to programmes that emphasize CS resistance in faba bean.

DECLARATION

I, Asnakech Tekalign Beyene, declare that;

The research reported in this thesis, except where otherwise indicated, is my original research.

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Signed:

…………………………………………………………………Date……………………………..

Asnakech Tekalign Beyene

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

……………………………………………………….........…Date………………..…………..

Professor John Derera (Supervisor)

……………………………………………………………….Date……………………………

Dr Julia Sibiya (Co-Supervisor)

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DEDICATION

This work is dedicated to the my beloved father

Tekalign Beyene Woldemikael

and to my uncle

Haweltu Ayenew Jembere

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INTRODUCTION TO THESIS

1. Background

Faba bean is a diploid (2*n* = 12 chromosomes) crop which is one of the most important food legumes ranking in the world fourth after garden pea, chickpeas and lentil. It is cultivated in the temperate and subtropical regions of the world (Zohary and Hopf, 2000; Maxted and Bennett, 2001; Torres et al., 2006). It was reported as one of the best performing crops under global warming and climate change because of its unique ability to excel under most types of climatic conditions and wide adaptability to a range of soil environments (Singh et al., 2013). According to the United Nations Food and Agriculture Organization's (FAO) the world area of faba bean production is 2.5 million ha, while common bean is 29 million, chickpea was 13.5 million ha and dry peas 6.4 million ha (FAOSTAT, 2014). However, faba bean exceeds both common bean and chickpea in terms of productivity. For instance, the world faba bean productivity in 2013 was 1.6 t ha^{-1} while that of dry bean was 0.8 t ha^{-1} and chickpea 0.9 t ha^{-1}. The world's leading producing countries for faba bean are China, Ethiopia, Australia, France and the United Kingdom (FAOSTAT, 2014). The world faba bean productivity in 2013 was 34 million metric ton. The share of Africa's faba bean productivity was 7 million metric ton of world production. Ethiopia is the leading producer of faba bean in Africa with a share of 1.5 million metric ton in 2013 (FAOSTAT, 2014).

In Ethiopia, faba bean is widely grown in the mid-altitude and highland areas and serves as a multi-purpose crop leading the pulse category in area and production (CSA, 2013). It is an important component of crop production in smallholders' agriculture as an alternative source of protein, starch cellulose and minerals; cash income and plays a significant role in improving the productivity of the soil in the cereal-based rotations where it serves as a break crop (Schmidtke and Rauber, 2000; Haciseferogullari et al., 2003; López-Bellido et al., 2006; López-Bellido et al., 2011). In addition, yields of cereal crops following faba bean are improved and the need for artificial nitrogen fertilizer applications is reduced for subsistence farmers (Lindemann and Glover, 2003; Agegnehu and Fessehaie, 2006). Faba bean total harvested area is increasing which is attributed to the demand of the crop in the country. Nevertheless, faba bean yield of 1.8 t ha^{-1} has remained below its potential (>5 t ha^{-1}) to meet the demand for food security of the growing population. This is a challenge to breeders (CSA, 2013), therefore, management should be prioritized for the major problem to improve faba bean productivity.

Despite its importance, constraints contributing to significant yield losses of faba bean in many countries, including Ethiopia, comprises; diseases, weed, and insect pests (Torres et al., 2006; Pe´rez-de-Luque et al., 2010). Faba bean is adversely affected by numerous fungal diseases, such as chocolate spot (*Botrytis fabae*), rust (*Uromyces viciae fabae*), black root rot (*Fusarium* spp.) and downy mildew (*Pernospora viciae*) which can cause great damage to the crop (Muehlbauer and Tullu, 1997).

Among diseases, faba bean chocolate spot caused by *Botrytis fabae*, is the most important disease throughout Europe, South East Asia, South America, Korea, India, Canada, Norway, the Middle east, north east and southern Africa, and Australia (Akem and Bellar, 1999; Bouhassan et al., 2004; Tivoli et al., 2006). Yield losses as high as 90% and total crop failure in severe epidemics of *Botrytis fabae* have been reported from areas where extended periods of wet weather conditions prevail (Elad et al., 2004; Singh et al., 2013). In Ethiopia, chocolate spot is one of the main constraints contributing to the low productivity of faba bean resulting in yield losses of up to 67% (Gorfu and Yaynu, 2001). The disease exists across all the agro-ecological zones but is more serious in areas of high-rainfall (>900 mm) and high elevation of >2000 m. a. s. l (Sahile et al., 2008).

Currently, some of the available improved genotypes and local landraces in Ethiopia are susceptible to chocolate spot and most landraces are associated with undesirable characteristics such as small seed size (Kemal et al., 2006). Several control measures for chocolate spot are available. These include cultural, chemical, use of resistant varieties and integrated disease management. However, resource poor farmers cannot afford the use of chemical which is considered to be one of the most effective control measures (Sahile et al., 2010). The use of resistant genotypes would be the cheapest and most favourable method for farmers. There is need to identify and evaluate adapted and new faba bean germplasm for resistance to disease and assess their usefulness in high yielding and resistant variety development.

Faba bean research in Ethiopia has given less emphasis on improving quality traits preferred by farmers and focused mostly on developing varieties that are high yielding. Though, there are some released faba bean varieties farmers mostly prefer to grow their own landrace. Moreover, analysis of farmers' perceptions and view on faba bean diseases and control methods utilised by farmers has been minimal. Farmers' insights and understanding of crop diseases plays an important role for sustainable disease management. Plant breeding should be carried out with the participation of farmers (Ceccarelli and Grando, 2007). Faba bean variety development has to concentrate on the farmers preferred traits to grantee the adoption of the newly developing varities. Therefore, there is a need to assess farmers' priority problem for faba bean production, their variety preference and their perception for the chocolate spot disease.

Breeding for chocolate spot resistance has been in progress and some inbred lines exhibiting partial resistance to chocolate spot have been identified (Villegas-Ferna´ndez et al., 2009). In Ethiopia currently the hybridization programmes used the few inbred lines from ICARDA such as ILB 938, ILB 4726, ILB 4615, ILB 4725, and ILB 4709 as source of resistance to chocolate spot and large–seeded (Gemechu et al., 2006). Therefore, it is necessary to identify potential germplams from the locally available faba bean landraces for proper utilization and of additional genetic source in the breeding programmes.

Breeding progress is profoundly dependant on genetically diverse germplasm. In Ethiopia there is no comprehensive information on the potential genetic diversity of faba and this is a major challenge for systematic use of faba bean in genetic breeding programmes. It is imperative to know the genetic diversity of the locally available germplasm to exploit the available trait of interest. Number of molecular marker systems have been utilized for the genetic diversity study of faba bean include; restriction fragment length polymorphism (RFLP), sequence specific amplification polymorphism (SSAP) markers, inter sequence repeats (ISSRs), genomic microsatellites (SSRs), Sequence related amplified polymorphism (SRAP) markers and target region amplification polymorphism (TRAP). However, SSR markers have many advantages over the other marker systems to applied plant breeding; co-dominant nature of SSR polymorphisms, easiness to assay, high reproducibility and high polymorphic genetic information contents (Mondini et al., 2009). Genetic diversity study using SSR markers would provide distinct genetic background of the locally available genotypes for diversifying sources for chocolate spot breeding. Therefore it is crucial to investigate the potential and genetic diversity of the local landraces collections of faba bean in Ethiopia as source for further breeding work.

Another problem that has made difficult for chocolate spot resistance breeding is lack of information regarding the mechanisms controlling the inheritance of chocolate spot resistance. Information on genetics and inheritance of resistance is basic for devising the strategy for faba bean breeding. Much has not been done on the inheritance of chocolate spot resistance. Moreover, no information available on maternal effect, which is potentially reduce the precision of genetic studies, for the inheritance of chocolate spot. Development of high yielding varieties that are resistant to chocolate spot is a feasible option to reduce yield losses. Therefore more sources of resistance have to be identified and inheritance of chocolate spot resistance needs to be investigated. It is also necessary to investigate the genetic control and the direct and indirect correlation of chocolate spot, grain yield and yield component characters of faba bean genotypes for faba bean breeding. Heterosis provides important information for improving yield of faba bean (Obiadalla-Ali et al., 2013). Nevertheless, there is no information on heterosis for chocolate spot resistance. Therefore, it is relevant to quantify heterosis for disease resistance and yield in faba bean.

Fluctuating yield in different crop growing situations necessitates the use of stable performing genotypes for higher and stable yields (Christopher et al., 2014). The performance of a crop variety is the resultant effect of its genotype and the environment in which it is grown. The genotype × environment interactions are of major importance to the plant breeders in developing improved varieties. Hence, evaluation to identify stable genotypes of wider adaptability or productive genotypes for a specific environment is important. Although faba bean is grown in diverse environments in Ethiopia, there is currently inadequate information on the stability and response of different cultivars in different environments. Therefore, there is a need to determine the nature of G X E interactions and study the response and stability for yield and disease resistance of baba bean genotypes using different parameters.

2. Problem statement

Although faba bean (*Vicia faba* L.) is the most important food legume cultivated in Ethiopia, yields have remained low (1.8 t ha^{-1}) compared to the yield potential of 5 t ha^{-1} (CSA, 2013). Chocolate spot (*Botrytis fabae*) disease has contributed to these low yields. Since use of chemicals is beyond the reach of most smallholder farmers, use of resistant varieties remains the cheapest method for farmers. Thus, there is a need to improve the resistance of available faba bean germplasm to this disease. However, crucial information such as farmers' requirements, inheritance of resistance and combining ability are required to devise an appropriate breeding strategy.

Ethiopian mid-altitude and high-altitude areas are dominated by small-scale and resource-poor farmers producing faba bean as main crop for different porpoises. Although, there are several improved faba bean varieties released by research centres, farmers still depend on a few varieties and raise the lack of improved seed as major problem for faba bean production (Mulualem et al., 2012). Participation of farmers in problem identification has been proposed as a solution for developing variety for users' preference and involvement of them at the initial stage towards research for the demand of the farmers (Ceccarelli et al., 2000). This suggested the incorporation of farmers' preference for the success of cultivar development.

Cultivar development for chocolate spot resistance relies on the presence of genetically variable genotypes. The success of breeding for disease resistance and yield depend on a clear understanding of availability, diversity and type of genetic resistance involved. This makes imperative to study the genetic diversity and evaluate germplasm landrace collections for thier disease resistance and grain yield and for appropriate selection of parental lines for the breeding programme. However, the behaviour of the genes and mode of inheritance in faba bean genotypes for chocolate spot resistance is not well understood. This information is important in scheming breeding strategy. It is therefore crucial to study the gene action for chocolate spot and grain yield in faba bean cultivars for use in formulating the approaches of breeding. Potential of heterosis in faba bean breeding using genetically divergent germplasm for yield increment is practiced. The future of faba bean breeding in Ethiopia will therefore incorporate this heterosis potential through finding heterotic sources from locally adapted germplasm that are efficient to develop chocolate spot resistant varieties and increase yield.

3. Research objective

The overall objective of this study was to contribute to food security in Ethiopia by improving resistance to chocolate spot (*Botrytis fabae*) and productivity of farmer-preferred faba bean varieties using conventional plant breeding approach**.**

The specific objectives of this study were;

1. To assess faba bean production, constraints, farmer criteria for varietal preferences and perceptions on chocolate spot disease.
2. To investigate genetic diversity of 50 faba bean genotypes using simple sequence repeat (SSR) markers and morphological characters in order to establish genetic diversity information.
3. To evaluate faba bean germplasm for chocolate spot resistance and yield.
4. To assess the gene action and the role of maternal effects for chocolate spot resistance, yield and yield components.
5. To estimate general and specific combining ability of some selected faba bean lines for chocolate spot resistance and grain yield.
6. To assess the heterosis, correlation and the direct and indirect effect of chocolate spot disease and yield component character on grain yield.
7. To assess the stability of yield and chocolate spot resistance of selected faba bean genotypes across environments.

4. Research hypothesis

This study was carried out to test the following hypotheses:

1. Smallholder farmers in Ethiopia recognise the key faba bean production constraints and have specific preferences for faba bean varieties.
2. Considerable genetic diversity and high levels of variation are available in locally adapted faba bean collections of Ethiopia, which can be exploiting in breeding productive and stable cultivars for the smallholder sector in Ethiopia.
3. Adequate genetic variation exists in the Ethiopian faba bean populations for resistance to chocolate spot disease and grain yield.
4. The additive-dominance model is adequate in explaining the resistance of faba bean to chocolate spot disease and maternal effect contributes to the inheritance of chocolate spot resistance in faba bean.
5. There is a high combining ability among faba bean lines for chocolate spot resistance and yield.
6. There is high and exploitable heterosis for chocolate spot resistance and yield in faba bean varieties and positive correlation and direct effect of yield character on yield.
7. The selected chocolate spot resistant materials yield potential is stable across different environments.

5. Thesis Outline

This thesis consists of nine separate chapters that addressed specific objectives previously mentioned. Each chapter is an independent, potential manuscript for journal publication containing all the necessary information. Some overlap and unavoidable repetition may exist between the chapters and references. The referencing system in the chapters is based on the journals of the Crop Science Society of America.

The chapters are divided as follows:

Chapter 1: Literature review.

Chapter 2: Farmers' perception on faba bean production constraints, and chocolate spot (*Botrytis fabae*) disease for assessing resistance breeding needs for faba bean in three districts of Ethiopia.

Chapter 3: Molecular genetic diversity study of faba bean (*Vicia faba* L.) landrace collections from the Ethiopian highlands using SSR markers

Chapter 4: Phenotypic diversity among faba bean (*Vicia faba* L.) landraces from the Ethiopian highlands and ICARDA

Chapter 5: Genetic variability of faba bean genotypes for resistance to *Botrytis fabae* resistance

Chapter 6: Gene action determining grain yield and chocolate spot (*Botrytis fabae*) resistance in faba bean diallel cross.

Chapter 7: A diallel cross analysis of yield components in faba bean

Chapter 8: Heterosis and path analysis for grain yield and chocolate spot disease resistance in faba bean

Chapter 9: GGE biplot and AMMI analysis of genotype x environment interaction in faba bean genotypes for resistance to chocolate spot (*Botrytis fabae*) and yield

Chapter 10: An overview of the research findings, summary and concluding remarks of the completed research.

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CHAPTER 1

Literature review

1.1 Introduction

This review covers the faba bean production limiting factors and the importance of chocolate spot (*Botrytis fabae*), discusses different control strategies and provides background information on possible disease management options, with emphasis on breeding for resistance to chocolate spot. The inheritance of disease resistance, yield and yield components is emphasised in the review. It also highlights information on farmer's variety preference and perception on disease of faba bean. The interaction between genotype and environment in faba bean is also assessed. This will help to formulate a proposal aimed at a study of the application of resistance breeding for sustainable management of chocolate spot via development of resistant varities and improved faba bean production in smallholder farming systems.

1.2 Importance and production of faba bean

Faba bean (*Vicia faba* L.), production ranks fourth in the world as an important food legume after garden pea chickpea and lentil (Bond et al., 1985; Zohary and Hopf, 2000; Torres et al., 2006). The world's leading producing countries for faba bean are China, Ethiopia, Egypt and the United Kingdom (FAOSTAT, 2014). The average productivity of faba bean since 1999 to 2008 is 1.7 metric t ha^{-1} and 1.2 metric t ha^{-1} in China and Ethiopia respectively. Ethiopia is the leading producer of faba bean in Africa accounting for 56% of the production (FAOSTAT, 2014). In Ethiopia, faba bean is widely grown in the mid-altitude and highland areas and serves as a multi-purpose crop leading the pulse category in area and production (CSA, 2013). It is an important component of crop production in smallholder agriculture that provides an economic advantage to farming communities as an alternative source of protein and cash income and also serves as a break crop in the cereal-based rotations thus playing a significant role in improving the productivity of the soil by fixing atmospheric nitrogen. Schmidtke and Rauber (2000) reported that the amount of atmospheric nitrogen fixed through symbiosis in faba bean exceeds that from peas. Yields of cereal crops following faba bean are therefore improved, thus reducing the need of artificial nitrogen fertilizer for subsistence farmers (Lindemann and Glover, 2003; Agegnehu and Fessehaie, 2006).

1.3 Origin and characterization of genetic diversity of faba bean

The geographic origin of faba bean (*Vicia faba L.*) is accepted as the Near East (Cubero, 1974; Duc, 1997), with the Mediterranean basin as the most important centre of diversity for *Vicia faba* (Maxted and Kell, 2009). China, Afghanistan and Ethiopia have also been reported as secondary centres of diversity for the crop (Zong et al., 2009). Globally, faba bean is genetically diverse with more than 38,000 accessions with approximately 37 collections (Duc et al., 2010). Selection in response to diverse environments and human needs, natural inter-crossing among the different faba bean races, and genetic drift have contributed to increased faba bean diversity and wide variability (Duc, 1997; Maxted and Kell, 2009). The potential for improvement of a plant is determined by the extent of genetic diversity; hence their use in breeding which results in enhanced food production (Khodadadi et al., 2011).

Characterization and quantification of the genetic diversity and of germplasm collections of a crop is essential for a rational use of genetic resources and ensuring long-term success of crop improvement programmes (Gwak, 2008). It is also important to understand the genetic diversity and population structure of faba bean for its conservation, management and exploitation of the variation for its improvement. Genotyping gives essential information on genetic diversity of the materials under consideration. Genetic variation assessments at the DNA level became simple after the development of molecular markers (Reif et al., 2003). The number of molecular marker systems that have been utilized for the genetic diversity study of faba bean include; restriction fragment length polymorphism (RFLP) (Torres et al., 1993b), sequence specific amplification polymorphism (SSAP) markers (Ouji et al., 2012), inter sequence repeats (ISSRs) (Wang et al., 2012) genomic microsatellites (SSRs) (Zeid et al., 2009), sequence related amplified polymorphism (SRAP) markers (Alghamdi et al., 2012) and target region amplification polymorphism (TRAP) (Kwon et al., 2010). Of these, SSR markers have many advantages over the other marker systems applied plant breeding. The advantages includes, co-dominant nature of SSR polymorphisms (Slavov et al., 2005; Mondini et al., 2009), easiness to assay, high reproducibility, which would be the most important in genetic analysis, high polymorphic genetic information contents (Powell et al., 1996), their abundance and distribution in genomes (Varshney et al., 2005), and that they are preferentially associated with non-repetitive DNA (Morgante et al., 2002).

Genetic diversity studies have been conducted on local collections of faba bean in different parts of the world. Terzopoulos and Bebeli (2008) confirmed the existence of different germplasm pools in Mediterranean faba bean (*Vicia faba* L.) using ISSR markers. Molecular assessment on genetic diversity of 151 faba bean collections from different part of the world using TRAP marker revealed large amount of variation within each landraces of faba bean compared to the inbred lines (Alghamdi et al., 2012). In contrast, narrow genetic diversity was observed in faba bean from China using EST-SSR markers (Gong et al., 2010). Accordingly, studies have been done on genetic diversity of faba bean in China, Asia, Europe and Egypt and Tunisia (Zeid et al., 2003; Zong et al., 2009; Kwon et al., 2010; Zong et al., 2010; Alghamdi et al., 2012; Ouji et al., 2012). In Ethiopia the existence of potential genetic diversity of faba bean that has been reported was based on morphological characterization (Gemechu et al., 2006). However, limited germplasm characterization is a major challenge for systematic use of faba bean in genetic breeding programmes in Ethiopia. Moreover, analysis of the genetic diversity and population structure of the Ethiopian faba bean landraces using markers has not been reported. As a result, the faba bean improvement programme depends on genotypes from other sources mainly the International Centre for Agricultural Research in the Dry Areas (ICARDA) (Gemechu et al., 2006). The potential of the local landraces as sources of breeding material has not been exploited. Therefore, it is important to investigate the genetic diversity among conserved germplasm landrace collections of faba bean in Ethiopia for further breeding work.

1.4 Production constraints of faba bean

There is a reduction in the cultivated area and productivity of faba bean in many countries. Several adverse factors have been reported to this decline of which; diseases, weeds, and insect pests are the main biotic yield limiting factors in its production (Torres et al., 2006; Pe´rez-de-Luque et al., 2010). Frost is one of the abiotic stresses contributing for its low production. For example, in Ethiopian highlands 100% yield losses can be experienced especially with late planting as the plants are exposed to frost damage (Mola, 1996). Among the biotic factors, the most damaging parasitic weed of faba bean is *Orobanche crenata* which germinates in response to chemicals released by faba bean (Joel et al., 2007). It is widespread in Mediterranean basin especially in northern Africa, Asia and southern and eastern Europe, attacking dicotyledonous crops, and losses of 50 to 80% have been reported in faba bean fields (Gressel et al., 2004).

In addition, faba bean is adversely affected by numerous fungal diseases. The major diseases are rust (*Uromyces viciae fabae*), black root rot (*Fusarium* spp.), downy mildew (*Peronospora viciae*), ascochyta blight (*Ascochyta fabae* Speg) (Hanounik and Robertson, 1989) and chocolate spot (*Botrytis fabae*). These diseases resulting up to 90% yield loss in faba bean (Muehlbauer and Tullu, 1997). Chocolate spot (*Botrytis fabae*) is one of the economically important diseases that damage the foliage, limiting photosynthetic activity and reducing faba bean production (Bouhassan et al., 2004; Torres et al., 2006). In Ethiopia in spite of its great importance, the productivity of faba bean is about 1.8 t ha^{-1} still far below the crop's potential (>5t ha^{-1}) and *Botrytis fabae* is one of the main constraints contributing to this low productivity (Agegnehu et al., 2006; CSA, 2013; FAOSTAT, 2014)

1.5 Distribution and importance of chocolate spot (*Botrytis fabae*)

Chocolate spot (*Botrytis fabae*) of faba bean has a worldwide distribution and has been reported in Europe, the Middle East, Australia, South East Asia, South America, Korea, India, Canada, Norway, and North East and Southern Africa (Akem and Bellar, 1999; Bouhassan et al., 2004; Tivoli et al., 2006). The disease is more important in humid regions than in the arid climatic zones. Yield loss from *Botrytis fabae* infection is attributed to the reduced pod set and subsequent reduction in number of pods per plant (Griffiths and Amin, 1977). Severe epidemics of *Botrytis fabae* can lead to total crop failure and over 90% yield loss has been reported on faba bean in Australia, 59% in the UK, over 50% in China and losses as high as 61% have been recorded in Ethiopia (Elad et al., 2004).

Mean disease incidence of 47 to 100% and disease severity index ranging from 17 to 49% for chocolate spot have been reported from surveys conducted in 2004 and 2005 in northern Ethiopia (Sahile et al., 2008). High disease prevalence was observed across the lower highland, and the upper midland agro-ecological zones, at altitude ranges of 1980 to 3319 m above the sea level with high incidence and severity at altitude above 2500 m a. s. l. Hence, chocolate spot is considered a major problem in Ethiopia across all the agro-ecological zones and altitudes where faba bean is under production.

1.5.1 Taxonomy and epidemiology of chocolate spot (*Botrytis fabae*)

Chocolate spot (*Botrytis fabae* Sardiña), is classified in the Eukaryota, Kingdom Fungi, phylum *Ascomycota,* subphylum *Pezizomycotina,* class *Leotiomycotina*, order *Helotiales,* family *Sclerotiniceae,* and belongs to the genus *Botrytinia* which is an imperfect fungi (CPC, 2005). The disease is caused by *Botrytis cinerea* Pers. and *Botrytis fabae* Sard., the latter being the most important, since its action can result in serious plant damage which is not the case for *B. cinerea*. *Botrytis* species have a necrotrophic life style occurring as pathogens infecting a single specific host or closely related host (Holz et al., 2007). Faba bean is the major host for*Botrytis fabae*. It also affects, soya bean (*Glycine max*), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris*), and pea (*Pisum sativum*) but these are minor host for the pathogen.

Initial infections of faba bean can result from wind-dispersed *Botrytis fabae* conidia produced under fevered mild, humid weather and temperature 20 to 23°C and with high relative humidity (RH) (>80%) (Harrison, 1988). In dry weather the fungus remains dormant in the host tissues, while under humid conditions there is rapid inter- and extracellular spreading of the disease. The aggressive stage of chocolate spot is also favoured by acid soils, low levels of P and K in the soil, water logging and high plant density (Parry, 1990; Sahile et al., 2008).

Even though, *Botrytis fabae* is detected on leaves and seeds of faba bean, seed-borne infections by *Botrytis* have never been shown to play a major role in the epidemiology of chocolate spot (Harrison, 1988). The conidia of *Botrytis fabae* are commonly airborne, contributing to the ease spread of the disease. The conidia infect new tissue, later giving rise to more conidiophores and starting another cycle of infection (Figure 1.1). Significant damage occurs at the flowering stage of the plant, thus, yield reductions may be serious due to spread of the pathogen from the flowers into the developing pods (Stoddard et al., 2010).

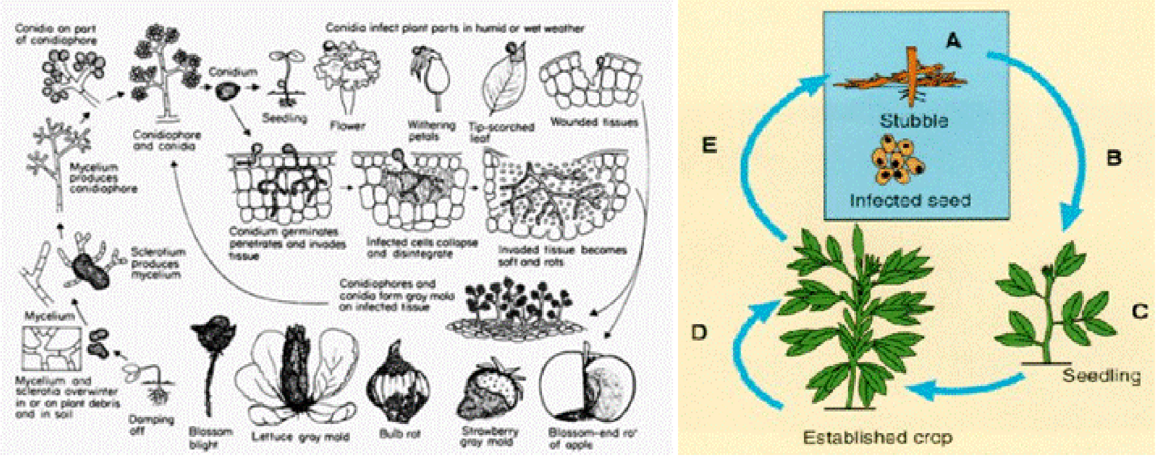

Figure 1.1 Generalised life cycle of chocolate spot disease (*Botrytis fabae*) in faba bean

Where A: The disease-causing fungus survives over summer in crop stubble and infected seed in a semi-dormant state; B: Spores produced on stubble are blown by wind and rain to infect seedlings. C: Small lesions form on healthy leaves; D: As the crop grows, spores produced on dead or dying leaves and flowers spread the disease in the canopy; E: Disease infested stubble remains after harvest. Infected seed is harvested with healthy seed. Source: http://www.grainlegumes.com

There are various methods by which *Botrytis fabae* overwinters and is introduced into a new crop. The fungus can survive and sporulate as a saprophyte on necrotic tissue, or produce long-term survival structures called *sclerotia* (Holz et al., 2007). Since the pathogen has a restricted host range, *sclerotia* are an important means of overwintering. Moreover, the fungus can overwinter in the form of chlamydospores on volunteer bean seedlings, off season crops and crop debris (Simay, 1994; Holz et al., 2007).

1.5.2 Symptoms of chocolate spot disease on faba bean

The symptoms of infection by *Botrytis fabae* on faba bean vary from minor necrosis to extensive destruction of tissue and leaves, stems, flowers and pods can be infected, with flowers and pods being the most susceptible parts (Griffiths and Amin, 1977). *Botrytis fabae* infection is divided into two distinct phases: non-aggressive and aggressive to describe limited and rapidly expanding lesions, respectively (Harrison, 1988). Non-aggressive infection can be detected as small scattered, discrete red-brown lesions on the foliage, stems and pods at any stage of crop development. *Botrytis fabae* induces lesions equally on the upper and lower leaf surface of faba bean. At the beginning of the life cycle of the pathogen, large numbers of spots called “chocolate spots” appear; 5-10 mm in diameter, circular on the leaflets, petals and pods, elongated on the stems, and tend to be evenly distributed of the lesions (Harrison, 1988).

When humidity is high, the non-aggressive stage changes to the aggressive stage. At this stage lesions begin to sporulate and the spots increase and coalesces turning into rust-coloured necrotic lesions. The lesions become much darker and get covered in fluffy grey-white mycelium of irregular form on all the plant organs. During this stage, large areas of leaf tissue of faba bean may die, leading to defoliation (Figure 1.2). Later infection of young pods leads to stunting and ovules may not develop. Infected flowers and pods may abort also causing discoloration of the faba bean seed. Consequently, dark brown streak symptoms on the stems may become noticeable. The highly infected stems can lead to lodging of the plant as a whole and *sclerotia* can be found in the dead stem (Figure 1.3) (CPC, 2005)

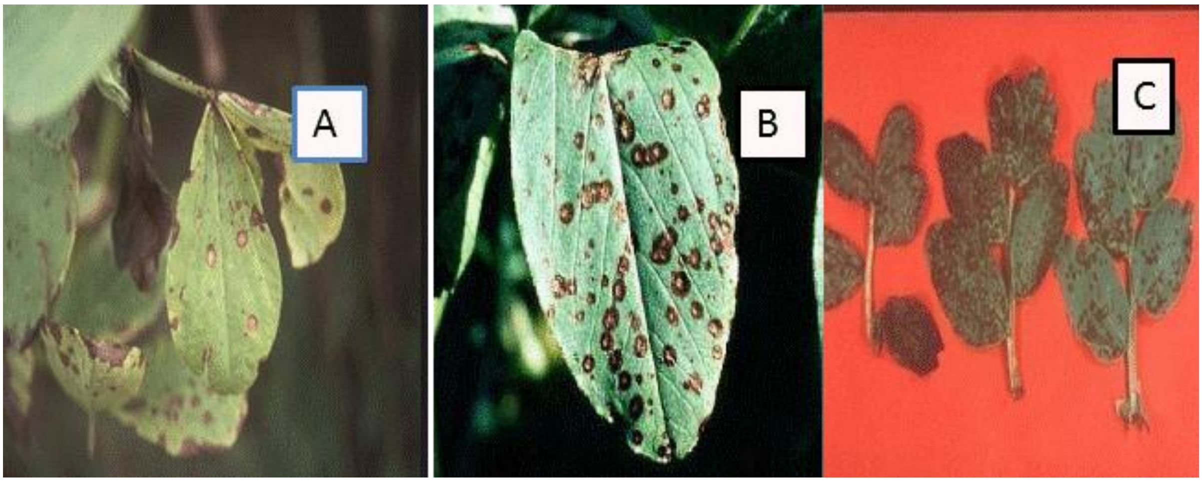

Figure 1.2 Chocolate spots on faba bean leaves: necrosis of the leaf and defoliation: (B and C aggressive phase of *Botrytis fabae*)

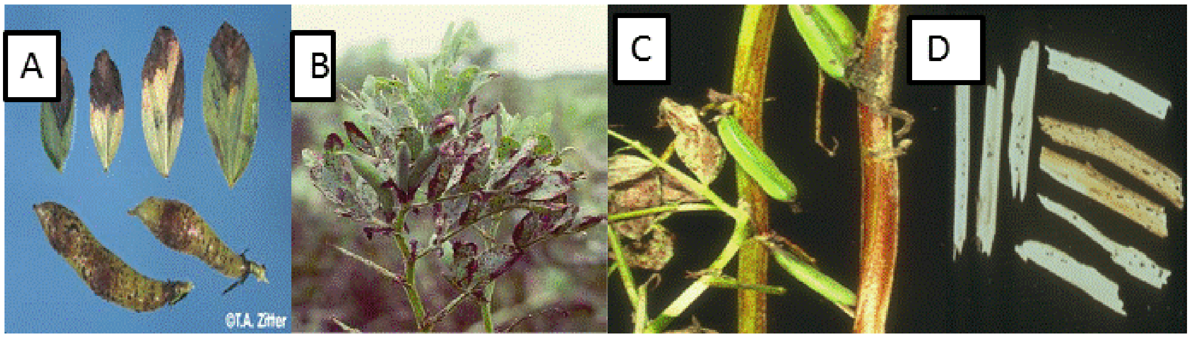

Figure 1.3 Chocolate spots on faba bean pods (A) leaves (B) and stem (C), reddish of a faba bean stem and sclerotia of *Botrytis fabae* on infected faba bean stems (D)

1.5.3 Biology and morphology of *Botrytis fabae*

Botrytis fabae can be cultured on growth medium. High sporulation of *Botrytis fabae* has been obtained when cultured on bean leaf extract agar medium with high concentrations of inorganic salts of sodium nitrate (Leach and Moore, 1966). Spores are produced by conidiophores that are visible with the naked eye in senescent leaves of diseased plants (Stoddard et al., 2010). Conidia are the means of asexual reproduction for the pathogen and these are detectable with a light microscope, while sexual reproduction takes place by means of ascospores produced in apothecia (CPC, 2005). Conidia of *Botrytis* are generally short-lived propagules in the field but they have been observed to survive up to 14 months when stored as dry at room temperature. However, microbial activity, temperature extremes, moisture availability, and sunlight exposure determined their survival (Coley-Smith et al., 1980). The conidia range between 14-29 µm x 11-20 µm in size (CPC, 2005). Since *Botrytis fabae* may be confused with *Botrytis cinerea*, especially if both are isolated from chocolate spot lesions, it is useful to note that conidia of *Botrytis fabae* are considerably larger than those produced by *Botrytis cinerea* (usually 6-18 µm x 4-11 µm). Moreover, *sclerotia* of

Botrytis fabae are always smaller in size and are produced abundantly, whereas *sclerotia* produced by *Botrytis cinerea* are larger and fewer (Harrison, 1988)

Botrytis fabae produces *sclerotia* in the field and more abundantly in culture on bean leaf extract agar, which can survive adverse conditions. The internal structure and histochemistry of sclerotia of *Botrytis fabae* and *Botrytis cinerea* are similar. The rind walls contain melanic pigments, the medullary hyphae are surrounded by a continuous matrix of β-glucans and the intra cellular nutrient reserves are protein, glycogen, polyphosphate and lipid (Elad et al., 2004). It can produce aprothecia after a sexual process and possess a considerable capacity for producing successive crops of conidia in many *Botrytis* species.

1.5.4 Variability of the chocolate spot pathogen (*Botrytis fabae*)

Although differences in the virulence of isolates and existence of races have been reported (Hanounik and Maliha, 1986), no comprehensive description of races of *Botrytis fabae* was carried out. In Ethiopia, study on morphological, cultural and pathogenic variability among nine isolates of *Botrytis fabae* found an isolate from Inewari to be the most virulent (Gorfu, 1996). Variability of the *Botrytis fabae* isolates in all faba bean growing areas of Ethiopia has not been intensively studied (Sahile et al., 2008). Thus there is a need to evaluate the virulence of a collection of isolates of *Botrytis fabae* from different parts of the country.

1.5.5 Management of chocolate spot of faba bean

Several methods may be applied to manage the chocolate spot of faba bean. These include cultural practices, chemical control, and integrated pest management.

Cultural Control

Use of crop rotation, avoiding infected debris and volunteer plants can minimize an epidemic of chocolate spot in subsequent faba bean crop (Harrison, 1979). It is also important to reduce the disease by ensuring adequate drainage and using optimum fertilizer rates, particularly of P and K (Parry, 1990). In Europe avoiding early sowing is recommended to prevent spring frost damage and hence predispose the crop to the disease (Jellis et al., 1998). A positive association between early sowing and chocolate spot severity was also noted in Syria (Hanounik and Hawtin, 1982). Agegnehu et al. (2006) reported late planting as an option of chocolate spot control in Ethiopia. Faba bean intercropped with cereals resulted in significant reduction of chocolate spot due to host biomass reduction, altered microclimate and physical barriers to spore dispersal (Fernández-Aparicio et al., 2011).

Chemical Control

Chemical control is the most widely used means of controlling chocolate spot in faba bean (Khaled et al., 1995). Mancozeb gave good control of chocolate spot and increased seed yield (Marcellos et al., 1995). Studies of integrated chocolate spot management in Ethiopia indicate that the disease epidemics can be managed by combining early sowing, spray of mancozeb, chlorothalonil fungicide, use of moderately resistant cultivars and cereal intercropping (Gorfu, 2000; Sahile et al., 2008). However, smallholder farmers cannot afford to buy fungicide, and complete resistant cultivars for chocolate spot have not been developed in Ethiopia.

Host-plant resistance

Breeding for disease resistance is the most cost efficient method of control (Sillero et al., 2009). Cultivar with only a single (major) gene for resistance provides only a short-term solution since new virulent pathotypes overcomes the resistance. Comprehensive description of races of *Botrytis fabae* and lack of good sources of resistance are reported as major constraint for faba bean breeding for chocolate spot resistance (Stoddard et al., 2010). Therefore, using the high level of out-crossing nature of faba bean cultivars, stable resistance can be developed by recurrent selection that combines minor genes at different loci (Hanounik and Hawtin, 1982).

1.6 Breeding progress in faba bean

Hybridization is the predominant means of generating genetic variation in breeding of faba bean for improvement of its productivity (Muehlbauer et al., 1988). Improvement includes a random-mating population or pure line for the effectiveness of selection based on the utilization of additive gene effects (Nassib and Khalil, 1982). The “diallel” selective mating system procedures showed a dynamic gene pools in the breeding populations of faba bean (a) to which new sources of germplasm are added whenever feasible, (b) in which the frequencies of favourable alleles are progressively increased via recurrent selection, (c) in which genetic recombination is enhanced by mass hybridization among selected genotypes, and (d) from which cultivars inbred or parental lines can be extracted at any stage (Nassib and Khalil, 1982).

Breeding of *Vicia faba* for pure line development decreases yield performance with an increase in homozygosity. In contrast, cross-pollination results in plants which are more vigorous than the population mean (Picard et al., 1982). The genetic gain of hybrids can reach 75% compared to the mid-parent performance. In addition to yield, winter hardiness and high protein content can be obtained with F_1 progenies. If pollination is a limiting factor for yield stability, hybrids should be more stable than natural populations (Picard et al., 1982).

1.6.1 Breeding for chocolate spot resistance and sources of resistance

Resistant cultivars are an ideal management tool for reducing yield losses caused by diseases. So far complete resistance of faba bean to chocolate spot has not been reported and only incomplete resistance is being used (Garcia et al., 2008). Several authors have tested and identified inbred lines with resistance to chocolate spot. Some inbred lines exhibiting partial resistance to chocolate spot include; BPL710, BPL 1179 (most consistently resistant accessions developed by ICARDA), BPL 1763, ILB 4726, LPF 120, ILB 4709, ILB 5284, Sel.97 Lat.97 132-1, Sel.97 Lat.97 132-3 (Villegas-Ferna´ndez et al., 2009). Cultivars Icarus and Nura, which were identified by Raynes (2008), are some of the recent faba bean germplasm reported as resistant to *Botrytis fabae*.

Chocolate spot is a major production constraint of faba bean in Ethiopia, but little breeding progress has been made to achieve adequate levels of resistance (Dereje and Yaynu, 2001; Gemechu et al., 2006). The development of faba bean varieties with resistance to faba bean chocolate spot disease is important for improvement of its productivity. In Ethiopia currently, the hybridization programmes focus mostly on the large-seeded, chocolate spot resistance, and high yielding materials using the bi-parental crosses (Gemechu et al., 2006). Sources of resistance to chocolate spot and large–seeded faba bean were identified from introductions from ICARDA of which ILB 938, ILB 4726, ILB 4615, ILB 4725, and ILB 4709 are considered resistant; Moti, Gebelcho, Tumsa, Hachalu, and Wolki that were released from HARC are moderately resistant (Gemechu et al., 2006). However, further screening is needed in order to identify more resistant materials that are adapted to different environments. Landraces have potential for breeding in marginal areas having genes for stable yield over a range of environments than introduced materials. Evaluation for resistance using a highly virulent pathogen is very important in developing resistant varieties (Azza et al., 2002; Vail et al., 2012). Moreover, higher disease infection will help to differentiate genotypes better (Carson et al., 2002). Thus it could be crucial to look for resistant materials to chocolate spot diseases from local landraces using virulent pathogen.

1.6.2 Vertical and horizontal resistance to chocolate spot

Vertical resistance is governed by major genes or simply inherited, while horizontal resistance is controlled by polygenes. The disadvantage with the vertical resistance is that a previously resistant genotype may become susceptible with the appearance of a new race of the pathogen (Beebe and Corrales, 1991). Horizontal resistance on the other hand, has been shown to be more stable than the vertical resistance. However, polygenic traits are more difficult to handle during breeding, because of complex segregation patterns that necessitate larger population size, greater environmental effects, and more possibility of negative linkage problems (Beebe and Corrales, 1991). In Ethiopia, a study on the interaction of faba bean (Kuse and S83103-1-1) and nine isolates of *Botrytis fabae* indicated resistance governed by major genes (Gorfu, 1996). A different study on resistance of faba bean to chocolate spot indicated that the faba bean lines BPL710, 1196, and 1179-1 revealed no differential reaction when inoculated with 12 isolates of *Botrytis fabae*. This indicated that they carry genes for a broad-based horizontal resistance. In contrast, lines BPL1763, 1821, and ILB1814 revealed significant differential reactions for the isolates of *Botrytis fabae* tested, implying they genes for a narrow based vertical resistance to *Botrytis fabae* (Hanounik and Maliha, 1986).

1.6.3 Diallel mating and estimation of gene action

Selection of mating designs is important in the study of genetic inheritance mechanisms. Diallel analysis developed by Hayman (1954) and Jinks (1955) provides reliable mechanism to properly understand the nature of gene action involved in the development of complex genetic characters of economic value. Moreover, it provides useful information that can be used to select best parental combinations that will create sufficient genetic variability for the purpose of developing superior segregants (Johnson and King, 1998; Sharma, 2006). This is helpful for breeders to identify the best combiners which can be hybridized to build up good fixable (Arunga et al., 2010). In the population improvement strategy knowing the relative amount of the genetic variation caused by additive gene effects or non-additive gene action is important (Johnson and King, 1998). An inference can be made from diallel cross mating technique about general combining ability (GCA) of parents and specific combining ability (SCA) of hybrids (Mar´ılia et al., 2001). This differentiates between the average performances of parents in crosses and the deviation of individual crosses from the average of the parents (Oscar et al., 2006). Analysis of a diallel design partitions the total variation of population data into the GCA, which provides estimates of additive gene effects, and SCA estimates non-additive gene action (Falconer and Mackay, 1996). The GCA/SCA variance ratio indicates the relative importance of heritable additive genetic effects against the non-additive (dominant) genetic effects and large ratio indicates the importance of additive gene effects and a low ratio entails the presence of dominant and/or epistatic gene effects (Baker, 1978). Information on maternal effects of the parental crosses also can be acquired from the diallel analysis.

The concepts of GCA and SCA are extensively used in plant breeding and have particular significance to the diallel mating design which allows obtaining inference on gene action for the trait under study (Hallauer et al., 2010). Hence the diallel mating design has been extensively used to study the genetics of different traits for faba bean which allows crosses among all possible combinations and estimates of GCA, SCA and their effects (Bond, 1967; Alghamdi, 2009; Ibrahim, 2010; Farag and Afiah, 2012). Attia et al. (2006) and El-Hady et al. (2006) reported that both GCA and SCA variance were important for yield and yield components in faba bean.

1.6.4 Gene action and inheritance of chocolate spot resistance in faba bean

Knowledge of the genetics for resistance to chocolate spot is of paramount importance. The mode of inheritance and type of gene action for this disease is not clearly known as most of the studies have focused on other disease of faba bean. Availability of information on the genetics of the target trait facilitates progression of any breeding programme and allows breeders to choose the most adequate working strategy (Lüders et al., 2008). Knowledge of the inheritance of resistance to disease is necessary for the development of effective breeding programmes for resistant cultivars development. Studies on the genetic analysis of the inheritance of resistance to stem rot (*Sclerotium sclerotiorum*) in the faba bean populations indicated it was controlled by a single dominant gene (Lithourgidisa et al., 2005). In contrast, Fuller et al. (1984) reported that inheritance of resistance to *S. sclerotiorum* in dry beans was quantitative. Similarly, Kohpina et al. (2000) identified major dominant gene in genotype ILB 752 which can be potentially used in breeding and minor genes in NEB 463 with the potential for accumulating resistance through mass selection for ascochyta blight resistance.

In other genetic studies of resistance to ascochyta blight, additive gene effects were found predominant to non-additive effect (Kharrat et al., 2006). In faba bean populations and diallel F_1 progenies, additive gene action was more important in determining the response to rust disease resistance (Stoddard and Herath, 2001). El-Bramawy and El-Beshehy (2011) reported that additive gene effect was more important than dominance effect for the inheritance of resistance to bean yellow mosaic virus of faba bean. In a study of the inheritance of chocolate spot and rust disease tolerance in faba bean, the mode of inheritance was suggested as dominant character controlled by a single gene (Noorka and EL-Bramawym, 2011). This study generated information which is applicable for the two diseases, but only two germplasms were considered. In addition, there is no information on inheritance of chocolate spot resistance in faba bean in Ethiopia using divergent breeding lines.

1.6.5 The role of maternal effects in disease resistance, yield and yield components

The contribution of the maternal parent to its offspring beyond the equal chromosomal contribution expected from each parent i.e. maternal effect can cause variation in phenotype of an individual like the genotype and environment do (Roach and Wuliff, 1987). Cytoplasmic genetic maternal effects are directly transferred from the maternal to the offspring independent of nuclear genes and its inheritance persists through generations. The most direct quantitative evidence for unequal contribution by maternal and paternal parents is reciprocal crosses where pairs of individuals serve as both maternal and paternal parent (Gurur and Stefansson, 1977). If there are no parental influences, variance between family groups will be similar, whereas maternal effects will be perceived if there are greater differences between maternal families than paternal families (Roach and Wuliff, 1987). Cytoplasmic or nuclear genetic maternal effects will inflate the amount of genetic variance, but may slow the response to selection if the trait is completely under maternal control. Maternal effects are generally considered to reduce the precision of genetic studies. The maternal effects can have a significant effect on the phenotype of an individual, therefore for precise interpretations of experimental results; it is worth to consider variation due to maternal effects (Roach and Wuliff, 1987).

Significant maternal effects among the parents were observed in the inheritance of bean yellow mosaic virus (BYMV) in faba bean *(Vicia faba* L.) (El-Bramawy and El-Beshehy, 2011). Similarly, identical performance of the reciprocal crosses was observed in a study of genetic expression of ascochyta blight resistance in faba bean but there were no maternal effects involved (Kohpina et al., 2000). Both nuclear and cytoplasmic genes have been suggested to be responsible resistance for faba bean rust (Stoddard and Herath, 2001). However, no information is available regarding maternal inheritance of faba bean resistance to chocolate spot.

Significant maternal effects for days to flowering and 100-seed weight were reported for chickpea (Tubabicer and Sakar, 2008). In contrast, no maternal effects for inheritance of resistance to pod borer and grain yield was observed in the study of the nature of gene action and maternal effects in chickpea (Narayanamma et al., 2013). Nevertheless, no information is available about the maternal effects for yield and its components in faba bean.

1.6.6 Gene action and inheritance of yield and yield components in faba bean

Grain yield is the result of the development and growth of yield components during the life of the crop. The need to understand the relationships between yield components and its genetics is vital for the improvement of yield (Slafer et al., 2009). Nature and magnitude of genetic variability determines the genetic improvement of a crop and the inheritance of desired traits (Alghamdi, 2009). Estimates of heritability of faba bean for different characters using different materials and methods indicated that heritability values were high for 100-seed weight and low to moderate for seed yield along with number of branches, pods, and seeds/plant (Attia and Salem, 2006; El-Hady et al., 2006; El-Hady et al., 2007). Genetic studies for some selected faba bean genotypes indicated highest estimates of broad sense heritability (h^2) for flowering date (0.99), number of pods (0.96), number of seeds per plant (0.96) and maturity date (0.91). Moreover, significant positive correlations were detected between seed yield (t/ha) and each of plant height, number of pods per plant, 100 seeds per plant, and 100 seed weight per plant (Alghamdi and Ali, 2004; Alghamdi, 2007). However, negative and significant correlations were observed between seed yield (t ha^{-1}) and flowering, maturity date, number of branches per plant and 100-seed weight. Study of the inheritance of purple seedling colour in faba bean indicated that it is controlled by a single gene which is dominant over green seedling colour. Since this trait is simply inherited and expresses itself at an early growth stage, it can be used as a marker to assay the extent of out crossing (Metz et al., 1992).

1.6.7 Heterosis and combining ability in faba bean

Best combiners to exploit heterosis or to build up the favourable fixable genes can be identified using combining ability analysis (Islam et al., 2009). Diallel cross technique can be used to exploit new genetic combinations where the performance of the hybrids is compared to that of the parents (Alghamdi, 2009). Significantly positive to negative heterotic effects were reported for different traits in F_1 progenies of faba bean, especially crosses among widely divergent materials (Alghamdi, 2009). Superiority of hybrids over the mid and better parents for seed yield was found to be associated with manifestations of heterotic effects in main yield components i.e., number of branches, number of pods, and number of seeds per plant and seed index. Kao and Mcvetty (1987) suggested that increases in yield and total dry matter could be achieved by exploiting over-dominance using F_1 progenies or synthetics from significant over-dominance for yield and number of pods per plant in both the F_1 and F_2 generations. In other inheritance studies of 14 crosses between faba bean pure lines, strong heterosis was observed for traits, number of pods per node and seeds per pod (Torres et al., 1993a). Melchinger et al. (1994) identified substantial heterosis in seed yield without significant heterosis in their component traits in parents, F_1, F_2, Bc1 and Bc2 generations from crosses between small and large seeded faba bean cultivars.

1.6.8 Path coefficient analysis

Grain yield of faba bean depends on many related traits. Identifying the extent and nature of relationship between traits, especially effect of each trait on yield, is the first step towards selecting the best plant breeding components. Interrelationships among traits help in formulating a scheme of multiple trait selection. Correlation coefficient could show linear relationship between them. However, path analysis would elucidate direct and indirect relationships among these traits, and hence on the basis of that the breeder could select the most effective traits to evaluate and release varieties. Indirect selection is also important when desirable characters have low heritability. Tadesse et al. (2011) using path analysis indicated that the number of pods per plants, seeds per pod, thousand seed weight, stand per cent and plant height had high positive direct effect for grain yield of faba bean. Azarpour et al. (2012) reported in the study of path coefficient analysis of seed yield and its components in faba bean that the maximum value of direct effect was related to plant height on seed yield. Path analyses have been observed to differ among the genotypes. Since yield is controlled by a number of traits, phenotypic path analysis of these component characters is essential for increasing the grain yield performance.

1.7 Farmers' participation for assessment of faba bean production constraints

Farmers are the final users of the research output thus their involvement in research from problem identification to variety development is crucial. Participatory rural appraisal tools enable for better understanding of farmer needs, diagnose, identify and prioritise key constraints of crop under study. This includes use of semi-structured interviews, focus group discussions; transect walks, and ranking exercises which can be useful to facilitate direct interaction between farmers and researchers for a common understanding on constraints and needs (Narayanasamy, 2009). Consequently, diverse opinions from farmers can be generated which contribute in developing selection guides for variety development and good basis for planning for various traits of interest.

Farmers' preferences have arisen as a major factor in the improvement of crops for smallholder farming systems. Apart from high yield and disease resistance, breeders may not know farmer's complex requirements. Further, breeding programmes focus on the needs of conventional agriculture where selection is carried out for superior genotypes that fit into high potential environment. Farmers have a long term and direct interaction with their environments and cropping systems. In addition, they have some specially preferred traits which may not be considered by breeders. Thus, it is important for farmers to participate in identification and determining specific breeding priorities that can be used to improve crop breeding strategies.

Participatory plant breeding in which users are involved in the development of new technology offers farmers to choose varieties suitable for their needs and in their own environment (Ceccarelli and Grando, 2007). It can also enable farmers to maintain the genetic resources they value (Chiffoleau and Desclaux, 2006). In addition to shortening time of releasing new varieties compared to conventional breeding, participatory plant breeding has an advantage of low investment by targeting varieties that can be adopted by farmers. In Ethiopia, participatory variety selection of faba bean was found effective and reliable for identifying appropriate cultivars through partnership with resource-poor farmers (Mulualem et al., 2012). Ayimut and Abang (2008) reported that farmers' awareness in the northern part of Ethiopia for diseases such as faba bean chocolate spot and chickpea ascochyta blight was extremely low. Lack of perception for diseases prevalence could delay the application of appropriate management. Therefore, assessing farmers' situations, farmers' participation in research problem identification and developing varieties with their involvement is essential for effective and sustainable agricultural research.

1.8 Genotype by environment interaction

Genotype by environment interaction refers to the differential response of genotypes to different environments. A study conducted to compare AMMI and Principal Coordinate models to analyse the genotype-by-environment (G × E) interaction of 11 genotypes of *Vicia faba* L., across environments in Spain indicated that AMMI was more sensitive in discriminating (six genotypes) across environments than Principal Coordinate Analysis (three genotypes) (Flores et al., 1996). GGE biplot is a method that displays the main genotype effect (G) and the genotype X environment interaction (G X E) of multi-environment tests (Villegas-Ferna´ndez et al., 2009). Study on the stability of resistance for chocolate spot using GGE biplot method in seven environments showed that G x E interaction accounted for 22% of the sum of squares of the multi-environment evaluation, revealing instability of the phenotypic expression across environments which hamper the efficiency of selection (Villegas-Ferna´ndez et al., 2009).

Karadavut et al. (2010) used only the Eberhart and Russel (1966) method to evaluate performance of faba bean genotypes across different environment. However, Attia et al. (2007) could reliably classify genotypes using, in addition to the genotypic stability of Tai

(1971), stability variance of Shukla (1972) and yield stability statistic of Kang and Magari (1995). This implies that more than one stability analysis methods may be needed to adequately analyse genotypes across environments. Ethiopia is a country of great environmental variation (EMA, 1988) which contributes to genotype by environment interaction, and yield instability. Mussa et al. (2001) reported that G x E interaction effect on faba bean yield is of major importance to the faba bean improvement. Conversely, limited information is available about stability analysis of faba bean genotypes for yield and chocolate spot resistance across environments in Ethiopia.

1.9 Summary

Generally, this chapter reviewed important issues such as importance of faba bean, position of resistance breeding and management options for chocolate spot (*Botrytis fabae*) and breeding strategies for the improvement of faba bean. It may serve as background information for faba bean breeding towards chocolate spot resistance and breeding for higher grain yield, using the conventional approach and molecular markers. Breeding information obtained out of Ethiopia might not have direct application, as the environment in which the application is going to be made, as well as the whole farming systems is quite different. It was also observed that there is limited information on the genetics of inheritance of faba bean resistance to the disease. It is therefore, important to assess the genetics of inheritance to set different breeding strategy. In this review it was noted that there is an enormous gap between grain yield potential and the actual yield in the farmers' fields, suggesting the presence of opportunities for breeders to bridge the gap. This review also suggested that genotypes with high resistance levels and good stability would be obtainable by selecting germplasm directly, followed by evaluation across the target environments. The importance of farmers involvement who are the end users of research output in the breeding process is emphasized as it influences adoption of new varieties.

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Chapter 2

Participatory assessment of production threats, farmers' desired traits and selection criteria of faba bean (*Vicia faba* L.) varieties: opportunities for faba bean breeding in Ethiopia

Abstract

Faba bean (*Vicia faba* L.) is an important legume crop used as a major source of dietary protein for subsistence farmers and as a source of foreign currency in Ethiopia. However, yield and productivity have remained low due to the type of varieties used, most of which are susceptible to plant diseases, thus threatening food security. The objectives of this study were to assess the major threats to faba bean production, determine farmers' varietal preferences and selection criteria, and assess farmers' perceptions of faba bean diseases with special reference to chocolate spot (*Botrytis fabae*) disease. Data were collected using participatory rural appraisal methodologies which included semi-structured questionnaires, matrix ranking, focus group discussion, and observation through a transect walk. A stratified random sampling method was employed to interview 240 households selected from 12 villages of three administrative zones within two regional states. The major threats to faba bean production were chocolate spot disease and lack of improved faba bean seed. Many farmers (>85%) were able to describe the symptoms of chocolate spot and had various names for the diseases but they were not aware of its causative agent. Results showed that farmers predominantly grew local faba bean landrace varieties with yields ranging from 0.56 to 2.8 t ha^{-1} and very few (10%) used improved varieties. On average 66.4% of the farmers preferred the local landraces for their adaptability to the environment, tolerance to frost, early maturity, good food taste and straw yield. On the other hand, 64.4% of the farmers preferred improved varieties for their high grain yield and bigger grain size. The farmers use these preferences to select varieties. It is concluded that faba bean chocolate spot disease is a persistent problem in all faba bean growing areas of Ethiopian highlands with high disease severity associated with growing susceptible local varieties. Thus, breeding opportunities do exist for improving the yield and disease resistance of the preferred local faba bean varieties and involving the farmers in the selection process at different stages of the breeding.

Keywords: Chocolate spot; faba bean; farmers' perception; farmers' preferences; production constraints.

2.1 Introduction

Globally, faba bean (*Vicia faba* L., 2n =2x =12) is the fourth food legume in production after garden pea, chickpea and lentil (FAOSTAT, 2014; Kaur et al., 2014). In Ethiopia, faba bean is a food security crop that is predominantly grown in the mid-altitude and highland areas as a multi - purpose crop and leads the pulse category in terms of area and production (CSA, 2013). Faba bean is a major source of dietary protein (Sarah et al., 2009), and staple food used in different forms by the majority of small-scale, subsistence farmers in Ethiopia. Besides its contribution to food and nutrition security in the households, it plays an important role in management of soil fertility through crop rotation in cereal production hence contributing to agricultural sustainability (Agegnehu and Fessehaie, 2006; FAOSTAT, 2014). It is also a foreign currency earner for the national economy. Ethiopia is the fourth largest exporting country of faba bean next to France, Australia and United Kingdom (FAOSTAT, 2014).

However, in spite of its importance, the productivity of faba bean is about 1.8 t ha^{-1} still far below the crop's potential >5 t ha^{-1} (CSA, 2013). This has been attributed to different biotic and abiotic stresses of which *Botrytis fabae* is a major constraint (Gorfu and Beniwal, 1987; Agegnehu and Fessehaie, 2006; Jarso et al., 2008). Faba bean research in Ethiopia has focused mostly on developing varieties that are high yielding with less emphasis on improving characteristics such as disease resistance and quality traits preferred by farmers, that is, taste and flavour. Moreover, there are no detailed studies involving the participation of smallholder farmers in establishing the extent to which they perceive the importance of foliar diseases in the production of faba bean, in particular chocolate spot. Ceccarelli and Grando (2007) underlined the importance of farmer participatory crop improvement by using and acknowledging their knowledge on essential quality characteristics as this ensures the adoption of new varieties.

Surveys conducted on faba bean in Ethiopia have mainly focused on field pests with the primary objective of establishing the status and distribution of the diseases and insect pests for exploring control measures (Assefa and Gorfu, 1985; Sahile et al., 2008a). In all these surveys, an analysis of farmers' perceptions and outlook on the diseases and control methods utilised by farmers has been minimal. Farmers' perceptions and an understanding of ecological principles related to or affecting crop diseases plays a fundamental role for sustainable disease management, thus ensuring food security and should therefore be an essential part of a long-term strategy for enhancing agricultural productivity (Ayimut and Abang, 2008). This study was therefore designed to determine farmers' perceptions on faba bean production threats, with special focus on chocolate spot (*Botrytis fabae*) disease and to assess farmers' preferences and varietal selection criteria in selected highland areas of Ethiopia.

2.2 Materials and methods

2.2.1 Study area

The study was conducted in two regional states of Ethiopia; Amhara and Oromia (Figure 2.1) in a total of three administrative zones; North Shewa zone, Arsi zone and Finfine Special zone. From each zone, one district with four villages was selected as indicated in Table 2.1. The three study zones represent *Dega* (cool, humid, highlands): where the annual rainfall ranges from 1200-2200 mm (Phama et al., 2010; USDA, 2013). These areas were chosen based on the coverage of faba bean production and where farmers have reported disease problems.

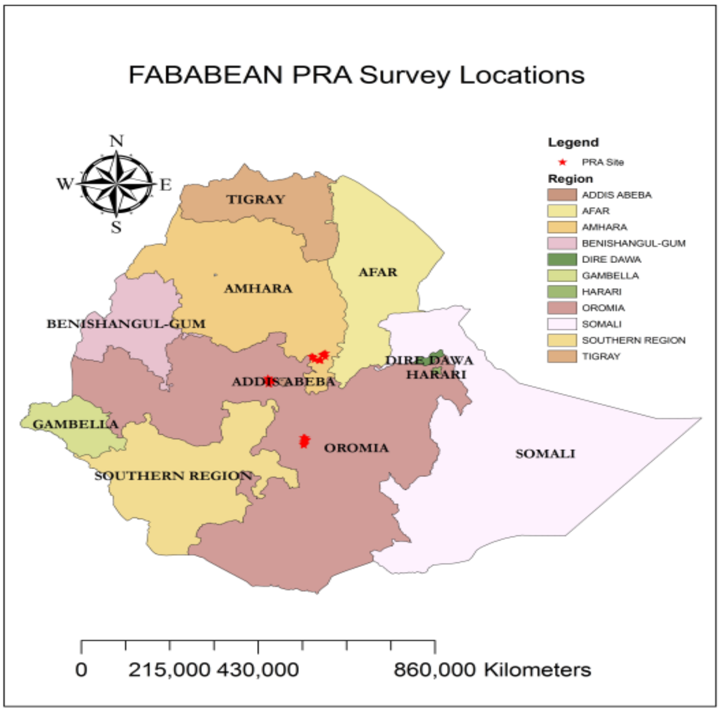

Figure 2.1: Map of Central highland areas of Ethiopia where the study was conducted: * North Shewa zone (Amhara), Arsi zone (Oromia) and Finfine special zone (Oromia)

Table 2.1 Description of the study area

Region	Zone	District	Village	Altitude(m.a.s.l)	Latitude	Longitude
Amhara	North Shewa	Basona Worana	1.Wushawushign 2.Gudoberet, Baso 3.Bengora 4.Goshe Bado	2754-3213	07^{o}22'49'' to 07^{o}34'79'' N	39 ^{o}14'69'' to 39 ^{o}17'038''E
Oromia	Arsi zone	Limuna Bilbilo	1.Lemu Dima 2.Chiba Michael 3.Koma Katar 4.Dawa Bursa	2698-3169	07^{o}22'49'' to 07^{o}34'79'' N	39 ^{o} 14'698'' to 39 ^{o}17'038''E
Oromia	Finfine Special zone	Wolmera	1.Bekekana Kore Oddo 2.Welmera Choke 3.Telecho Gebriel 4.Wajitu Harbu	2268-2751	09^{o}00'57'' to 09^{o}09'51'N	38 ^{o} 27'300'' to 38 ^{o}32'599''

2.2.2 Data collection

Structured questionnaires and other participatory rural appraisal tools including focus group discussions, matrix ranking and transect walk were used for data collection. The survey was made up of a team of researchers led by the principal investigator, enumerators [all from technical staff of Holetta Agricultural Research Centre (HARC)] and extension officers from the respective zones who also facilitated the interviews and focus group discussions. Before conducting the survey, a pre-test of the questionnaire was done at two villages. Training was also given to the team on the objective of the survey and how to carry out effective interviews.

Stratified random sampling was used to select the 240 faba bean farmers from the three districts. The farmers differed in age (range 31-60) and gender (88% male and 12% female). Further, 20 farmers were selected in each village resulting in 80 participating farmers per district. A questionnaire was used to gather the following information on faba bean; farming systems practiced in the study areas, utilisation, major production constraints, disease perceptions, control options used for faba bean disease and varietal preferences. To assist farmers in identifying diseases, pictures showing symptoms of different faba bean diseases were provided to all respondents during the interview.

In each district, focus group discussions involving 5-9 faba bean growers differing in age and gender were conducted. These discussions were facilitated by the use of an interview guide and matrix ranking for faba bean constraints. A transect walk was conducted during the main cropping season of 2012 in the three zones with some representative farmers who had been interviewed. During the transect walk, the area allocated to faba bean was observed. Moreover, awareness of farmers to plant diseases especially faba bean chocolate spot was assessed. Secondary data which included the total number of households, total number of farmers growing faba bean, areas covered with faba bean in 2011 growing season were collected from the crop department of the Ministry of Agriculture office of the respective districts. All the data collected were coded and subjected to analysis and computed for their relationships, frequencies and descriptive statistics. Statistical analyses for qualitative data were performed using SPSS Inc. version 19 (SPSS, 2010). Chi-square test was performed for determination of association between qualitative variables.

2.3 Results and discussion

2.3.1 Demography

Of the total interviewed farmers 88.3% were males, while 11.7% were females (Table 2.2). The mean household age in the study areas was 45, with the maximum and minimum age of 80 and 18, respectively, and on average the respondents had 18.2 years of experience in farming and only 62 respondents had farming experience of < 10 years. There was variation among the respondents for education level. Among the total respondents; 75.5% were educated (23.8% adult education, 4.2% religious and 45.8% up to secondary education). However, 26.2% were without education. This has an implication on the adoption degree of new technologies to increase production and productivity for the improvement of the livelihood of farmers.

Table 2. 2 Farmers and household information for the three districts in Ethiopia

General information		**Zone**				**P-Value**
		North Shewa	Arsi	Finfine Special	Total	
Number of household interviewed		80	80	80	240	
Number of PA sampled		4	4	4	12	
		% farmers responding				
Age range of farmers	< or equal to 30	21.3	15	16.3	17.5	0.27
	31-40	26.3	36.3	21.3	27.9	
	41-50	17.5	18.8	32.5	22.9	
	51-60	21.3	15.2	20	18.8	
	61-70	10	11.3	5	8.8	
	71-80	3.8	3.8	5	4.2	
Sex of respondents	Male	93.8	88.8	82.5	88.3	0.09
	Female	6.3	11.3	17.5	11.7	
Education level of the respondents	None	20	21.3	32.5	24.6	0.00
	Adult education	35	16.3	20	23.8	
	Religious education	11.3	0	1.3	4.2	
	Formal education	31.3	60	46.3	45.8	
	Both religious and formal education	2.5	2.5	0	1.7	
Households head	Male headed	95	92.5	83.8	90.4	0.04
	Female headed	5	7.5	16.3	9.6	
Religion	Christian (Orthodox)	100	76.3	98.8	91.7	0.00
	Christian (Protestant)	0	6.3	1.3	2.5	
	Muslim	0	17.5	0	5.8	
Marital states	Married living with spouse	78.8	91.3	88.8	86.3	0.02
	Married but spouse away/died	2.5	1.3	2.5	2.1	
	Divorced	3.8	1.3	3.8	2.5	
	Widow	2.5	5	3.8	3.8	
	Never married	13.8	1.3	1.3	5.4	

2.3.2 Major crops grown and their productivity in the study area

The major crops grown in all the three study zones included cereals; barley, wheat and tef and pulses; faba bean, field pea, chickpea and lentil. Landholdings were generally small, ranging between 1.39 ha and 2.47 ha per household across the three zones. The average size of cultivated landholdings per farmer planted to different crops in 2011 cropping season was about 2.09 ha in Finfine Special zone, 2.22 ha in Arsi and 1.47 ha in North Shewa zone. There was valuable variation in productivity of faba bean with average faba bean yields in 2011 season as follows: Arsi (28 q ha^{-1}), North Shewa zone (15.3 q ha^{-1}) and 5.6 q ha^{-1} at Finfine special zone. Farmers in Arsi and North Shewa districts realised higher yields than Finfine special zone. This could be attributed to the different biotic and abiotic stresses such as disease, soil acidity and poor soil fertility around Finfine special zone (Gorfu and Beniwal, 1987; Jarso et al., 2008). Overall, productivity of faba bean was higher in Arsi among the three study zones (Figure 2.2). Arsi zone showed better productivity and could be categorized as high agricultural potential zone. Most of the farmers in Arsi zone responded that they strictly implement crop rotation practices of pulse after cereals production system which has an impact on the improvement of soil fertility. Low productivity in North Shewa zone could be due to the nature of soils in most parts of the zone which are vertisoil. Erkossa et al. (2006) also reported that 7.6 million hectares of central highlands of Ethiopia is covered with vertisols which constrained crop production due to its effect on the soil physical and hydrological properties. Similarly, soil degradation was reported as major a constraint of crop productivity in the highlands of Ethiopia (Kebede and Yamoah, 2009).

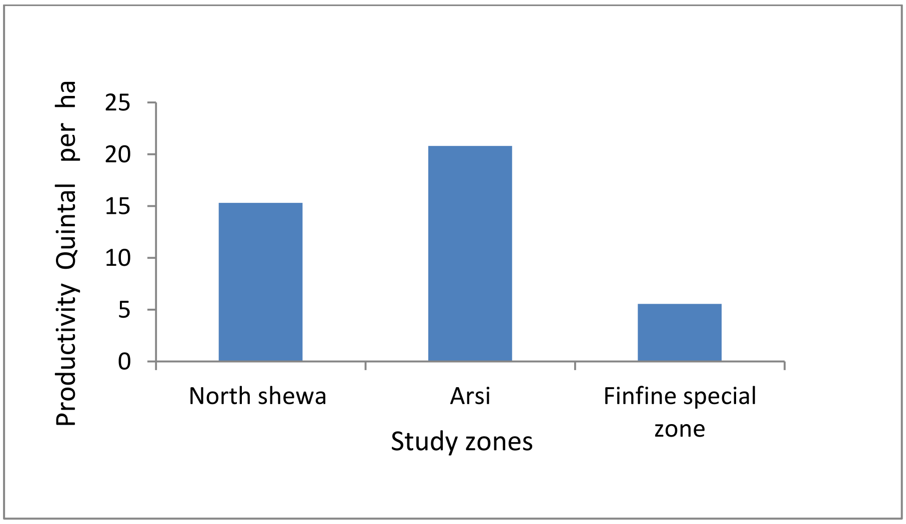

Figure 2.2 Productivity (q ha^{-1}) of faba bean grown by the sample farmers' in the three zones in 2011 cropping season

2.3.3 Land allocated to faba bean production

There were significant differences (P<0.001) in the farmers' response among the three districts regarding the area allocated to faba bean production during the last ten years. In Arsi zone, 75% of the respondents had increased their land allocation for faba bean production, while in North Shewa zone and Finfine special zone only 41.3% and 8.8%, respectively had increased their allocation (Table 2.3). Of all the interviewed farmers across the zones, 41.7% had increased their land allocation to faba bean production, while 37.1% and 13.3% of the respondents had decreased or not changed their land allocation, respectively. In contrast, the majority (78.8%) of the respondents in Finfine special zones had decreased the land allocation to faba bean production followed by Arsi (17.5%) and North Shewa (15.0%). The reasons given for the decrease of land allocation for faba bean were shortage of land, lack of improved varieties and biotic and abiotic problems. Farmers' allocated 28.8%, 19.8% and 20.7% of cultivated land for faba bean production in North Shewa, Arsi and Finfine special zones, respectively, in 2011 cropping season. More than 76% of the respondents from the three zones used their own land for faba bean production, with only 3-10% renting land in 2011 cropping season (Table 2.4). Most of the respondents planted faba bean on ranges from 0.16 - 0.26 ha followed by 0.49 – 0.59 ha of land in the 2011 cropping season (Figure 2.3).

Table 2. 3 Percentage (%) of farmers responding on decrease or increase of land allocated for faba bean production in the last ten years (2001-2011)

Response type	Zone (% farmers responding)			Total
	Finfine Special	Arsi	North Shewa	
Increasing	8.8_c	75_a	41.3_b	41.7
Decreasing	78.7_a	17.5_b	15.0_b	37.1
Constant	5.0_b	3.8_b	31.3_a	13.3
Depending on the season (some years decrease and some years increase)	7.5_{ab}	3.8_b	12.5_a	7.9
Total	100	100	100	100

Means with same letter across the zones are not significantly different at 0.05 probability level

Table 2. 4 Number of farmers responding to using a type of land holding for faba bean production across the three zones in 2011 cropping season.

Type of land holding by farmers for faba bean	% farmers responding per each zone			Total
	Finfine Special zone	Arsi	North Shewa	
Own	76.3	83.8	86.3	82.1
Rented in	10.0	6.3	3.8	6.7
Shared in	5.0	0.0	3.8	2.9
Both own and rented in	3.8	5.0	2.5	3.8
Both own and shared in	0.0	1.3	3.8	1.7
Both rented in and shared in	0.0	2.5	0.0	0.8
Did not produce in this year	5.0	1.3	0.0	2.1
Total	100	100	100	100

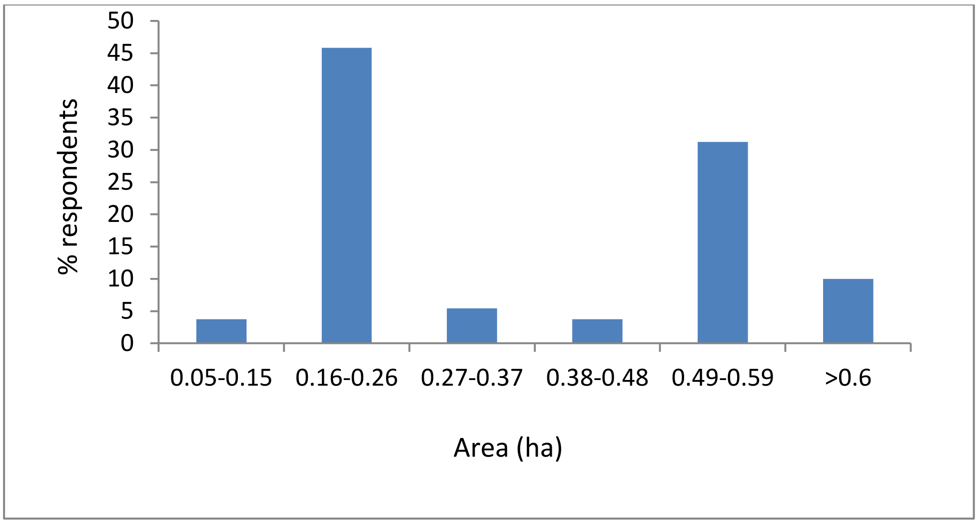

Figure 2.3 Response of farmers to total area (ha) used for faba bean production across all studied three zones of the central highland of Ethiopia in 2011 cropping season

2.3.4 Faba bean production practices in the study area.

Crop rotations that were predominant among the farmers were faba bean followed by wheat or tef (33.8%) in Finfine; and barley followed by potato in Arsi (82.5%) and North Shewa (36.3%) (Table 2.5). On average, 43.8% of the respondents grew barley followed by faba bean. During focus group discussions, farmers confirmed that faba bean was used as a break crop and planted after barley at Baso Dengora and Wushawushign villages, wheat at Gudoberet village, and wheat or barley at Goshe Bado village of North Shewa zone. Similarly, in Arsi zone farmers from Chiba Michael, Koma Katar, Dawa Bursa and Limu Dima preferred to plant malt barley after faba bean to get a better yield of barley, which is a major agro-industrial cash crop of the subsistence farmers. In Finfine special zone, at Telecho village, farmers planted wheat after faba bean, wheat or barley at Bekekana-Kore Oddo village, tef or wheat at Wajitu village and barley at Wolmera Choke village. The main reason for the rotations was for the intensification of crop productivity by increasing soil fertility for the increasing population pressure and scarcity of suitable land. The benefit of this practice was also reported by Grando and Macpherson (2005). They confirmed that faba bean was a precursor crop on unfertilized vertisol resulting in yield advantage of barley over fallow in North Shewa.

Table 2. 5 Crop rotation sequence practiced by the farmers in the study area (% of respondents)

Rotation with faba bean	per zone (% farmers responding)			Total
	Finfine Special	**Arsi**	**North Shewa**	
Barley-chickpeas			1.3_{a}	0.40
Barley-potato	1.3_{a}			0.40
Barley	20.0_{c}	82.5_{a}	36.3_{b}	46.30
Barley/Field pea-Wheat			1.3_{a}	0.40
Barley/wheat	16.3_{b}	1.3_{c}	25.0_{a}	14.20
Potato	2.5_{a}		1.3_{a}	1.30
Potato/wheat/tef	1.3_{a}			0.40
Tef	2.5_{a}			0.80
Tef/wheat	1.3_{a}			0.40
Wheat	3.8_{b}	13.8_{a}	$7.5_{a, b}$	8.30
Wheat/barley			1.3_{a}	0.40
Wheat/tef	16.3_{a}			5.40
Wheat/tef/barley	33.8_{a}			11.30
Field peas-barley		1.3_{a}		0.40
Pulse-cereals		1.3_{b}	23.8_{a}	8.30
No rotation	1.3_{a}		2.5_{a}	1.30
Total	100	100	100	

Means with same letter across the zones are not significantly different at 0.05 probability level

Although, in all the study areas farmers were aware of the potential of faba bean in the improvement of soil fertility, they used faba bean only as a break crop. Farmers reported that intercropping of faba bean with highland small cereals resulted in difficulties in the management of the crops. However, a study on the mixed cropping of faba bean with tef

(*Eragrostis tef*) and barley in the central highlands of Ethiopia showed yield improvement of tef, barley and wheat compared to sole cropping, increased land use efficiency and reduced the weed biomass below compared to that observed in sole tef (Agegnehu et al., 2006a, b, 2008). Other studies showed a reduction in chocolate spot disease severity when faba bean was mixed with barley, wheat, oats or triticale, compared with sole faba bean (Sahile et al., 2008b; Fernández-Aparicio et al., 2011).

However, from North Shewa, 24% of the respondents did not use faba bean as a break crop, instead they left the land fallow and burnt the soil to increase soil fertility. This was mainly due to the effects of frost on faba bean in this area, which can cause 100% crop loss (Mola, 1996). Most (60%) of the farmers at Wajitu village in Finfine special zone did not practise crop rotation. They used mono cropping production systems for tef after wheat or wheat after tef. Farmers in these areas indicated this was because they preferred to grow tef or wheat for its high market value, but they recognized the productivity of the land was becoming poor year after year. Nigussie and Kissi (2012) also indicated that this system of production was depleting the soil nutrients. It is thus important for the extension officers to demonstrate the benefit of the rotational system which includes legumes to the villagers.

2.3.5 Other faba bean cultural practices

Significant differences among the study zones were observed for planting and harvesting time of faba bean (Table 2.6). Planting time was an important factor for crop productivity. Farmers planted most of their crops immediately after the onset of rainfall to avoid terminal low moisture stress. The planting date for faba bean varied slightly from district to district, but in general in 2011/12 cropping season, all the respondents planted between early-June to end of June. However, some farmers (10%) in North Shewa planted faba bean at end of May. Most of the respondents (85.1%) in Finfine special zone harvested faba bean in November from early to end of November. Harvesting was late in Arsi zone, that is, end of November (47.5%) and some respondents (18.5%) from this zone harvested in early December. This was due to the area being located relatively at high altitude and the rainfall period was longer unlike the other sites. In North Shewa, harvesting was from end of October to early November and a few (8.8%) harvested early December (Table 2.6).

Table 2. 6 Time of planting and harvesting for faba bean at the three zones in 2011 cropping season

Planting time	Zone (% of farmers responding)			Total (%)
	Finfine Special zone	Arsi zone	North Shewa zone	
Early July	8.8_a	0.0_b	5.0_a	4.6
Early June	0.0_b	15.0_a	22.5_a	12.5
End of June	33.8_a	25.0_b	21.3_b	26.7
End of May	0.0_b	0.0_b	10.0_a	3.3
June	42.5_a	3.8_c	20.0_b	22.1
Mid June	15.0_b	56.3_a	21.3_b	30.8
Total	100	100	100	100
Harvesting time				
Early December	3.8_b	18.8_a	8.8_{ab}	10.4
Early November	32.5_a	5.0_c	16.3_b	17.9
End of December	0.0_b	1.3_b	8.8_{ab}	3.3
End of November	2.5_b	47.5_a	6.3_b	18.8
End of October	2.5_b	1.3_b	20_a	7.9
Mid December	0.0_b	3.8_a	0.0_b	1.3
Mid November	6.3_a	8.8_a	5.0_a	6.7
Mid October	0.0_b	0.0_b	8.8_a	2.9
November	43.8_a	13.5_b	12.5_b	23.3
October	8.6_b	0.0_b	13.5_a	7.5
Total	100	100	100	100

Means with same letter across the zones are not significantly different at 0.05 probability level

There were significant differences among the three zones in application and type of fertilizer utilised for faba bean production (Table 2.7). Although farmers did not use fertilizers on faba bean as much as on wheat and barley, about 86.3% of the farmers in Arsi applied Diammonium phosphate (DAP) fertilizer for faba bean at the rate which depended on the availability of the fertilizer, and very few (9.6%) used natural fertilizer (compost). In North Shewa zone, most of the sampled farmers (93.4%) used compost for their faba bean, while 50% in Finfine special zone used a mixture of DAP and UREA fertilizer and 25% of the respondents in this zone used compost (Table 2.7).

In this study, 79.2% across the zones of farmers' did not mix faba bean with field pea during planting. In contrast, 20.8% mixed faba bean with field pea depending on the type of soil (Table 2.6). Most of the farmers (85.3%) at Finfine special zone planted faba bean as a mixture with field pea for different reasons (Table 2.7). On fertile soils they grew faba bean as a sole crop. In addition, farmers planted the mixture on black soil to support the field pea. Since it is difficult to separate mixed faba bean from field pea, all farmers' harvested, threshed and took the mixture to the market. Moreover, they indicated that the mixture had a better price on the market than the sole faba bean. Though the percentage of farmers growing the mixture of faba bean and field pea was small, the study by Ghizaw (1996), Abera and Feyisa (2008) demonstrated that the higher grain yield was attained at proportion of 75:25 of faba bean: field pea.

Table 2. 7 Farmers response to the use of fertilizer and mixing of faba bean with field pea in the study area

Response of farmers for utilization of fertilizer for faba bean	**Zone (% farmers responding)**			**Total**
	Finfine Special	**Arsi**	**North Shewa**	
Yes	30_c	91.3_a	76.3_b	65.8
No	70_a	8.8_c	23.8_b	34.2
Total	**100**	**100**	**100**	**100**
Type of fertilizer used				
DAP	25_b	86.3_a	3.3_b	44.93
Compost	25_b	9.6_b	93.4_a	44.3
UREA and DAP	50_a	0_b	0_b	7.6
DAP and Compost	0_a	4.1_a	3.3_a	3.2
Total	**100**	**100**	**100**	**100**
Response of farmers for planting mixture of faba bean with field pea				
No	56.9_b	97.5_a	82.5_b	79.2
Yes	43.0_a	2.5_c	17.5_b	20.8
Total	**98.8**	**101.3**	**100**	**100**
Reason for mixing faba bean with field pea				
Supporting the field pea	26.5_b	100	100_a	48
To increase soil fertility	47.1_a	0_b	0_b	32
No idea	17.6_a	0_a	0_a	12
For controlling weeds	8.8_a	0_a	0_a	6
To increase yield	2.9_a	0_a	0_a	2
Total	100	100	100	100

Means with same letter across the zone is not significantly different at 0.05 probability level

2.3.6 Faba bean varieties grown and traits preferred

Almost 87-93% of the farmers from the three zones grew faba bean local landraces (Table 2.8). The rest of the respondents have been using improved varieties of faba bean in the last ten years. The faba bean local landraces were preferred by most of the farmers (64.8%) for their good food quality and better price on the market. Approximately 13.4% of the respondents preferred the local landraces for their good biomass, and 19.4% for their resistance to disease. Very few respondents (2.4%) preferred the landraces due to the unavailability of improved faba bean varieties. Acknowledging that the landraces were low yielding, 79.6% of the farmers indicated they continued growing them mainly due to inadequate information regarding the improved varieties. On the other hand, agricultural development encourages farmers to adopt crop varieties with greater yield potential than local landraces (Ceccarelli, 1994). This, however, can result in genetic erosion that leads to loss of biodiversity (Wouw et al., 2009). Cubero (2011) also categorized the Ethiopian faba bean landraces in Vavilov recollections as minor. It is, therefore, essential to conserve the local landraces while transferring farmers' preferred traits to improve faba bean production.

The majority of farmers grew small seeded faba bean. This might explain the low productivity of their varieties. The demands of the consumers determined the size of faba bean variety grown by farmers. For instance, small seed size was preferred for household consumption for its good test of *‘Shero’* (a sauce for *‘Enjera’*) and *‘Siljo’*, (a national food made from faba bean flour). On the other hand, large seeded faba bean was preferred for its high yield and market demand for preparation of traditional food such as *Nifro (boiled), ‘Ashuke’ (*roasted and soaked*) and ‘Endushdush’* (sprouted and roasted). In addition, consumers also utilised the large seeded faba bean for making *‘Enjera’* (flat bread used as staple food in Ethiopia) by mixing it with other cereals like wheat, barley or sorghum. It was also used for making Ful (dish of cooked and mashed faba bean served with oil, chopped onion garlic and tomato), a popular breakfast in towns of Ethiopia.

There was variation in faba bean seed size preference by consumers across the three zones (Table 2.8). Farmers from Finfine special zone (58.8%), Arsi (87.5%) and North Shewa (22.5%), indicated that large seeded faba bean was preferred by the consumers and had a high market demand. In general 56.3% of respondents across the zones indicated consumers preferred large seeded faba bean. In contrast, only 12.5% indicated that small seeded faba bean was preferred by the consumers. However, consumer preferences depended on the purpose of the faba bean. For instance, for “Shero” consumers preferred the small seeded faba bean for its good taste. Farmers' preference for the faba bean seed size depended on the demand by consumers and most of the farmers (62.1%) responded they preferred the large seeded faba bean (Table 2.8). Medium sized seed of faba bean was preferred by consumers because it was easy to process. In addition, the amount of flour *‘Shero’* obtained was much better from the medium sized than from the small seeded faba bean. It is, therefore, important to consider seed size in the faba bean breeding programme and how to transfer the good taste trait to the large and medium seeded faba bean. This will increase and improve the value of the latter.

Table 2. 8 Faba bean varieties grown, seed size preference by consumers and farmers' in the three study zones

	Zone (% of farmers responded)			Total
Faba bean variety grown	**Finfine Special**	**Arsi**	**North Shewa**	
Improved	12.5	6.3	11.3	10
landrace	87.5	93.7	88.7	90
Total	100	100	100	100
Seed size preference by consumers				
Large	58.8	87.5	22.5	56.3
Small	3.8	2.5	31.3	12.5
Medium	23.8	3.8	17.5	15.0
Based on purpose	2.5	3.8	3.8	3.3
not answered	11.3	2.5	6.3	6.7
medium and large	0.0	0.0	3.8	1.3
All seed size	0.0	0.0	6.3	2.1
small and medium for different purposes	0.0	0.0	8.8	2.9
Total	100.0	100.0	100.0	100.0
Seed size preference by farmers				
Large	61.3	88.8	36.3	62.1
Small	5.0	1.3	31.3	12.5
Medium	31.3	3.8	21.3	18.8
All seed size	0.0	0.0	3.8	1.3
Both small and large for different purposes	2.5	6.3	1.3	3.3
small and medium for different purposes	0.0	0.0	5.0	1.7
Large and medium	0.0	0.0	1.3	0.4
Total	100.0	100.0	100.0	100.0

As shown on Table 2.9, there were no significant differences (P>0.05) among the three zones for most of the faba bean traits preferred by the farmers. The local landraces were preferred for their adaptability to the local environment, tolerance to frost, early maturity, good 'Shero' making quality, good storability and good straw yield and palatability. The improved faba bean varieties were preferred for their high grain yield, bigger grain size and tolerance to water logging.

Table 2. 9 Farmers' trait preferences and their response (%) to different characteristics of the local landrace and improved faba bean varieties in the study areas

Characteristics	Farmers' response (%) (n=240)		Preference	χ^2*
	Local landrace	**Improved**		□
Adaptable to the locality	58.1_a	41.9_b	Local	0.01*
High grain yield	38.2_b	61.8_a	Improved	0.17
Good tillering ability	47.9_a	52.1_a		0.03*
Good grain color	43.2_b	56.8_a		0.02
Good grain size	33.1_b	66.9_a	Improved	0.32
Resistance for shattering	58.9_a	41.1_b		0.86
Straw yield	55.6_a	44.4_b		0.00*
Palatability of straw	65.5_a	34.5_b		0.00*
Easy for threshing	68.8_a	31.2_b		0.00*
Need high fertilizer	43.4_b	56.6_a		0.01
Tolerant to frost	62.6_a	37.4_b		0.05
Tolerant to water-logging	34.3_b	65.7_a		0.07
Early maturing	75.2_a	24.8_b	Local	0.00*
Tolerant to disease	49.0_a	51.1_a	Improved	0.78
Tolerant to insect pest	68.8_a	31.3_b		0.35
For Nifro, Ashuke, Endushdushe	37.1_b	62.9_a	Improved	0.27
Good 'Shero' making quality	80.5_a	19.5_b	Local	0.09
Short cooking time	62.1_a	37.9_b		0.11
Good storability	72.4_a	27.6_b		0.00*
High market demand	59.6_a	40.4_b	Local	0.003*
High market price for food	55.5_a	44.5_b	Local	0.002*
High seed market price	60.0_a	40.0_b	Local	0.014

*χ^2 significant differences at $P < 0.05$ among responses from farmer for each character among the three zones, Means with same letter for the characteristics are not significantly different at 0.05 probability level between landraces and improved

2.3.7 Sources of seed

Faba bean seed planted by most of the respondents were from local landraces which the farmers had kept for a long time. The main source of seed was farmer to farmer seed exchange as indicated by 42.9% of the respondents, while 27.2% obtained the seed from their parents, 11.6% purchased from the market, and 10.8% saved seed from previous faba bean production (Figure 2.4). Unlike for crops such as barley and wheat which were acquired from Government Agricultural Office, only 7.5% of the farmers reported that they sourced from the faba bean seed from Ministry of Agriculture (MOA). In spite of limited access to the improved faba bean seed, farmers were willing to purchase and use improved seed to solve the problem of low yield in faba bean. Some farmers also expressed their fear for yield deterioration of improved faba bean varieties after three consecutive years if they used the same seed.

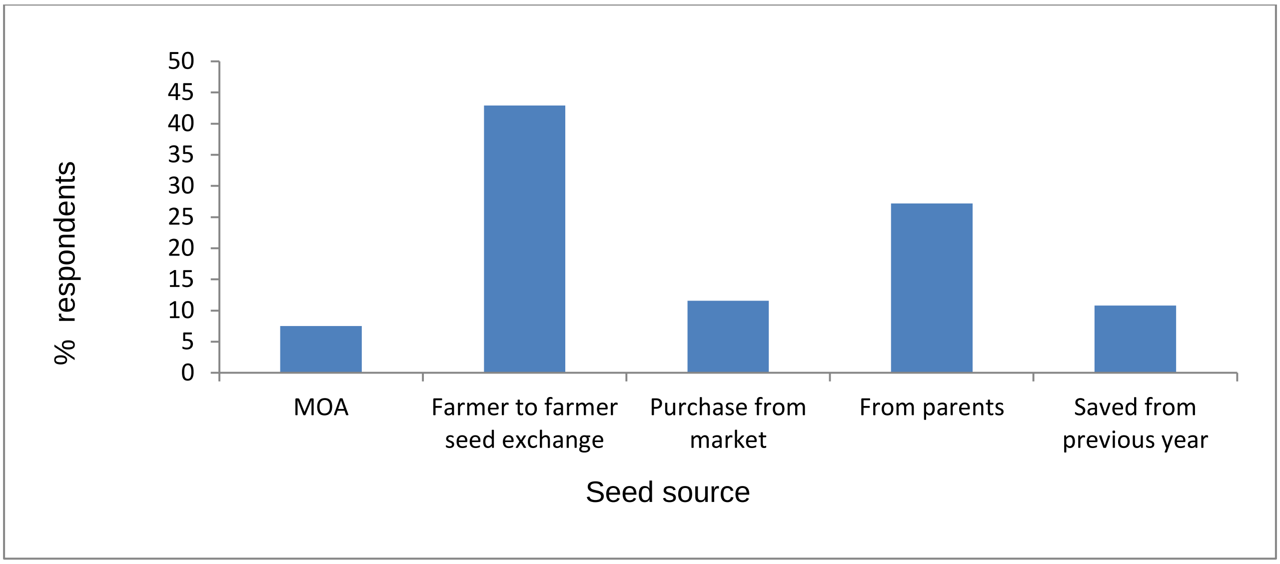

Figure 2.4 Farmers response for faba bean seed source in the three study zones

2.3.8 Threats to faba bean production

Most of the respondents (87.9 %) in the three zones reported faba bean chocolate spot disease as a major problem (Table 2.10). Other constraints included lack of improved varieties, shortage of land, and insect pests. During focus group discussions, among the biotic problems, faba bean chocolate spot disease was ranked first in all villages of Finfine special zone and two villages of North Shewa zones, and second in all villages of Arsi Zone (Table 2.11). This is in agreement with the report of Sahile et al. (2008a).

Table 2. 10 Faba bean production constraints and the response of farmers' (%) in the study areas of the three zones

	Zone (% of farmers response)				
Constraint	**North Shewa**	**Arsi**	**Finfine Special**	**Overall mean**	**P-value**
Faba bean chocolate spot	86.3	85.0	92.5	87.9	0.39
Insect pest	15.6	50.8	33.6	33.3	0.00
Shortage of land	38.8	30.3	30.9	33.3	0.09
Poor soil fertility	27.8	35.6	36.7	33.4	0.19
Lack of improved variety seed	27.6	39.6	32.8	33.3	0.00
Lack of seed of local variety	7.6.0	41.5	50.8	33.3	0.00
Frost	49.4	23.4	27.3	33.4	0.01
Moisture stress	29.3	30.4	40.3	33.3	0.05
High rainfall	13.0	41.3	45.7	33.3	0.00
Hail	53.1	13.6	33.3	33.3	0.00
Bird and other vertebrate	40.9	9.1	50.0	33.3	0.00

* Percentage of respondents who answered ‘No’ have been omitted, however are provided in the chi-squared value

Table 2. 11 Focus group matrix- ranking of faba bean production constraints across the three zones and twelve Peasant Associations in the studied areas 2011

Faba bean production constraints	**Finfine special zone**				**Arsi zone**				**North Shewa zone**			
	A	B	C	D	E	F	G	H	I	J	K	L
Faba bean chocolate spot	1	1	1	1	2	2	2	2	1	2	2	1
Faba bean insect pest	-	-	-	-	3	3		3	3	3	-	3
Shortage of rainfall	2	-	4	-	1	-	4	-	-	1	-	-
Frost	3	-	-	2	5	-	1	-	2	-	1	2
Lack of improved faba bean variety	4	2	2	-	4	1	3	1	-	-	3	-
Shortage of land	-	-	3	-	-	-	-	-	-	-	-	-

Where: A-L are list of villages: A= Bekekanakoro, B= Wajitu, C= Wolmera choke, D= Telecho, E= Koma ketara, F= Limu dima, G= Cheba Michael, H= Dawa bursa, I= Wushawushegn, J= Gudoberet, K= Basodengora, L= Goshebado; *the lower the rank the most important the problem; constraint specified as’ - ‘indicates it was not mentiond as a problem for the respective village.

Although in Ethiopia there are some faba bean cultivars released by the Ethiopian Agricultural Research Institute for different purposes, the main focus has been on high yielding varieties (Ali et al., 2003). More than 40% of the farmers from the three zones indicated they had never grown improved faba bean varieties in the last ten years of production. This is mainly (51.2%) due to lack of access to improved faba bean variety seed. However, some of the farmers (38%) preferred to grow their own local varieties though they had access to seed of the improved varieties. On the other hand, some farmers have never grown improved faba bean varieties due to a lack of awareness (Figure 2.5).

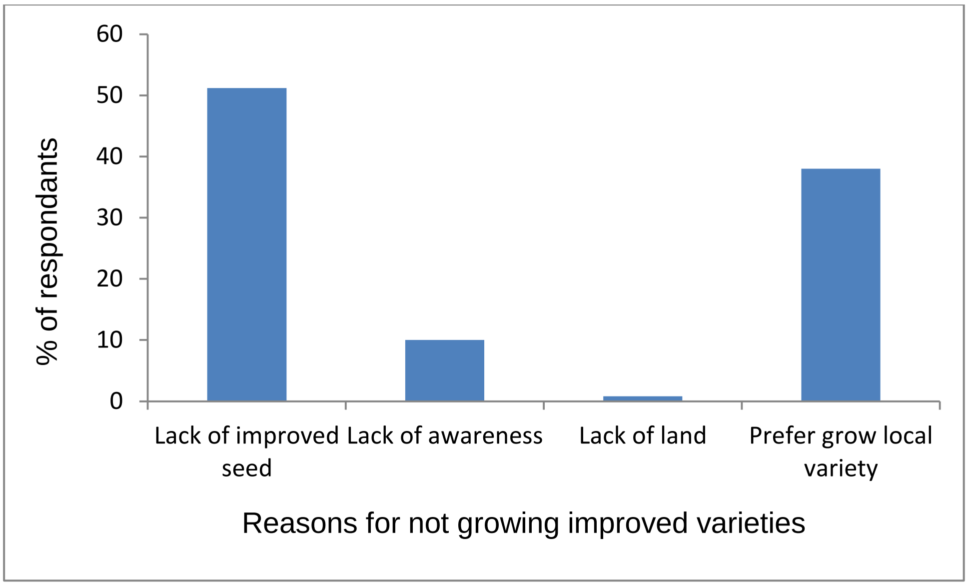

Figure 2.5 Farmers' reasons for not growing improved faba bean varieties in the study areas

2.3.9 Farmers' perception for faba bean chocolate spot (*Botrytis fabae*) disease

There were no significant variation (P>0.05) among the three zones on faba bean disease perception and most of the respondents were aware of the disease but named it differently; "Emde", in North Shewa and Wag/rust in Finfine and Arsi (Table 2.12). On the whole, 85-92% of the farmers from the three zones were familiar with the faba bean disease. Farmers, however, were not aware of the causal organism although they were able to describe the symptoms. When provided with pictures showing different faba bean disease symptoms, most of the respondents in all the districts confirmed the symptoms of chocolate spot (*Botrytis fabae*) as the one which occurred in their faba bean farms (Table 2.12). In addition, farmers who participated in the transect walk confirmed the symptoms of faba bean chocolate spot through observations in the field.

Table 2. 12 Faba bean disease symptoms mentioned by the farmers in their faba bean farm 2011

Disease symptom of faba bean described by farmers (N=240)	**% farmers responding**	**Disease named by farmers**	**% farmers responding**
Black spot on leaf, steam and pod	5.2	"Faki"	1.6
Brown spot on leaf, steam and pod	3.2	Chocolate spot	0.4
Burning of leaf, pod and then plant as a whole	2.4	Do not know name	5.4
Discoloration and dropping of leaf and flowers	4.4	“Emde”	22.9
Drying and shattering of leaves and flowers	15.1	Frost	1.2
Brown spot on leaves, stem, pod, flower and shattering	44.8	Wage / rust	55.8
Wilting, drying and shattering of leaf, flower	9.4	Leaf spot	0.4
Yellow spot on leaves	0.8	Worm	0.4
No answer	12.9	No answer	12.1
Total	**100**	**Total**	**100**

Note: Farmers descriptions of the disease symptom as Emde, and Wage/rust are local names for chocolate spot; N=number of farmers responding

The majority (61.7%) of the respondents did not have any idea of how the disease spread from field to field, while 30.8% indicated that it was disseminated by wind (Figure 2.6). About 54.9% of the respondents indicated the disease was severe in their field, while 39.3% as moderately severe and 5.7% as low in severity. There was significant variation ($P< 0.001$) among the three zones regarding faba bean disease control. Among the three zones studied, most respondents from Arsi (49.2%) controlled faba bean disease followed by North Shewa (29.7%) and Finfine special zone (21.5%) (Table 2.12). This is mainly due to the fact that most of the farmers from Arsi zone are literate, had higher allocation of land to faba bean and used faba bean - cereal rotations. Moreover, the farmers in this zone sold most of their produce both locally and exported to other countries. Of all the respondents from the three zones who observed the disease in their field, 69.2% did not use any disease control method for the faba bean disease. The major reason was lack of knowledge about the disease followed by lack of chemicals.

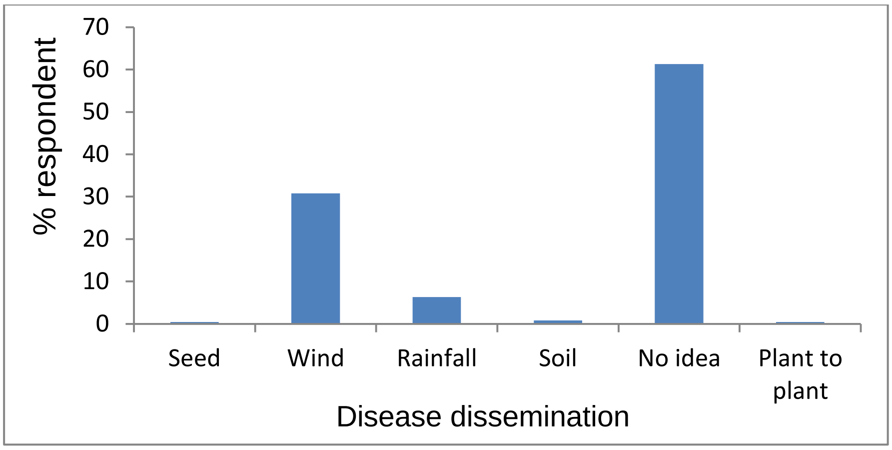

Figure 2.6 Farmers response for the way chocolate spot disease was spread

Those farmers that controlled the disease used chemicals including the fungicide mancozeb, which they sprayed at different rates. Most of the respondents applied one coffee cup of mancozeb per quarter of a hectare, which is equivalent to 0.16 kg/ha. In addition, most of them sprayed only once during the growing period of the faba bean. This is below the recommended rate for this fungicide (2.5 kg/ha) and frequency, which is at least three times starting from the onset of chocolate spot disease (Sahile et al., 2008b). This has an implication on the need to train farmers about the right time and rate of chemical application. On the effectiveness of the chemical, 9.2, 12.1, and 4.2% of the respondents indicated the chemical to be effective, moderately effective and not effective, respectively, in controlling the faba bean disease. Some of the farmers used other cultural practices (garlic and holy water) to control faba bean disease, but none of them found these methods to be effective. Farmers reported different effectiveness of the chemical used to control faba bean disease. This variation was due to the differences in the rate and time of application of the chemical.

Faba bean disease outbreaks were reported to start from mid-August (flower initiation stage of the plant) but most of the respondents from all districts (78.7 %) indicated in September (flowering stage), while a few farmers pointed out that it can extend up to October (podding stage of the plant) (Table 2.13). Across the zones 69% of the respondents had the same opinion that the disease was severe at the flowering stage of the faba bean and caused early flower dropping. This observation is in agreement with reports by Gorfu and Beniwal (1987). However, most of the farmers who used chemicals for this disease applied late after flowering which resulted in the non-effectiveness of the chemical. According to the farmers, the distribution and seasonal incidence of chocolate spot disease of faba bean was increasing year after year and this observation was also reported by the International Center for Agricultural Research in the Dry Areas (ICARDA, 2006). Most of the respondents mentioned that high rainfall was the favourable weather condition for faba bean chocolate spot disease outbreak.

Table 2. 13 Responses of farmers for disease outbreak in their field, severity, control and method of control for the faba bean disease.

Response of farmers for:	Zone (% farmers responding)			Total
	Finfine Special	Arsi	North Shewa	
Presence of the disease in their field				
Yes	92.5	85.0	86.3	87.9
No	7.5	15.0	13.7	12.1
Total	100	100	100	
Stage of the plant disease observed				
Seedling	1.3	0.0	3.8	1.7
Flowering	75.0	76.3	56.3	69.2
Podding	6.3	8.8	8.8	7.9
Flowering and podding	10.0	0.0	16.3	8.8
Seedling, podding	0.0	0.0	1.3	0.4
Total	92.6	85.1	86.5	
Level of damage				
Severe	60.0	28.8	56.3	48.3
Moderate	28.8	50.0	25.0	34.6
Low	3.8	6.3	5.2	5.0
Total	92.6	85.1	86.5	
Control of disease				
Yes	17.5	40.0	23.8	27.1
No	75.0	45.0	62.5	60.8
	92.5	85	86.3	
Type of control				
Chemical	16.3	38.8	21.3	25.4
Cultural (Holy water)	1.3	1.3	2.5	1.7
Cultural (garlic)	0.0	1.3	0.0	0.4
Total	17.6	41.4	23.8	

2.4 Conclusion and recommendations

Faba bean chocolate spot disease was a major problem in all the study zones and most famers were familiar with the disease. Lack of improved varieties, the poor seed system and shortage of land were also some of the major problems reported. Farmers preferred local landraces for their adaptability to the local environment, tolerance to frost, early maturity, good food taste and better straw yield and palatability. The improved faba bean varieties were preferred for their high grain yield, bigger grain size and tolerance to water logging. Generally, farmers revealed strong preferences for varieties with high and stable yield, resistance to disease and pest problems, good taste *'Shero'* making quality, early maturing and adaptable to the environment. Thus, there is great need to improve the yield and disease resistance of farmers' local varieties through different breeding strategies and also involve the farmers in the selection process at appropriate stages of evaluation.

Given that the majority of the respondents did not have any idea of the causes and disease dissemination, practiced poor disease control practices, and had limited training on faba bean available production technologies; it is imperative that extension services be strengthened to assist farmers and support them in understanding more about the chocolate spot disease. This should include educating the farmers on the disease dissemination mechanisms, conducting integrated management and control and enabling the farmers to contribute to the faba bean resistant variety development programme. Although, there are a limited number of faba bean varieties released for high yield and large seed from the National Agricultural Research Institute, the problem of availability of improved seed was a major production constraint for faba bean. It is therefore, essential to encourage and provide incentives to engage the private sector in seed multiplication. Moreover, most of the available improved varieties lack genes for chocolate spot disease resistance. Setting of breeding priorities should be supported and determined with substantial consideration of farmers' preferences and constraints, and be based on their resources.

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Chapter 3

Molecular genetic diversity study of faba bean (*Vicia faba* L.) landracees from the Ethiopian highlands using SSR markers

Abstract

Knowledge of genetic diversity plays an important role in devising breeding strategies. However, levels of genetic diversity have not been quantified in the faba bean landraces from the Ethiopian highlands which can be exploited in the breeding programme. This study was carried out to determine the extent of genetic diversity and population structure among faba bean landraces collected from different parts of the Ethiopian highlands. Thirty Simple Sequence Repeat (SSR) markers were used to assess genetic diversity of 50 faba bean landraces. The data were analyzed using SAS V9.3, Arlequin ver-3.1, STRUCTUR ver.2.3.4, PAST V 3.0 and Power Marker V.3.0. There were 222 alleles and the number of alleles per microsatellite locus in the 50 faba bean germplasm ranged between 1 and 24 and averaged 7.4. The greatest number of alleles was found with VfG67 SSR marker. The polymorphism information content (PIC) ranged between 0.0 and 0.87 with an average of 0.62 per locus, indicating the suitability of the microsatellite markers for detecting genetic diversity among these faba bean genotypes. The PIC and genetic diversity value averaging 0.62 and 0.63, respectively, indicated that genetic diversity among faba bean germplasm was high. It was revealed that 68.5% of molecular variation in the faba bean landraces was due to variation within population and 31.5% accounted for the variation among populations. The genotypes were divided into three major distinct clusters. Clusters I and II comprised accessions collected from farmers and improved varieties and Cluster III comprised of genotypes from ICARDA. The large genetic polymorphism within populations may have haplotypes which can be exploited by breeding programmes to broaden the genetic diversity. The results have implications for breeding new faba bean varieties using the available potential genetic resources for the highland ecologies.

Keywords: Faba bean landrace, genetic diversity, molecular breeding, principal component analysis, SSR; Structure.

3.1 Introduction

Faba bean (*Vicia faba* L.) is the fourth most important annual grain legume after garden pea, chickpea and lentil in many tropical and subtropical regions of the world. It belongs to the family Fabaceae (Leguminosae) with an estimated genome size of ~13000 Mb (Maxted and Bennett, 2001; Torres et al., 2006). It is a diploid (2n = 2x = 12) and predominantly a cross-pollinating species (55%) depending on the genotype (Metz et al., 1993; Suso et al., 2001). Occurrence of at least 50% cross pollination indicates that there could be some high levels of diversity in faba bean, which should be quantified. The faba bean is a significant crop due to its adaptation ability and therefore deserve attention and improvement.

In the Ethiopian highlands, faba bean landraces are used by resource poor farmers, who practice subsistence farming. The faba bean can be grown in the mid altitude to highland areas with high rainfall and various types of soil. It is cultivated for multiple usages because of its high nutritional value, which includes being as excellent source of protein (27 - 34%) (Hove et al., 1978). Apart from being a potential food and cash crop for resource poor farmers, faba bean has been used as a major break for cereal mono cropping system which can increase the soil fertility by atmospheric nitrogen fixation through symbiosis (López-Bellido et al., 2006). Although less productive, the faba bean landraces have shown good adaptation to local conditions and they are preferred by the farmers and consumers for their good taste in the traditional food preparation.

The landraces might be an important source of new alleles which are required to improve agronomic performance of faba bean varieties. They are known to harbour great genetic potential for faba bean improvement, particularly for biotic and abiotic stress tolerance and their ability to escape drought. Although the landrace might not necessarily have a high level of tolerance to diseases, but being heterogeneous they have some level of protection against extreme agronomic conditions. Therefore, there is potential to develop new varieties by selecting for the desired traits within the landrace populations (Gnanasambandam et al., 2012). However, the actual level of genetic diversity has not been quantified in the Ethiopian highland faba bean landraces which can be exploited in the breeding programme. Knowledge of genetic diversity plays an important role in devising a breeding strategy.

Farmers either adopt new varieties or retain traditional landraces for various reasons. On the other hand, breeders entail farmers to adopt crop varieties with greater yield potential than local landraces for the demand of increasingly growing human population. Selected germplasm used as parents for successive breeding leads to greater genetic uniformity and germplasm vanishing due to a narrow germplasm base (Duvick, 2005). Many farmers, however, retain landraces to conserve local diversity by practicing conservation (Brush, 1995). The major constraint in utilizing faba bean landraces in Ethiopia is lack of information about its genetic variability.

Genetic diversity study in faba bean can be performed using morphological, molecular and biochemical markers (Chahal and Gosal, 2002). However, the morphological characters are influenced by environmental factors and they often do not dependably depict genetic diversity (Fufa et al., 2005). Moreover, the morphological differences are usually determined by a small number of genes and may not be representative of genetic divergence in the entire genome (Brown-Guedira et al., 2000). Further, for effective breeding and management of genetic diversity, germplasm collections need to be well-characterized and genetic pools should be classified into distinguishable clusters based on genetic diversity. The objectives of this study were two-fold; to estimate the genetic diversity, population structure and gene flow of faba bean landraces from major faba bean growing areas of Ethiopian highlands; and to investigate the relationships between improved and foreign cultivars and the landraces.

3.2 Materials and methods

3.2.1 Plant material

A total of 50 genotypes of faba bean (*Vicia fabae*) including typical 40 landraces and 10 inbred lines were used for this study. The landraces were collected from different major faba bean growing areas of Ethiopian highlands (Figure 3.1), 7 inbred lines from breeding programmes of Holetta Agricultural Research Centre (HARC) and 3 inbred lines from International Centre for Agriculture Research in the Dry Area (ICARDA). More detailed information for each genotype is indicated in Table 3.1.

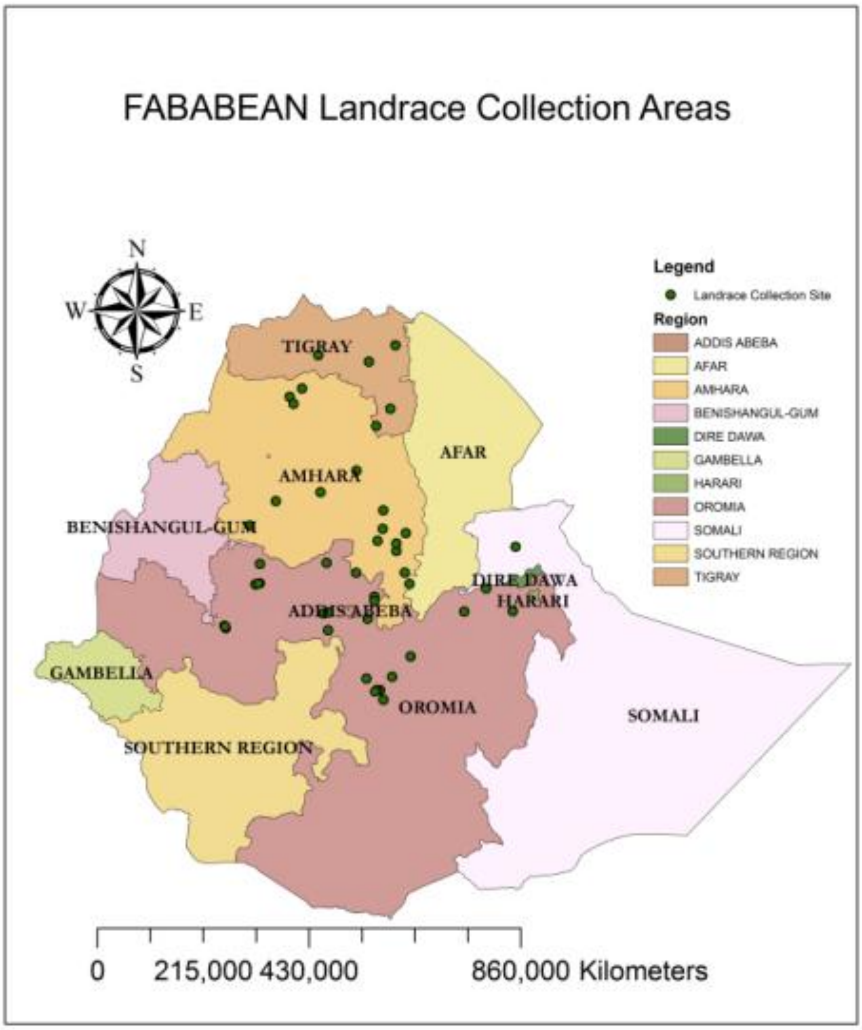

Figure 3.1 Map of the faba bean landrace collection areas in major growing areas of Ethiopian highlands in 2011.

Table 3.1 Description of faba bean genotypes used for the study

No	Code	Geographical Location	Population	Code-Pop	Biological status	Altitude
1	FBColl-001	Arsi/Bekoji	Arsi1	Pop1	Landraces	2784
2	FBColl-002	Arsi/Arsi Robe	Arsi2	Pop1	Landraces	2410
3	FBColl-003	Arsi/Limu Jara	Arsi3	Pop1	Landraces	2853
4	FBColl-004	Arsi/Dawa Bursa	Arsi4	Pop1	Landraces	2908
5	FBColl-005	Arsi/Chole	Arsi5	Pop1	Landraces	3050
6	FBColl-006	West Gojjam/Yilmanadensa	Gojjam1	Pop2	Landraces	2240
7	FBColl-007	West Gojjam/ Awi	Gojjam2	Pop2	Landraces	2610
8	FBColl-008	East Gojjam/ Hulet-Eju Enebse	Gojjam3	Pop2	Landraces	2412
9	FBColl-009	North Gonder Zone/Wogera	Gonder1	Pop3	Landraces	2961
10	FBColl-010	North Gonder Zone/Debark	Gonder2	Pop3	Landraces	2739
11	FBColl-011	North Gonder Zone/Wogera	Gonder3	Pop3	Landraces	2951
12	FBColl-012	Harer/Borda	Harar1	Pop4	Landraces	2240
13	FBColl-013	Harer/Kulubi	Harar2	Pop4	Landraces	2380
14	FBColl-014	Harer/Arberkoti	Harar3	Pop4	Landraces	2266
15	FBColl-015	Harer/Gerawa	Harar4	Pop4	Landraces	2288
16	FBColl-016	North Shewa/MoretnaJiru	North Shewa1	Pop5	Landraces	2663
17	FBColl-017	North Shewa/Basonaworana	North Shewa2	Pop5	Landraces	3012
18	FBColl-018	North Shewa/Mehalmeda	North Shewa3	Pop5	Landraces	2667
19	FBColl-019	North Shewa/ Ankober	North Shewa4	Pop5	Landraces	3200
20	FBColl-020	North Shewa/ Molale	North Shewa5	Pop5	Landraces	3100
21	FBColl-021	North Shewa/ Tarmaber	North Shewa6	Pop5	Landraces	3058
22	FBColl-022	North Shewa/Hageremariyam	North Shewa7	Pop5	Landraces	2670
23	FBV-023	HARC	CS-20-DK	Pop6	Inbred lines	2300-3000
24	FBV-024	HARC	NC-58	Pop6	Inbred lines	1900-2000
25	FBV-025	HARC	Moti	Pop6	Inbred lines	1800-3000
26	FBV-026	ICARDA	ILB-938	Pop7	Inbred lines	Unknown
27	FBV-027	HARC	Kasa	Pop6	Inbred lines	1900-2300
28	FBV-028	ICARDA	ILB-4726	Pop7	Inbred lines	Unknown
29	FBV-029	HARC	Gebelcho	Pop6	Inbred lines	1800-3000
30	FBV-030	HARC	Bulga-70	Pop6	Inbred lines	2300-3000
31	FBV-031	ICARDA	BPL-710	Pop7	Inbred lines	Unknown
32	FBV-032	HARC	Dosha	Pop6	Inbred lines	1800-3000
33	FBColl-033	East Shewa/Chefe Donsa	Central highland1	Pop8	Landraces	2263
34	FBColl-034	South West Shewa/Tulu Bolo	Central highland2	Pop8	Landraces	2192
35	FBColl-035	South West Shewa/ Tole	Central highland3	Pop8	Landraces	2127
36	FBColl-036	West Shewa/ Chalya	Central highland4	Pop8	Landraces	2839
37	FBColl-037	West Shewa/ Dendi	Central highland5	Pop8	Landraces	2270
38	FBColl-038	South West Shewa/ Elu	Central highland6	Pop8	Landraces	2100
39	FBColl-039	Central Tigray/MayTimeket	Tigray1	Pop9	Landraces	1855
40	FBColl-040	Eastern Tigray/Saese Tsaedamba	Tigray2	Pop9	Landraces	2547
41	FBColl-041	Eastern Tigray/Atsiwemberta	Tigray3	Pop9	Landraces	2840
42	FBColl-042	East-southern Tigray/Degua Tembien	Tigray4	Pop9	Landraces	2770

No	Code	Geographical Location	Population	Code-Pop	Biological status	Altitude
43	FBColl-043	Horo Guduru Wollega	Wollega1	Pop10	Landraces	2451
44	FBColl-044	Horo-guduru wollega	Wollega2	Pop10	Landraces	2296
45	FBColl-045	East Wollega/Jima Arjo	Wollega3	Pop10	Landraces	2424
46	FBColl-046	Horo Guduru Wollega/Horo	Wollega4	Pop10	Landraces	2435
47	FBColl-047	East Wollega/Jima Arjo	Wollega5	Pop10	Landraces	2460
48	FBColl-048	South Wollo/ Ambasel	Wollo1	Pop11	Landraces	2972
49	FBColl-049	North Wollo/Wadla	Wollo2	Pop11	Landraces	2930
50	FBColl-050	South Wollo/Wereilu	Wollo3	Pop11	Landraces	2640

3.2.2 Deoxyribonucleic acid (DNA) extraction

Faba bean genotypes were planted in seedling trays at INCOTEC laboratory in South Africa, and genomic Deoxyribonucleic acid (DNA) of the 50 faba bean genotypes was extracted from the newly grown leaves based on the International Maize and Wheat Improvement Center (CIMMYT) protocols (CIMMYT, 2005). The concentration and quality of extracted DNA was examined using 0.7% TBE agarose- gel electrophoresis. The purified total DNA was verified for its quality using spectrophotometer. The final DNA concentration of all extracted DNA stock was adjusted to 10 ng/µl and the DNA samples were stored at 4ºC.

3.2.3 SSR markers and polymerase chain reaction (PCR) amplification

Thirty SSR markers used in the present study comprised 6 P (Gong et al., 2010) 5 M (Ma et al., 2011) and 19 VfG (Zeid et al., 2009). These markers have been recommended by previous researchers due to their polymorphism and close association with known function gene. They were recommended for the basic studies, diversity in population or germplasm collection, genetic mapping and marker-assisted breeding of faba bean. Details of the 30 SSR loci used in this study are presented in Table 3.2.

PCR amplification was performed using a GeneAmp PCR System 9700 (Applied Bio systems) thermal cycler. Reactions were executed using 12 µl of a reaction mixture containing 1 x PCR reaction buffer, 2.5 mM Mg^{++} 0.2 µl each of dNTPs (Bioline), 1 unit Taq 42 polymerase (Bioline) and 10 ng of genomic DNA. Primers were labelled with a 104 fluorescent dye; two primers were provided for the amplification of each SSR locus: one tailed forward primer (0.05 µmol), one normal reverse primer (0.25 µmol). The initial denaturation step was performed at 94ºC for 2 min, followed by 1 cycle at 94ºC for 30 s, 63ºC for 30 s and 72ºC for 45 s. The annealing temperature was decreased by 1ºC per cycle in subsequent cycles until it reached a temperature of 57ºC. Products were subsequently amplified for 33 cycles at 94ºC for 30 s, 57ºC for 30 s, and annealing of primer at primer specific temperature for 30 s, and 72°C for 45 s with a final extension for 20 min.

3.2.4 Data collection and analysis

Basic statistics were calculated using the genetic analysis package. Summary statistics, the number of alleles (NA), major allele frequency (MAF), the heterozygosity, polymorphism information content (PIC), and gene diversity (Alghamdi et al., 2012) were calculated using Power Marker (ver.3.23) (Liu and Muse, 2005). Variance components within and among groups and populations were calculated using analysis of molecular variance (AMOVA) with the software ARLEQUIN V3.1 (Excoffier et al., 2006). As a measure of genetic differentiation between sub-populations a test for significance between population pairs were computed with the F-Statistics estimation (*Fst*) with AMOVA. Genetic relationships among individuals were assessed by multivariate principal component analysis (PCA), which is used to detect patterns of variation in complex data sets based on Jaccard's similarity coefficient (Jaccard, 1908) using PAST software V 3.0 (Hammer et al., 2001). The pattern of diversity among the genotypes was identified based on eigen vectors. The genetic distance based clustering was performed with the unweighted pair group method with arithmetic mean (UPGMA) and the dendrogram showing the relatedness among the 50 faba bean obtained by WADR clustering method using SAS software V. 9.3. (SAS Institute, 2012).

3.2.5 Population structure

The population structure was analysed using STRUCTUR 2.3.4 which implements a model-based clustering algorithm using the genotype data (Pritchard et al., 2000). It assumes a model in which there are K populations (where K may be unknown), each of which is characterized by a set of allele frequencies at each locus. Individuals are assigned to populations according to their 'membership coefficients' for each cluster. A series of K, from 1 to 20, was used to estimate the number of clusters under the admixture model with allele frequencies correlated. For each K, 20 independent runs of 10000 iterations were processed following a burn-in period of 50000 iterations. The optimum K value, which indicates the number of genetically distinct clusters in the data, was determined from 20 replicate runs for each of K (Evanno et al., 2005). The ΔK was calculated based on the rate of change of the log-likelihood between successive K values. Software program Structure Harvester V 0.6.93 (Earl and vonHoldt, 2012) was used for calculating parameters of Evanno et.al. (2005). Following the method of Evanno et al. (2005), the ΔK were plotted against the K numbers of the groups. The software package CLUMPP was used to combine the STRUCTURE group-membership output data for each population from 20 replicates run from the molecular data for K=3 (Jakobsson and Rosenberg, 2007). The optimal number genetic structure (the maximum value of *ΔK* was graphically displayed using DISTRUCT (Rosenberg, 2004).

3.3 Results

3.3.1 Marker characterisation

Number of alleles

Twenty eight of the 30 microsatellites were polymorphic with a varying degree (Table 3.2). VfG87 and M10 were monomorphic loci. The fragment size of the alleles ranged from 120 bp (VfG31 and VfG55) to 326 bp (VfG11). A total of 222 alleles were detected with 30 SSRs markers. The number of alleles detected by a single SSR locus varied from 1 (for VfG87 and M10) to 24 (for VfG67) with an average of 7.4 per primers. Heterozygosity (He) ranged from 0.0 (M10 and VfG87) to 1.00 (M17, VfG3, VfG 10, VfG27, and VfG 81), with a mean of 0.85. The major allele frequency ranged from 0.14 (VfG28) to 1 (M10 and VfG87) with a mean of 0.48. The number of alleles detected showed that out of 28 polymorphic SSR markers, 9 were polymorphic among all the improved cultivars and 7 among all the faba bean landrace collections.

Gene diversity

Gene diversity, or expected heterozygosity per locus in a population, is used to quantify the genetic variation, evaluate genetic divergence and population relationship and detect inbreeding. The marker detecting the highest number of alleles also showed the highest gene diversity (Table 3.2). The gene diversity scores of the 28 polymorphic SSR loci ranged from 0 (M10 and VfG87) to 0.91 (VfG28) with mean of 0.63. Observed heterozygosity across all loci was very high ranging from 0 (M10 and VfG87) to 1 (M17, VfG3, VfG10, VfG27 and VfG81) with a mean of 0.85.

Polymorphic information content

The PIC value for 30 SSR loci varied from 0.0 (M10 and VfG87) to 0.91 (VfG28). All except four loci (P41, M10, VfG87, VfG19) showed high PIC values (>0.5) with an average of 0.62 (Table 3.2).

Table 3.2 Summary statistics for the 30 SSR markers used in this study:

SSR Markers	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Size range (bp)	Alleles number	PIC value	He	Major allelic frequency	Gene Diversity	Genotype number
P27	< HEX > CGGGTTTATTCCATCATTT	CGTTATTGTTGTCGCTATTT	210-235	3	0.59	0.92	0.46	0.62	50
P28	< HEX > CCATCTCCACCACCAG	CACAACGGCTTCAAAT	195-215	8	0.81	0.98	0.24	0.82	49
P41	< HEX > ACACTGTTCAACGGTAT	GGAGTGATTAGAAGGTAG	152-166	2	0.38	0.92	0.54	0.50	50
P99	< FAM > AGATGGGAGCTGAGAATGAT	TTTAAACCAAACACCAGAGT	192-212	3	0.68	0.98	0.40	0.69	49
P139	< FAM > AACCCATCTGGAAGAAACA	TTGAGAATCCGAAGAAACC	195-222	8	0.67	0.94	0.44	0.69	50
M10	CCATGGTTACTGCAGTCGAA	GCCGTCGATTGATTCGTATT	202-216	1	0.00	0.00	1.00	0.00	50
M17	CTCCAACGAAGGCAGAGAAG	CATGATTCCCATAGCCTTGC	220-260	3	0.46	1.00	0.50	0.55	50
M41	CAACGCGGCAGTTAAAGAAT	CAGGTATGGCTGACACCTCA	180-255	7	0.66	0.94	0.47	0.65	50
VfG1	TTTCAGCAAACTAGAACCAATC	GGCATTCAGTTTTTACCTTGTA	218-272	13	0.76	0.93	0.50	0.72	45
VfG3	TTCTTTGGTCCTCTCTCTATC	GCACTGTTGTTGCTGATACAA	164-184	5	0.76	1.00	0.45	0.72	49
VfG4	AAGGGGAGGGCATAACAGAA	AATCCGCAAGGGTCTTCTTT	225-250	5	0.62	0.98	0.48	0.65	46
VfG10	ACCAAAACGCGCACTTATCA	AAGAGAGAGAAGAGAGCTTC	205-240	14	0.77	1.00	0.40	0.78	43
VfG13	GGTTGGGATCTTTTAGGTTGAA	TGGCCTTATATCCGTCCAAT	195-217	9	0.73	0.92	0.45	0.71	48
VfG87	AGGGCCAGCGTGATCCAATA	TGGGTTGGGATCTTTTGGTTG	232-245	1	0.00	0.00	1.00	0.00	50
VfG19	AGCGATGGTGCTCATGCTTA	TCTCTCACGGAATCACATCTTT	180-210	3	0.32	0.39	0.83	0.28	47
VfG28	AGAGTCCCAAAGAGTGGGTT	CCAAAGGCAAAAATGAGGGCTT	215-257	18	0.91	0.97	0.14	0.91	36
VfG31	ATAAGAGAGAACGAGGGAGAA	TTATGGTGGGACGTCTTACAT	120-150	9	0.71	0.96	0.44	0.70	50
VfG34	GCACTCGAAGGAATTAATTTT	GAACAGTTGTTTCGTGTCGTA	202-215	4	0.55	0.98	0.51	0.59	47

SSR Markers	**Forward primer sequence (5'-3')**	**Reverse primer sequence (5'-3')**	**Size range (bp)**	**Alleles number**	**PIC value**	**He**	**Major allelic frequency**	**Gene Diversity**	**Genotype number**
VfG53	GGTTCATGAAAAGAGGTTAG	CATTTTCCGTTCTCTCTCTA	234-266	5	0.58	0.87	0.54	0.61	45
VfG55	ATCATCCAGGAGGGAGAAAA	ATGGGCAGAGAGGATAAAAA	120-180	12	0.81	0.98	0.33	0.82	50
VfG11	GCAAAAGGAGAGCAAGGGAA	CGAAAGAGGGGGACATTTTGT	310-326	7	0.74	0.95	0.36	0.73	42
VfG67	GTTCATCAAGCACCAATCTAAAC	TCAATTTGGTTTATCTCTCTCTCT	122-190	24	0.84	0.96	0.33	0.85	47
VfG27	CCCAAAAAGAGACGAACTGTAT	AGGGTTCATACGTTTGGCTT	196-236	10	0.87	1.00	0.35	0.80	49
P11	< FAM > CGTGGTTGATTTGCTTC	ACCTCCATCTTCGCCTC	166-192	5	0.66	0.90	0.43	0.65	48
VfG9	GGTTTTGAATAGAAATGCAA	AAGATGTGTCAATATTGTTTT	148-196	12	0.87	0.96	0.22	0.87	45
VfG41	CAAGCTTGTTGAGAGCCAAA	GAACGAGGCTCACGAAAATA	142-170	2	0.38	0.96	0.52	0.50	50
M49	GCGTTATTAGGCCGCTGTAA	AAAACCGTGGCTCGAATATTTA	240-310	4	0.59	0.67	0.64	0.51	49
VG47	CGATTGTTTGCAGAGGAGATA	ACAGAGAGGGACAGAGAGAA	290-306	4	0.59	0.67	0.52	0.61	48
M57	TGCAGAGAAGCTAAGCACCA	TCGCATGGTACAGTAGCAAAA	225-272	17	0.74	0.92	0.45	0.75	48
VfG81	GTCCTGGAAAAAAGAAAGAGA	AAAGAAACCTCTCTCTCCAT	141-207	4	0.62	1.00	0.45	0.67	49
Total				222	18.67	25.58	14.38	18.97	1429
Mean				7.4	0.62	0.85	0.48	0.63	47

3.3.2 Analysis of molecular variance (AMOVA)

There was high and significant (P<0.001) distribution of genetic variation within populations (Table 3.3). There was 68.54% of the molecular variation within populations and 31.46% among populations. This variation among groups further partitioned 11.81% among groups and 19.65% among populations within groups. A value of *Fst* 0.31460 was recorded showing the extent of differentiation within the populations.

Table 3.3 Analysis of molecular variance (AMOVA) of 50 faba bean genotypes in the population grouped based on their geographical location collected.

Source of variation	d.f	Sum of squares	Variance component	Percentage of variation	Probability
Among groups	7	310.767	1.31399 Va	11.81	0.2932
Among population within groups	3	74.823	2.18583 Vb	19.65	0.1407
Within populations	89	678.61	7.62483 Vc	68.54	0.0000
Total	99	1064.2	11.12464		
Fixation Indices					
FST: 0.31460					
FSC: 0.22280					
FCT: 0.11811					

3.3.3 Genetic differentiation analysis

The pair wise *FST* value revealed the lowest genetic differentiation (0.087) between Tigrai and Harer populations (Table 3.4). Alternatively, it was highest between the faba bean genotypes from ICARDA and all the other populations (Wollega 0.77, Wollo 0.72, North Shewa 0.69, Gojjam 0.69, Tigrai 0.68, Central highland 0.58 and HARC 0.55). All the pair-wise *FST* values were very high suggesting that there is more evidence for genetic differentiation. Significant variation (P<0.001) of population differentiation was recorded between population of Arsi and Central highland, Arsi and Wollo, Arsi and North Shewa, Arsi and ICARDA materials, Central highland and North Shewa, improved materials from HARC and Central highland, North Shewa and Harar, Wollega and Central highland, Wollo and Central highland population (Table 3.4).

Table 3.4 Population pair wise FSTs, (below diagonal) and FST P value on 99 permutations (above diagonal)

	Arsi	**Gojjam**	**Gonder**	**Harer**	**North shewa**	**HARC**	**ICARDA**	**Central highland**	**Tigray**	**Wollega**	**Wollo**
Arsi	**0.000**	0.189	0.739	0.315	0.018	0.108	0.009	0.027	0.315	0.018	0.000
Gojjam	0.202	**0.000**	0.450	0.468	0.018	0.081	0.054	0.090	0.333	0.054	0.153
Gonder	0.107	0.263	**0.000**	0.901	0.009	0.225	0.072	0.072	0.622	0.009	0.081
Harer	0.143	0.165	0.108	**0.000**	0.009	0.054	0.045	0.036	0.892	0.036	0.153
North shewa	0.169	0.269	0.254	0.222	**0.000**	0.009	0.000	0.000	0.577	0.009	0.018
HARC	0.144	0.175	0.165	0.181	0.178	**0.000**	0.018	0.000	0.162	0.009	0.027
ICARDA	0.650	0.687	0.668	0.675	0.687	0.551	**0.000**	0.018	0.009	0.018	0.090
Central highland	0.225	0.242	0.247	0.243	0.216	0.217	0.575	**0.000**	0.045	0.009	0.018
Tigray	0.147	0.177	0.156	0.087	0.090	0.148	0.677	0.207	**0.000**	0.685	0.955
Wollega	0.283	0.338	0.310	0.210	0.191	0.272	0.769	0.302	0.092	**0.000**	0.324
Wollo	0.307	0.345	0.305	0.252	0.247	0.243	0.716	0.295	0.098	0.166	**0.000**

3.3.4 Population structure and cluster analysis

The dendrogram classified the germplasm into three major clusters. Cluster II and cluster III were further subdivided into sub-clusters. Cluster I comprised the landraces from different locations, and three old small seeded improved varieties (FBV-23=CS-20-DK, FBV-024= NC58, FBV-030 = Bulga-70 and FBV-027= Kasa) and two recently released varieties, Moti and Dosha (FBV-025= Moti and FBV-032 = Dosha). All faba bean landrace collections from different parts of Wollega (FBColl-43, FBColl-44, FBColl-45, FBColl-46 and FBColl-47) were grouped in cluster II. Cluster III comprised the exotic genotypes from ICARDA (FBV-026= ILB-938, FBV-028=ILB-4726, and FBV-031=BPL-710). FBColl-36, which is a faba bean collection from the Central highland, West Shewa, and FBV-029 (Gebelcho which is an improved variety from HACR) were grouped in cluster I.

3.3.5 Estimation of the optimal number of cluster in structure

There were three different genetic groups (GI, GII and GII) (Figure 3.2 A and B). The maximum *ΔK* occurred at K=3 (Figure 3.3 B). At K=3, the faba bean genotypes divided into three clusters (Figure 3.2 B). All ICARDA faba bean genotypes were fully grouped together in the DISTRACT plot (Figure 3.3 B) the rest of the population showed admixture. As shown in Figure 3.3 C some of the individual genotypes from populations of Gojjam, Central highland, Tigray, Wollega, and Wollo were pure. All the improved faba bean materials from HARC were admixtures.

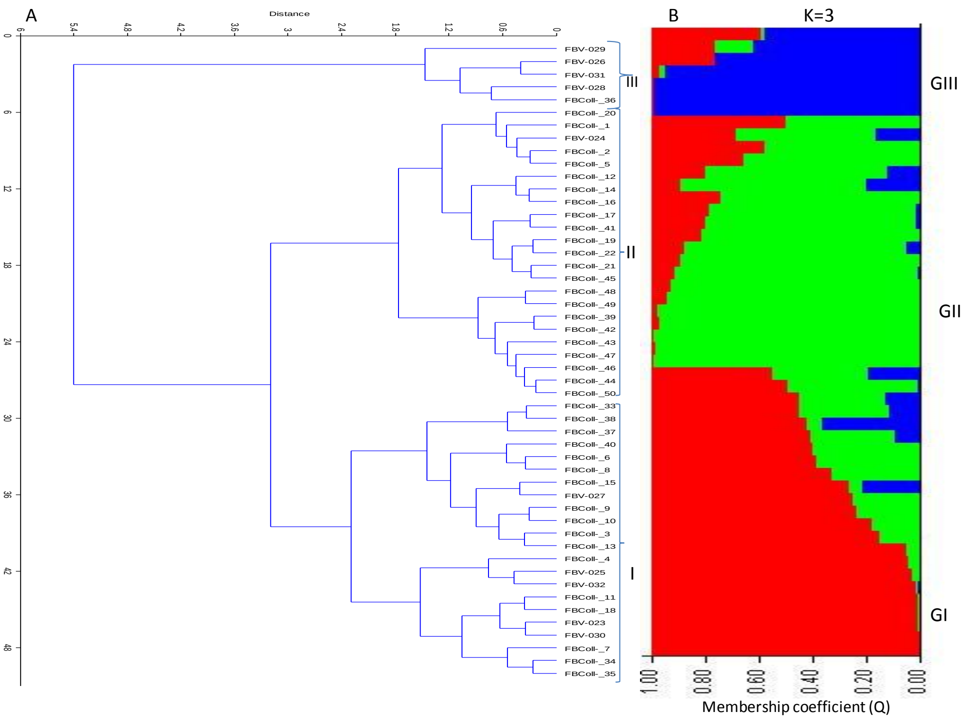

Figure 3.2 (A) UPGMA dendrogram for fifty faba bean genotypes based on the Jaccard coefficient as revealed using SSR markers. The number indicated cultivars and collection listed in Table 1. (B) Bar plot of K=3, estimates of membership coefficient, each vertical bar represents the membership coefficient (Q) for an individual genotype grouped into three GI, GII and G III.

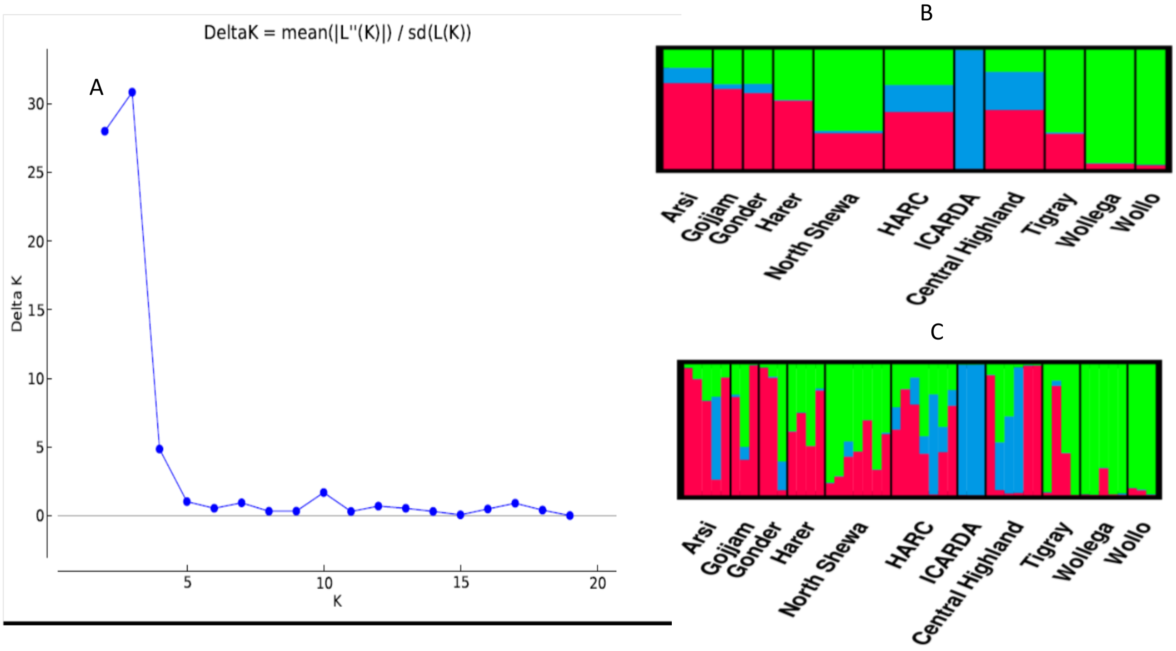

Figure 3.3 Inferred population structure of faba bean genotypes: Plot of (A) The relationship between ΔK and K showing the highest peak at K=3; (B) DISTRUCT plot for 50 faba bean genotypes based on the STRUCTURE analysis, each color represent a different cluster, and black segments separate the population, population names are below the figure (C) Individual genotypes from each population (collection site and source) is represented by a single vertical line partitioned in to K=3. Each color represents one cluster, and the length of the colored segment shows the genotype's estimated proportion of membership in that cluster as calculated by STRUCTURE. The color code for the inferred three cluster is 1=Red, 2=Green, 3= Blue.

3.3.6 Individual genotype identification

The individuals were assigned to populations (Table 3.5). Genotypes with membership coefficient > 0.800 were assigned to the respective cluster completely and individuals with < 0.800 indicated that they were admixed and were assigned to two or more population clusters. All genetic groups comprised individuals with a high estimated membership coefficient for the respective cluster.

Table 3.5 The average membership coefficients of individual faba bean genotypes in three genetic clusters

Location	Code	Population	Average probability of membership to cluster (K=3)		
			K1	K2	K3
Arsi1	FBColl-001	1	0.97	0.00	0.03
Arsi2	FBColl-002	1	0.89	0.00	0.11
Arsi5	FBColl-005	1	0.90	0.00	0.10
Arsi3	FBColl-003	1	0.72	0.00	0.28
Arsi4	FBColl-004	1	0.12	0.63	0.25
Gojjam3	FBColl-008	2	0.99	0.00	0.01
Gojjam2	FBColl-007	2	0.27	0.10	0.63
Gojjam1	FBColl-006	2	0.75	0.02	0.23
Gonder1	FBColl-009	3	0.97	0.00	0.02
Gonder2	FBColl-010	3	0.90	0.01	0.09
Gonder3	FBColl-011	3	0.04	0.22	0.74
Harar1	FBColl-012	4	0.49	0.00	0.51
Harar2	FBColl-013	4	0.63	0.00	0.37
Harar3	FBColl-014	4	0.38	0.00	0.62
Harar4	FBColl-015	4	0.80	0.02	0.18
North Shewa1	FBColl-016	5	0.09	0.00	0.90
North Shewa2	FBColl-017	5	0.14	0.00	0.85
North Shewa3	FBColl-018	5	0.29	0.12	0.59
North Shewa4	FBColl-019	5	0.33	0.00	0.66
North Shewa5	FBColl-020	5	0.57	0.00	0.43
North Shewa6	FBColl-021	5	0.19	0.00	0.80
North Shewa7	FBColl-022	5	0.47	0.01	0.52
NC-58	FBV-024	6	0.81	0.00	0.19
CS-20-DK	FBV-023	6	0.50	0.17	0.33
Moti	FBV-025	6	0.69	0.21	0.10
Kasa	FBV-027	6	0.32	0.13	0.55
Gebelcho	FBV-029	6	0.01	0.76	0.23
Bulga-70	FBV-030	6	0.33	0.19	0.48
Dosha	FBV-032	6	0.68	0.12	0.20
ILB-938	FBV-026	7	0.00	0.99	0.00
ILB-4726	FBV-028	7	0.00	0.99	0.00
BPL-710	FBV-031	7	0.00	1.00	0.00
Central highland1	FBColl-033	8	0.92	0.00	0.08
Central highland5	FBColl-037	8	0.99	0.01	0.01
Central highland6	FBColl-038	8	0.99	0.00	0.01
Central highland4	FBColl-036	8	0.02	0.96	0.02
Central highland2	FBColl-034	8	0.04	0.36	0.60
Central highland3	FBColl-035	8	0.01	0.59	0.40
Tigray2	FBColl-040	9	0.84	0.04	0.12
Tigray4	FBColl-042	9	0.00	0.00	1.00
Tigray1	FBColl-039	9	0.02	0.01	0.97

Location	Code	Population	Average probability of membership to cluster (K=3)		
Tigray3	FBColl-041	9	0.32	0.00	0.68
Wollega1	FBColl-043	10	0.01	0.00	0.99
Wollega2	FBColl-044	10	0.00	0.00	0.99
Wollega4	FBColl-046	10	0.01	0.00	0.99
Wollega5	FBColl-047	10	0.00	0.01	0.98
Wollega3	FBColl-045	10	0.21	0.00	0.79
Wollo1	FBColl-048	11	0.05	0.00	0.94
Wollo2	FBColl-049	11	0.04	0.01	0.96
Wollo3	FBColl-050	11	0.00	0.01	0.99

3.3.7 Principal component analysis

The first four principal components explained 30% (each 12.05% (Rohlf), 7.08% (PC 2), 5.16% (PC 3) and 4.67% (PC 4)) of the total diversity. The test genotypes were divided into three distinct groups with the ICARDA materials fully separated from the others (Figure 3.4). In the PCA plot, the faba bean germplasm were divided into three main groups and one admixture with 12, 16, 5 and 17 genotypes in Cluster I, II, III and admixture respectively. Group (III) consisted of faba bean exotic varieties from ICARDA (FBV26-ILB-938, FBV28-ILB-4726, and FBV31-BPL 710). This group also comprised of faba bean improved varieties Gebelcho-FBV29 and landrace collections (FBColl-36). The grouping obtained by unweighted pair-group method with arithmetic mean (UPGMA) dendrogram and plot from STRUCTUR analysis confirmed by principal component analysis (PCA).

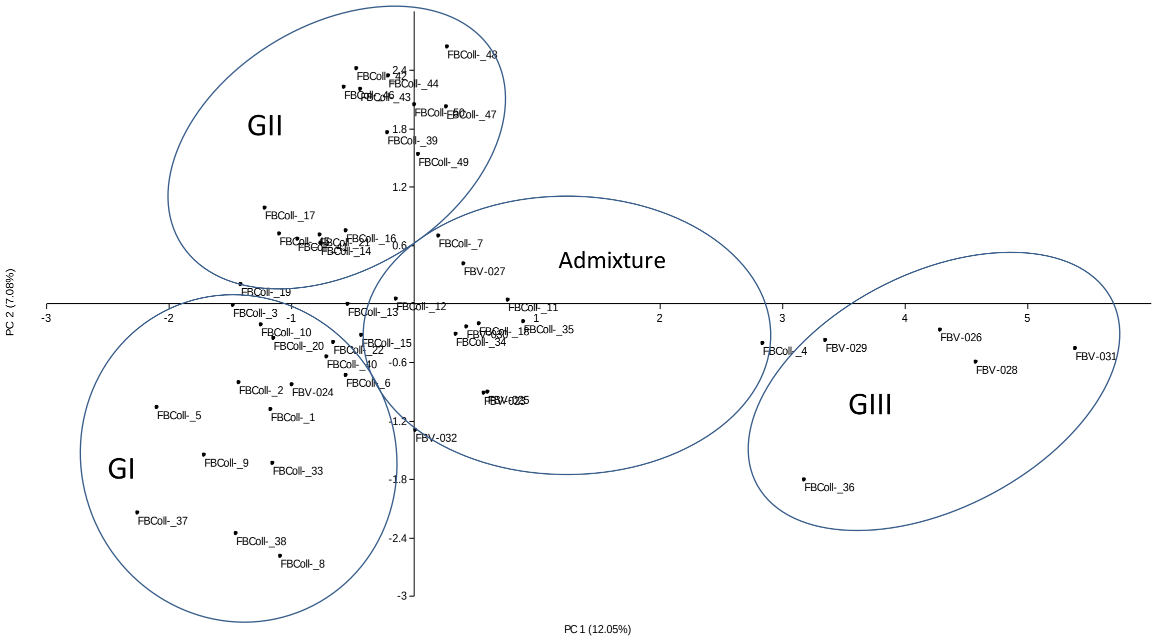

Figure 3.4 Scatter plot of PC 1 and PC 2 of the principal component analysis (PCA) based on the similarity of 30 SSR markers for 50 faba bean germplasms. Different genotypes group of three main groups and one admixture were delineated.

3.3.8 Genetic dissimilarity among genotypes

Genetic dissimilarity >50% was observed from about 86.94 % of the pair wise comparison among the faba bean genotypes (Figure 5). The highest genetic dissimilarity coefficient (93%) was depicted between faba bean collection from West Shewa (FBColl-036) and faba bean collection from Arsi Zone (FBColl-003).The lowest value of genetic dissimilarity coefficient (29%) recorded between faba bean varieties ILB-4726 (FBV-028) and ILB-938 (FBV-026).

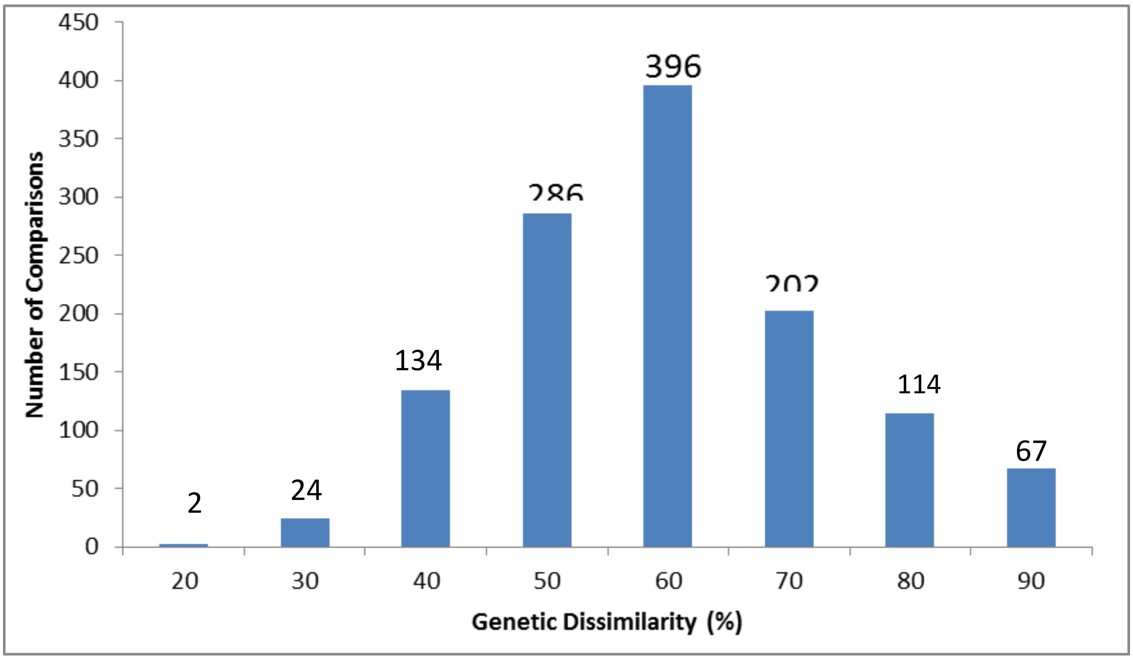

Figure 3.5 A histogram showing the distribution of 1245 pair wise genetic dissimilarity coefficients among 50 faba bean germplasms

3.4 Discussion

The number of alleles detected in the present study, on average 7.4 alleles per locus, is different from the faba bean diversity study using EST-SSR markers which had an average of 2.3 alleles per locus (Ya-ming et al., 2011). Such considerable differences in the number of alleles detected may arise from differences in the diversity of the test genotypes used, the number of genotypes examined and the genotyping method used. SSR markers exhibit relative abundance and co-dominant inheritance and are useful for estimating genetic relationship and diversity (Kumar et al., 2009). In this study, the SSR markers were able to detect considerable level of genetic diversity present among the tested faba bean genotypes. This set of markers would be recommended for use in future studies on faba bean.

High average heterozygosity 0.85 was detected in this study, which could reflect the partial cross-pollination nature of faba bean (Gnanasambandam et al., 2012). This heterozygosity is important in creating genetic variability in faba bean (Gregorius, 1987; Hegaya et al., 2012). The results have implications for breeding new varieties. There is potential for selection within the populations, which is consistent with previous studies. Link et al. (1996) reported the possibility of considerable potential for selection within populations for specific traits from highly heterogeneous and heterozygous plants in faba bean.

Polymorphism information content (PIC) value provides an estimate of discriminating power of a marker based on the number of alleles at a locus and relative frequencies of these alleles. In the present study nearly 86% of these markers had high PIC values (>0.5) with an average of 0.62. This indicates that the markers were highly polymorphic, informative and were useful in discriminating the faba bean genotypes. This is considered to be high based on the previous classification by Botstein et al. (1980). They cite that PIC value >0.5 is considered to be highly informative markers, 0.5>PIC>0.25 is informative marker, and PIC $\leq$ 0.25 is a slightly informative marker. A survey of the literature indicates that the results obtained in the current study are both consistent and in disagreement with previous studies on faba bean. For example, Terzopoulos and Bebeli (2008) reported PIC value of 0.3-0.5 and average of 0.21 using the Inter-Simple Sequence Repeats (ISSRs) markers. The PIC of 0.06-0.43 with average of 0.29 was reported in Chinese and European collections (Ya-ming et al., 2011), but a higher PIC of 0.96 was reported by Alghamdi et al. (2012) using the Sequence Related Amplified Polymorphism (SRAP) markers. The differences reflect the type and number of genotypes evaluated. In the present study the high polymorphic rate for most (86%) of the markers and PIC value, together with more than 50% genetic dissimilarity for about 87% pair wise comparison in the test faba bean genotypes, suggests a high level of heterogeneity. Furthermore, the SSR markers used in the present study were effective and highly polymorphic comparable from previous studies (Ya-ming et al., 2011). Therefore, the set of markers used are recommended in other future evaluation of faba bean germplasm.

The AMOVA results indicated high genetic variation which is consistent with previous studies on faba bean. The findings from this study indicate that faba bean populations from the Ethiopian highlands are highly variable. This is very much in line with the study by Kwon et al. (2010) who reported a large amount of variation within faba bean landraces. Recently, Ouji et al. (2012) reported 74.3% genetic diversity within nine populations of Tunisian faba bean than that of among population which is also congruent with findings from the current study. A previous study indicated a high level of genetic diversity within the populations of Mediterranean-type faba bean (Terzopoulos and Bebeli, 2008). The study, therefore, adds crucial evidence that variation within populations is large in faba bean. This has also been reported in soybean (Jin et al., 2006) and Lathyrus populations (Belaid et al., 2006). The higher genetic diversity within populations is expected (Smýkal, 2006) than that of among populations since faba bean is largely an out-crossing crop (Suso et al., 2006). The small differentiation, low percentage of variation (31.46%) partitioned among populations of faba bean from different locations observed in this study could be attributed to the exchange of faba bean seed among farmers. Ethiopian faba bean growers get their seed mostly by informal seed exchange system. In this line there has been a lot of genetic exchange among famers' landrace for seed (Alemu et al., 2010). However, in this study large *FST* value (0.17 - 0.23) between the population of Arsi, Central highland, and North Shewa suggests that although they are located geographically near each other, the collections from these locations have high genetic differentiation.

The clustering of genotypes into three major groups reflects the pedigree data and origin of the genotypes. For example, the improved variety Gebelcho grouped with the ICARDA materials because it is a hybrid of ILB-4726 x Tesfa released from HARC (Gemechu et al., 2006). The results showed well-defined distribution patterns of the materials, according to the genetic distance and the membership coefficient relationships among them (Rosenberg, 2004). In the PCA plot, the faba bean germplasm were divided into three main groups and one admixture. These groups reflect the breeding history and the relation between improved, exotic and landrace collection. Admixture situation in the genotypes shows good agreement with pedigree information. Both the PCA and STRUCTURE analysis also suggested the existence of three major groups. The admixture from small membership coefficient value in the PCA is explained as the presence of gene flow of the genotype which had infiltrated into other population clusters (Zhang et al., 2011). This observed admixture situation is due to the largely out-crossing nature of faba bean (Suso et al., 2005) and artificial crossing and hybridization.

The high percentage genetic dissimilarity coefficient for the majority of pair wise comparison among the faba bean genotypes suggests lesser amount of genetic relatedness and elucidates low genetic similarity. This result is different from Kwon et al. (2010) who reported that the majority of the pair wise comparison among worldwide collected faba bean entries exhibited high genetic similarity.

3.5 Conclusion

The present study suggests that the faba bean collections were genetically variable and there was considerable gene flow among the geographical locations where the materials were collected. Future germplasm collection strategy should focus on sampling a large number of plants in the populations. The same recommendation holds for the crosses in breeding programmes. The selection of parents within faba bean population would be recommended to explore the intra-population variability, followed by crosses among different populations to explore the inter-population variability.

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Chapter 4

Phenotypic diversity among faba bean (*Vicia faba* L.) landraces from the Ethiopian highlands and ICARDA

Abstract

Knowledge of phenotypic diversity is important for devising the breeding strategy for the faba bean programme in Ethiopia. This study was conducted to determine phenotypic diversity among 50 faba bean genotypes collected from the major faba bean growing areas of Ethiopia and ICARDA. The landraces were evaluated at three locations in the highlands of Ethiopia. The experiments were replicated three times at all locations. The data was analysed using ANOVA, principal component analysis and the Shanon-Weaver index, using SAS V9.3 and PAST V 3.0 software. There were significant differences among genotypes. The genotypes were categorized into three phenotypic clusters and different sub-groups. Six principal components were identified explaining more than 80% of the total variation. The Shannon-Weaver diversity index, which ranged from 3.82 for pod weight to 7.15 for number of basal branches per plant, revealed high diversity among and within the genotypes. The observed high variation among the faba bean genotypes would be exploited in breeding new varieties with desired traits for the target regions.

Keywords: Cluster analysis; faba bean; genetic diversity; morphological characterization; phenotypic diversity.

4.1 Introduction

Ethiopia is one of the Vavilovian centers of diversity for several grain legume and cereal crops (Vavilov, 1926). It is a secondary centre of diversity for faba bean (Zong et al., 2009). This makes Ethiopia an ideal place to search for diversity in faba bean. Globally, faba bean is genetically diverse with more than 38,000 accessions with approximately 37 collections (Duc et al., 2010). Selection in response to diverse environments and human needs, natural inter-crossing among the different faba bean races, and genetic drift have contributed to increased faba bean diversity and wide variability (Maxted and Kell, 2009).

Previously morphological traits were used as markers in germplasm management (Panthee et al., 2006; Stanton et al., 1994). They have limitations of low polymorphism, low heritability and are affected by environmental influences which may affect the estimation of genetic relationships (Muthusamy et al., 2008). However, morphological markers are still one of the choices for diversity studies for traits which are highly heritable and do not require complicated laboratory facilities and procedures for characterizing the germplasm. Phenotypic characterisation would be effective when it is done through replicated trials across multiple environments.

Landraces are used by subsistence farmers as a key component in their cropping systems and by breeders for developing modern varieties (Hammer and Teklu, 2008). The success of any breeding programme in general and improvement of specific traits through selection in particular, totally depends upon the genetic variability present in the available germplasm of a particular crop. The genetic variation in current Ethiopian faba bean germplasm has not been determined. However, it is expected to be large because the landraces have been preserved by the farmers by keeping seeds from the previous harvest every year or by farmer to farmer seed exchange and introduction of new seed from the market. If available, genetic diversity among and within landraces makes them a valuable resource for use in new variety development in the breeding programme (Soleri and Smith, 1995). Conversely, the genetic diversity could be small if farmers were selecting for a few traits since time immemorial in the Ethiopian highlands. Thus, it is not clear whether the diversity has not been compromised by farmers who have been selecting for a few common and desired traits for home consumption or the market.

Characterization and quantification of the genetic diversity and of germplasm collections of a crop is essential for a rational use of genetic resources and ensuring a long-term success of crop improvement programmes (Gwak, 2008), because selection depends on the levels of genetic variation The faba bean collections, from Wollo, Gonder, North Shewa and Arsi, exhibited variation for most phenotypic traits (Gemechu et al., 2006). However, a comprehensive study of genetic diversity has not been done. As a result, the faba bean improvement programmes in Ethiopia depend on genotypes from other sources, mainly from the International Centre for Agricultural Research in the Dry Areas (ICARDA) (Gemechu et al., 2006). The potential of the local landraces as sources of breeding material has not been exploited, yet they are likely to provide varieties that combine genes of adaptation to the highlands and the alleles for the consumer desired traits. Therefore, it is prudent to investigate the levels and patterns of genetic diversity among the faba bean landrace collections in Ethiopia.

4.2 Materials and Methods

4.2.1 Plant materials

The germplasm used in the study is presented in Table 4.1. The forty landraces were collected from the farmers' fields in the major faba bean growing areas in the Ethiopian highlands. Seven improved varieties were obtained from the Holetta Agricultural Research Center (HARC) and these comprised; three recently released varieties (Dosha, Gebelcho, Moti) and four old varieties (CS-20-DK, Kassa, Bulga-70 and NC58)). Additionally, three faba bean germplasm lines (ILB-47-26, ILB-938 and BPL-710) were obtained from the International Centre for Agricultural Research in the Dry Area (ICARDA).

Sampling of germplasm

The landraces were collected from the farmers' fields during the main cropping season of 2011. Ten samples were collected per location and represented different regions and different altitudes. The altitude classes (Dejene, 2003) were as follows:

a) the Wurch, the cold highlands, which are >3000 m . a. s. l;
b) the Dega, which is the cool humid highlands, 2500-3000 m. a. s. l;
c) Wiena Dega, which is the temperate cool and sub-humid highlands, 1500-2500 m a. s. l.

The genotypes from the altitudes <2000 m. a. s. l were classified as Altitude class I, 2001-2500 m. a. s. l altitude class II, 2501-3000 as altitude class III and from altitude >3000 classified as altitude class IV.

Table 4.1 Description of 40 faba bean landraces and 10 germplasm lines from Ethiopia and ICARDA

No	Code	Geographical Location	Population	Code-Pop	Biological status	Altitude
1	FBColl-001	Arsi/Bekoji	Arsi1	Pop1	Landraces	2784
2	FBColl-002	Arsi/Arsi Robe	Arsi2	Pop1	Landraces	2410
3	FBColl-003	Arsi/Limu Jara	Arsi3	Pop1	Landraces	2853
4	FBColl-004	Arsi/Dawa Bursa	Arsi4	Pop1	Landraces	2908
5	FBColl-005	Arsi/Chole	Arsi5	Pop1	Landraces	3050
6	FBColl-006	West Gojjam/Yilmanadensa	Gojjam1	Pop2	Landraces	2240
7	FBColl-007	West Gojjam/ Awi	Gojjam2	Pop2	Landraces	2610
8	FBColl-008	East Gojjam/ Hulet-Eju Enebse	Gojjam3	Pop2	Landraces	2412
9	FBColl-009	North Gonder Zone/Wogera	Gonder1	Pop3	Landraces	2961
10	FBColl-010	North Gonder Zone/Debark	Gonder2	Pop3	Landraces	2739
11	FBColl-011	North Gonder Zone/Wogera	Gonder3	Pop3	Landraces	2951
12	FBColl-012	Harer/Borda	Harar1	Pop4	Landraces	2240
13	FBColl-013	Harer/Kulubi	Harar2	Pop4	Landraces	2380
14	FBColl-014	Harer/Arberkoti	Harar3	Pop4	Landraces	2266
15	FBColl-015	Harer/Gerawa	Harar4	Pop4	Landraces	2288
16	FBColl-016	North Shewa/MoretnaJiru	North Shewa1	Pop5	Landraces	2663
17	FBColl-017	North Shewa/Basonaworana	North Shewa2	Pop5	Landraces	3012
18	FBColl-018	North Shewa/Mehalmeda	North Shewa3	Pop5	Landraces	2667
19	FBColl-019	North Shewa/ Ankober	North Shewa4	Pop5	Landraces	3200
20	FBColl-020	North Shewa/ Molale	North Shewa5	Pop5	Landraces	3100
21	FBColl-021	North Shewa/ Tarmaber	North Shewa6	Pop5	Landraces	3058
22	FBColl-022	North Shewa/Hageremariyam	North Shewa7	Pop5	Landraces	2670
23	FBV-023	HARC	CS-20-DK	Pop6	Inbred lines	2300-3000
24	FBV-024	HARC	NC-58	Pop6	Inbred lines	1900-2000
25	FBV-025	HARC	Moti	Pop6	Inbred lines	1800-3000
26	FBV-026	ICARDA	ILB-938	Pop7	Inbred lines	Unknown
27	FBV-027	HARC	Kasa	Pop6	Inbred lines	1900-2300
28	FBV-028	ICARDA	ILB-4726	Pop7	Inbred lines	Unknown
29	FBV-029	HARC	Gebelcho	Pop6	Inbred lines	1800-3000
30	FBV-030	HARC	Bulga-70	Pop6	Inbred lines	2300-3000
31	FBV-031	ICARDA	BPL-710	Pop7	Inbred lines	Unknown
32	FBV-032	HARC	Dosha	Pop6	Inbred lines	1800-3000
33	FBColl-033	East Shewa/Chefe Donsa	Central highland1	Pop8	Landraces	2263

No	Code	Geographical Location	Population	Code-Pop	Biological status	Altitude
34	FBColl-034	South West Shewa/Tulu Bolo	Central highland2	Pop8	Landraces	2192
35	FBColl-035	South West Shewa/ Tole	Central highland3	Pop8	Landraces	2127
36	FBColl-036	West Shewa/ Chalya	Central highland4	Pop8	Landraces	2839
37	FBColl-037	West Shewa/ Dendi	Central highland5	Pop8	Landraces	2270
38	FBColl-038	South West Shewa/ Elu	Central highland6	Pop8	Landraces	2100
39	FBColl-039	Central Tigray/MayTimeket	Tigray1	Pop9	Landraces	1855
40	FBColl-040	Eastern Tigray/Saese Tsaedamba	Tigray2	Pop9	Landraces	2547
41	FBColl-041	Eastern Tigray/Atsiwemberta	Tigray3	Pop9	Landraces	2840
42	FBColl-042	East-southern Tigray/Degua Tembien	Tigray4	Pop9	Landraces	2770
43	FBColl-043	Horo Guduru Wollega	Wollega1	Pop10	Landraces	2451
44	FBColl-044	Horo-guduru wollega	Wollega2	Pop10	Landraces	2296
45	FBColl-045	East Wollega/Jima Arjo	Wollega3	Pop10	Landraces	2424
46	FBColl-046	Horo Guduru Wollega/Horo	Wollega4	Pop10	Landraces	2435
47	FBColl-047	East Wollega/Jima Arjo	Wollega5	Pop10	Landraces	2460
48	FBColl-048	South Wollo/ Ambasel	Wollo1	Pop11	Landraces	2972
49	FBColl-049	North Wollo/Wadla	Wollo2	Pop11	Landraces	2930
50	FBColl-050	South Wollo/Wereilu	Wollo3	Pop11	Landraces	2640

4.2.2 Experimental sites

The field experiments were conducted at two sites in Ethiopia during the 2012 main cropping season. The sites were the Holleta Agricultural Research Centre (HARC) (09º03'N, 38º30'E, and 2390 m a. s. l.) classified as M2-5 agro ecology and Kulumsa Agricultural Research Centre (KARC) (08º00'N, 39º09'E, and 2730 m a. s. l) classified as ME2 agro ecology. The soil type of HARC experimental fields is Eutric Nitosols, 933.3 mm annual average precipitation, with average minimum and maximum temperature of 5.3ºC and 22ºC respectively. The KARC soils are xerosols and the annual average precipitation is 962.7 with average minimum and maximum temperature of 10ºC and 24ºC respectively.

4.2.3 Experimental design and management

The experiment was arranged in a randomised complete block design with three replications at each site. The experimental plots at the two sites had uniform slope. Fifty seeds of each genotype were planted on two rows of 2.5 m and 0.4 m inter-row spacing (with average of 25 seeds per row). A common border row was planted with the faba bean variety *Mesay*. The trials were managed in accordance with the standard practice for the area. This included regular hand weeding to keep field clean of weeds, application of the recommended fertilizer DAP at the rate of 100 kg/ha. Planting was done at the onset of the main season rainfall on the 22nd of June 2012 at the HARC and 28th of June 2012 at the KARC. The trials were rain-fed at both sites.

Data collection

Fifteen individual plants were randomly selected for each genotype per each replication from the central part of the row and were marked before flowering. All morphological data were collected from the 15 marked plants. The following data were recorded: days to emergence started one week after planting, days to 50% flowering when 50% of the plant per genotype flowerd, days to physiological maturity, plant height at maturity stage of the plant, number of basal and secondary branches/plant at grain filling stage, stem diameter of the main stem at grain filling stage, leaf length, leaf width, height to the first pod at grain filling period, pod length, pod weight, number of pod/node, number of pods/plant, number of seeds/pod at grain filling stage, number of viable nodules/plant at 50% flowering stage, seed width, seed length and seed breadth after harvest. The disease reactions of the genotypes were measured. The chocolate spot (*Botrytis fabae*) disease incidence was recorded at different growth stages (flowering, pooding and grain filling growth stage) of the crop. The disease severity based on a modified percentage leaf area infected was estimated at 1%, 3%, 6%, 12%, 25%, and 50%. At harvest the total biomass, 100 seed weight and grain yield were measured. All data except the days to emergence, days to 50% flowering and days to physiological maturity were recorded on an individual plant-basis and the mean of 15 plants was used for data analysis. Above ground biomass production rate calculated as total biomass weight divided by number of days to 90% physiological maturity; economic growth rate was calculates as total grain weight divided by grain fill duration and then multiplied by 100; harvest index (HI) was calculated as proportion of total biomass to that of grain yield and the number of days of vegetative growth derived from days from planting to days to 50% podding stage.

4.2.4 Statistical analysis

The data were first subjected to the individual site analysis (ANOVA), then tested for homogeneity of variance for the sites using Bartlett's homogeneity test (Snedecor and Cochran, 1989) in SAS/STAT 9.3 (SAS Institute, 2012). A combined analysis of variance was then performed. The Unweighted Pair-Group Method using Arithmetic average (UPGMA) was performed with the program PAST software V 3.0 (Hammer et al., 2001). The PROC CLUSTER macro was used for defining optimum cluster number based on the values of cubic clustering criterion (CCC), Pseudo-F statistics (PSF) and Hotelling's pseudo T^2 value resulted from similarity analysis of the 50 genotypes using the means of 29 traits. The Gower general similarity coefficient (Gower, 1971) was used to construct the dendrogram. The GENSTAT 12th Edition (Payne et al., 2009) was used for principal components analysis (PCA). PCs with eigen values greater than 1.0 were selected as proposed by Jeffers (1967). The diversity index (H') of Shannon and Weaver (1949) was calculated and used as a measure of phenotypic diversity of each trait using the PAST software. The index was estimated for each character over all genotypes. Pearson's phenotypic correlation coefficients were estimated using the PROC CANCORR subprogram of SAS. The step-wise regression was conducted to determine the most important phenotypic traits for grouping the genotypes using SAS/STAT 9.3 (SAS Institute, 2012).

4.3 Results

Phenotypic diversity

There were highly significant (P<0.001) differences among the genotypes for all phenotypic traits except for the number of basal branches per plant, number of days to physiological maturity seed length and seed width (Table 4.2). Significant genotype x location interaction effects were recorded for yield and most of its components. The descriptive statistics for each genotype is presented in Table 4.3. Highly significant (P>0.001) differences were recorded among the regions for most of the morphological traits (Table 4.3.). All regions showed highly significant variation (P>0.001) for hundred seed weight and Arsi, Gojjam, Harar, Norht Shewa, Tigray and Central highland showed significant variation for yield (Table 4.4). There was also significant variation (P>0.001) for faba bean chocolate spot disease severity in Gojjam, Harar and North Shewa. There was significant variation (P>0.001) among altitude classes for all traits except basal branches per plant, number of days to physiological maturity and seed length (Table 4.2). High genetic variation was observed within altitude classes II, III and IV (Table 4.4). Highly significant variation was observed between altitude class II, III and IV for number of days to 50% flowering, pod length, pod weight, total above ground biomass, hundred seed weight, chocolate spot disease severity and grain yield.

Table 4.2 Analysis of variance and variance components for phenotypic traits measured on 50 faba bean genotypes

Morphological traits	Mean	CV (%)	δ^2g	δ^2g x I	δ^2e	Region	Altitudes class
Number of basal branches per plant	0.9	40	0.002^{NS}	0.00^{NS}	0.1	NS	NS
Number of days to physiological maturity	135.5	7.1	2.338^{NS}	7.90**	93.8	NS	NS
Number of days to 50% flowering	56.6	4.9	13.464**	3.62^{NS}	8	**	**
Grain filling period	79	12.1	5.88**	2.6	90.8	**	**
Stem diameter (cm)	8.4	18	0.017**	0.29^{NS}	2.3	*	*
Number of days of vegetative growth	48.1	5.9	13.46**	3.62**	8	**	**
Leaf length (mm)	7.2	18.6	0.023*	0.06**	1.8	**	**
Leaf width (mm)	3.4	20	0.008**	0.01**	0.5	**	**
Plant height (cm)	136.4	7.4	37.25**	19.14**	102.5	**	**
High to the first pod (cm)	51.4	22	6.091**	3.39**	128.9	**	**
Number of pod per node	1.5	22	0.014**	0.04**	0.1	**	*
Number of pods per plant	16.9	30	1.952**	6.68**	26.6	**	**
Number of nodules per plant	104.3	40	26.198**	263.35^{NS}	1783.1	*	**
Grain production efficacy	627.5	40	31393.8**	2447.6**	63871	**	**
Pod length (cm)	6.8	9.5	0.216**	0	0.4	**	**
Number of Node with pod	9.6	24	0.924**	0.38*	5.8	**	**
Number of seeds per pod	2.7	9.3	0.001**	0.02**	0.1	**	*
Pod weight (g)	439.9	32	12277.2**	1887.2**	20716	**	*
Number of node per plant	28.7	10.6	0.206**	3.24**	9.3	**	**
Seed Length (mm)	4.8	22	0.016NS	0	2.2	NS	NS
Seed width (mm)	4.4	29	0.016NS	0.2	2.1	NS	**
Seed breadth (mm)	4.6	24	1.251**	0.2	1.7	**	**
Above ground biomass production rate	8.4	26	2.114**	1.67**	5	**	**
Economic growth rate	438.9	34	12849.9**	3082.1**	23371.3	**	**
Total above ground biomass	1132.5	26	44782.1**	18918**	87629.6	**	**
Grain yield (kg/ha)	2248.9	33	37103.1**	16353*	56387.8	**	**

Morphological traits	Mean	CV (%)	δ^2g	δ^2g x l	δ^2e	Region	Altitudes class
Hundred seed weight (g)	64.3	10	125.89**	54.80**	45.6	**	**
Harvest index	707.8	16.3	14968	248282**	134032.8	**	**
Disease Severity (%)	27.7	28	22.657**	19.40**	54.2	**	**

δ^2g, δ^2g xl, δ^2e genotype, genotype x location and error variance, respectively; CV (%): coefficient of variation; NS: data is not significant at P>0.05; *, **, *** data Significant at 0.05, 0.01 and 0,001, respectively.

Table 4.3 Descriptive statistics for selected traits of 50 faba bean landraces (only traits with significant data are shown)

Region	NDTF	SD	LL	LW	PH	HFP	NPPN	NPPP	GPF	PL	NNWP	NSPP	PWt	NNPP	SB	AGBP	EGR	BM	GY	HSW	HI	CHDS
Arsi	53.23ed	8.54abc	7.40a	3.5ab	140.5ab	49.0bcd	1.55abc	15.75b	845.4a	7.3ab	9.66bc	2.83a	550.5a	30.2a	5.7b	9.5ab	535.0ab	1251ab	2794.8a	79.1b	653cbd	25.4ef
Gojjam	59.17c	8.64abc	7.38a	3.5ab	140.1ab	55.4ab	1.49bc	18.59b	554.0ed	6.9bc	9.66bc	2.7abc	430.3bc	29.0a	5.3bc	8.5bc	452.2bc	1139bc	2188cde	65.2c	414cbd	26.2def
Gonder	53.17ed	8.95ab	7.57a	3.5ab	140.5ab	50.6bcd	1.62ab	18.16b	683.3cd	7.1abc	11.26a	2.62c	439.6bc	29.0a	4.5d	8.0cd	413.1cd	1092bcd	2238bcd	61.1ef	457cbd	28.1cde
Harar	52.29e	7.81cd	7.08a	3.3bc	134.4bc	46.7cd	1.45bcd	15.53b	604.0ed	7.2ab	9.35c	2.79ab	396.2cd	29.0a	4.7cd	7.1d	381.1cd	950.0d	1999de	67.9c	348cd	30.1cd
NShwa	54.26d	8.09bcd	7.22a	3.4ab	134.7abc	49.5bcd	1.62ab	17.31b	764.7abc	6.4de	9.95abc	2.7abc	494.0ab	28.6a	3.7ef	8.9abc	465.1abc	1217abc	2511abc	57.3fg	433cbd	31.1c
HARC	54.02ed	9.08a	7.73a	3.7a	137.5abc	52.7abc	1.52abc	15.39b	839.3ab	7.4a	10.32d	2.7abc	543.2a	29.3a	5.8b	9.1abc	556.0a	1230abc	2878.7a	78.4b	299d	27.9cde
ICARDA	70.00a	8.08bcd	7.75a	3.6ab	123.1d	59.1a	1.26d	9.45c	234.7g	7.0bc	7.19c	2.4abc	283.9ef	28.5a	6.6a	8.5bc	265.5ef	1179abc	1217.7f	83.9a	1117b	1.1G
Chighl	59.47c	8.47abc	7.25a	3.4ab	138.2abc	55.7ab	1.59ab	17.20b	690.5bcd	6.8cd	9.39ab	2.67bc	511.0ab	29.1a	4.2de	10.0a	523.9ab	1345.3a	2673ab	62.9de	1803a	27.9cde
Tigray	52.58ed	8.21abcd	6.23b	2.9cd	133.4c	47.2cd	1.55abc	17.65b	504.3ef	6.4de	11.20ab	2.58c	3234 ef	26.5b	4.0efd	7.0d	329.2de	947.5d	1732e	54.9g	796cbd	37.1b
Wollega	64.40b	8.51abc	7.40a	3.6ab	141.1a	54.7ab	1.74a	22.72a	363.4fg	6.1e	10.9abc	2.60c	368cde	28.7a	3.4ef	7.9cd	410.1cd	1053cd	1819de	46.3h	474cbd	23.1f
Wollo	53.22ed	7.38d	6.03b	2.8d	132.5c	44.1d	1.33cd	16.93b	371.3fg	6.2e	10.2abc	2.59c	246.7f	26.2b	3.3f	5.3e	231.8f	694.3e	1254f	46.1h	1098cb	42.4a
Summary statistics over all genotypes																						
Min	51.33	6.45	5.44	2.5	112.2	41.9	1.13	7.1	123	5.3	4.35	2.19	129.1	22.8	2.5	4.04	146.1	542	658	38.07	216	1
Max	71.33	10.27	8.2	4.2	150.2	65.1	1.99	25.3	1115	8.77	12.57	3.04	820	31.83	9.49	14.76	883.3	2017	4388	112.71	8676	45.8
Mean	56.58	8.39	7.23	3.4	136.4	51.4	1.54	16.93	627.56	6.81	9.96	2.67	439.95	28.68	4.61	8.42	438.92	1132.5	2248.96	64.29	707.8	27.7
SE	0.84	0.11	0.1	0.1	1.7	0.9	0.03	0.55	37.7	0.09	0.22	0.02	21.63	0.27	0.21	0.3	22.6	40.88	116.94	2.31	171.4	1.3
Var	34.86	0.66	0.48	0.2	69.5	37.2	0.03	15.02	71059.3	0.45	2.35	0.03	23382.5	3.65	2.12	4.59	25535.1	83554.1	683760	266.21	1.47E+05	85.8
SD	5.9	0.81	0.69	0.4	8.3	6.1	0.18	3.88	266.57	0.67	1.53	0.16	152.91	1.91	1.45	2.14	159.8	289.06	826.9	16.32	1211.81	9.3

Means with the same letter in each columns are not significantly different ($p<0.05$)

Where: NDTF=Number of days to 50% flowering; SD=Stem diameter (cm), LL= Leaf length (mm); LW= Leaf width (mm); PH= Plant height; HFP=High to the first pod (cm); NPPN= Number of pod per node; NPPP= Number of pods per plan; GPF= Grain production efficacy; PL= Pod length; NNWP= Number of Node with pod; NSPP= Number of seeds per pod; PW= Pod weight (gm); NNPP= Number of node per plant; SB= Seed breath (mm);AGBP= Above ground biomass production rate; EGR=Economic growth rate; BM=Total above ground biomass; GY= Grain yield (kg/ha); HSW=Hundred seed weight (gm); HI= Harvest index; CHDS= Chocolate spot Disease Severity (%); N=number of genotypes; Min=Minimum; Max=Maximum; SE=Standard error; Var=Variance; SD=Standard deviation; NShewa= North Shewa; Chighl= Central highland.

Table 4.4 The variance components of faba bean genotypes for selected traits over eleven collection regions/ source and four altitude classes

Region/Altitude class	NDTF	SD	LL	LW	PH	HFP	NPPP	PL	NNWP	NSPP	Pwt	NNPP	SB	BM	GY	HSW	CHDS
Reagion/source of genotypes																	
Arsi	6.79	0	0.04	0.04	31.21	5.1	6.94*	0	0.73	0.02**	9119.6	1.11	0.52	43961	41847**	144.91*	5.39
Gojjam	72.5**	0.03	0.12	0.03	8.28	70.7	1.68	0.5**	0	0.01	11436*	0	3.24	5000	312877*	135.5**	26.37*
Gonder	0	0.36	0.03	0	50.49	2.36	3.41	0	0	0	699.8	0	0.31	0	48475.5	76.97**	7.61
Harar	0	1.44	0	0.04	74.0**	0	0.93	0.1	0.03	4E-04	9291.2*	0	0.61	61167**	233713*	90.45**	16.18*
North Shewa	1.72	0.18	0.16	0.07	0	18.3	3.48	0.3**	0	0	14174**	0.63	0.15	64228**	397339**	117.92**	34.99**
HARC	12.0**	0.12	0.22**	0.08	19.68	0	2.04	0.6**	1.39	0.02	9368.2	0.55	2.43**	39027**	175959	255.33**	11.43
ICARDA	0	0.11	0.08	0.01	17.23	0	1.39	0.2**	0	0.02	2809.7	0.22	6.17**	20189	12965	631.12**	0
Central highland	29.6**	0.26*	0.36	0.08*	175.8**	10.5	14.4**	0	6.0**	0	50962**	2.70**	0.53*	204796**	1651943**	26.34*	17.26
Tigray	0	0	0.27	0.05	0	0	0	0.5**	0	0	4530.7	0	1.30*	0	50595.6*	151.33**	10.07
Wollega	0.6	0	0	0.02	29.48	12.5	6.91	0.2	0.96	0.007	0	0.85	0.05	1916.7	111823	31.03**	1.49
Wollo	0	0	0	0	22.33	0	2.48	0.3	0	0.012	0	3.52*	0.35	17500	0	43.78*	0
Altitude class for the source of the genotypes																	
I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
II	25.6**	0.24*	0	0.05	34.8**	9.78*	5.43**	0.48**	0.41	0.009*	16079**	1.013	1.59**	80647**	544739**	203.95**	10.75*
III	28.4**	0.74	0.22*	0.06	33.7**	14.7	6.78**	0.31**	1.86**	0	12116**	0	1.50**	29362**	375553**	229.48**	28.25**
IV	7.44**	0	0.22	0.07*	17.54	9.53	1.45	0.42**	0.59	0.003	13910**	0.31*	1.97**	50531**	422297**	213.36**	37.99**

Where: NDTF=Number of days to 50% flowering; SD=Stem diameter (cm), LL= Leaf length (mm); LW= Leaf width (mm); PH= Plant height; HFP=High to the first pod (cm); NPPN= Number of pod per node; NPPP= Number of pods per plan; PL= Pod length; NNWP= Number of Node with pod; NSPP= Number of seeds per pod; Pwt= Pod weight (gm); NNPP= Number of node per plant; SB= Seed breath (mm); BM=Total above ground biomass; GY= Grain yield (kg/ha); HSW=Hundred seed weight (gm); HI= Harvest index; CHDS= Chocolate spot Disease Severity (%)

Associations between traits

There was significant ($p<0.01$) positive correlation of grain yield with pod weight, total biomass, plant height, number of node per plant, number of pod per plant, number of node with pod, number of seed per pod (Table 4.5). There was significant negative correlation between yield and disease also with height of the first pod. There was also highly significant ($P<0.001$) positive correlation of hundred seed weight with pod length and seed breadth. Similarly highly significant ($P<0.001$) negative correlation was recorded for disease severity with number of pod per plant, seed breadth, pod weight, and grain yield.

Table 4.5 Phenotypic correlation coefficients (above diagonal) and the level of significance (below diagonal) among faba bean traits (n=50)

	NBB	SD	LL	LW	PH	GFP	HFP	NPPN	NND	GPF	PL	NPPP	NNWP	NSPP	PWt	NNPP	SL	SW	SB	AGBP	EGR	BM	GY	HSW	HI	DS
NBB	1	-0.01	-0.16	-0.17	-0.48	-0.42	-0.39	-0.39	-0.08	-0.39	-0.09	-0.42	-0.39	-0.34	-0.36	-0.43	-0.31	-0.15	-0.17	0.09	0.22	0.00	-0.37	-0.11	0.21	0.46
SD	0.8436	1	0.21	0.22	0.26	0.02	0.16	0.16	-0.02	0.27	0.21	0.19	0.21	0.22	0.28	0.13	0.04	0.11	0.20	0.31	0.14	0.32	0.29	0.21	-0.23	-0.06
LL	0.0032	0.00	1	0.93	0.34	0.21	0.34	0.34	0.05	0.41	0.27	0.25	0.26	0.19	0.40	0.56	0.21	0.10	0.22	0.32	0.08	0.35	0.42	0.28	-0.41	-0.32
LW	0.0032	0.00	<.0001	1	0.33	0.19	0.32	0.32	0.06	0.40	0.27	0.24	0.25	0.19	0.40	0.53	0.17	0.09	0.23	0.33	0.09	0.35	0.42	0.26	-0.37	-0.31
PH	<.0001	<.0001	<.0001	<.0001	1	0.42	0.60	0.60	0.06	0.64	0.21	0.65	0.60	0.50	0.65	0.69	0.38	0.25	0.22	0.33	-0.23	0.41	0.64	0.14	-0.34	-0.40
GFP	<.0001	0.73	0.00	0.00	<.0001	1	0.31	0.31	0.02	0.58	0.23	0.35	0.33	0.33	0.44	0.46	0.22	0.15	0.22	0.06	-0.38	0.15	0.41	0.24	-0.22	-0.29
HFP	0.1724	0.00	0.68	0.60	0.02	0.01	1	-0.17	-0.06	-0.20	-0.10	-0.22	-0.29	-0.30	-0.19	-0.17	-0.14	-0.14	-0.05	0.00	0.14	-0.02	-0.19	0.02	0.04	0.00
NPPN	<.0001	0.01	<.0001	<.0001	<.0001	<.0001	0.0026	1	0.03	0.54	0.14	0.62	0.55	0.40	0.56	0.64	0.50	0.15	0.06	0.28	-0.24	0.36	0.57	0.03	-0.26	-0.35
NND	0.1821	0.68	0.41	0.27	0.32	0.73	0.31	0.61	1	0.07	-0.02	0.06	0.11	0.02	0.08	0.08	0.05	0.01	-0.01	0.08	-0.04	0.09	0.08	-0.02	-0.02	0.06
GPF	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.00	<.0001	0.20	1	0.41	0.57	0.56	0.49	0.94	0.63	0.19	0.22	0.33	0.59	-0.10	0.66	0.98	0.36	-0.31	-0.40
PL	0.1252	0.00	<.0001	<.0001	0.00	<.0001	0.09	0.01	0.75	<.0001	1	0.05	0.13	0.26	0.40	0.38	0.06	0.19	0.48	0.31	0.40	0.33	0.40	0.62	-0.19	-0.23
NPPP	<.0001	0.00	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.32	<.0001	0.43	1	0.78	0.52	0.59	0.57	0.41	0.22	0.12	0.26	-0.42	0.35	0.59	-0.14	-0.25	-0.36
NNWP	<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0674	<.0001	0.02	<.0001	1	0.48	0.56	0.55	0.30	0.15	0.09	0.24	-0.32	0.32	0.57	-0.03	-0.33	-0.29
NSPP	<.0001	0.0001	0.0011	0.001	<.0001	<.0001	<.0001	<.0001	0.7199	<.0001	<.0001	<.0001	<.0001	1	0.49	0.45	0.26	0.23	0.19	0.20	-0.19	0.26	0.49	0.11	-0.41	-0.25
PWt	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0013	<.0001	0.1842	<.0001	<.0001	<.0001	<.0001	<.0001	1	0.63	0.19	0.21	0.32	0.66	-0.04	0.73	0.96	0.34	-0.30	-0.42
NNPP	<.0001	0.0201	<.0001	<.0001	<.0001	<.0001	0.0036	<.0001	0.1704	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1	0.36	0.17	0.27	0.32	-0.10	0.40	0.63	0.29	-0.43	-0.50
SL	<.0001	0.443	0.0003	0.0024	<.0001	0.0002	0.0167	<.0001	0.3567	0.0013	0.32	<.0001	<.0001	<.0001	0.00	<.0001	1	0.12	-0.10	0.04	-0.23	0.09	0.19	-0.08	-0.12	-0.14
SW	0.0096	0.0657	0.0819	0.1126	<.0001	0.0074	0.0181	0.0099	0.8321	0.0002	0.00	0.00	0.01	<.0001	0.00	0.00	0.04	1	0.24	0.12	-0.03	0.15	0.21	0.10	-0.10	-0.03
SB	0.0033	0.0005	0.0002	<.0001	<.0001	<.0001	0.377	0.2657	0.8252	<.0001	<.0001	0.04	0.12	0.00	<.0001	<.0001	0.09	<.0001	1	0.26	0.48	0.29	0.31	0.67	-0.07	-0.33
AGBP	0.1227	<.0001	<.0001	<.0001	<.0001	0.3328	0.9778	<.0001	0.1606	<.0001	<.0001	<.0001	<.0001	0.00	<.0001	<.0001	0.46	0.04	<.0001	1	0.26	0.99	0.64	0.33	-0.07	-0.17
EGR	<.0001	0.014	0.1722	0.1338	<.0001	<.0001	0.0127	<.0001	0.5015	0.0772	<.0001	<.0001	<.0001	0.00	0.44	0.09	<.0001	0.55	<.0001	<.0001	1	0.19	-0.05	0.77	0.15	-0.04
BM	0.9738	<.0001	<.0001	<.0001	<.0001	0.0087	0.6834	<.0001	0.1342	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.11	0.01	<.0001	<.0001	0.00	1	0.71	0.34	-0.10	-0.24
GY	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0012	<.0001	0.1783	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.00	0.00	<.0001	<.0001	0.40	<.0001	1	0.33	-0.32	-0.40
HSW	0.0477	0.0002	<.0001	<.0001	0.0156	<.0001	0.7065	0.6533	0.7117	<.0001	<.0001	0.0123	0.6252	0.0531	<.0001	<.0001	0.1816	0.0881	<.0001	<.0001	<.0001	<.0001	<.0001	1	-0.09	-0.26
HI	0.0003	<.0001	<.0001	<.0001	<.0001	0.0002	0.4719	<.0001	0.7092	<.0001	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	0.0344	0.0872	0.2587	0.2232	0.01	0.09	<.0001	0.10	1	0.16
DS	<.0001	0.3219	<.0001	<.0001	<.0001	<.0001	0.9623	<.0001	0.3364	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0166	0.5483	<.0001	0.0039	0.51	<.0001	<.0001	<.0001	0.01	1

Where: The color indicates the strength of correlation, Blue=positively correlated Dark blue to light= high to week correlation; Red color=negatively correlated and the red to light red high to weak correlation

NNB= Number of basal branches per plant; NDTM= Number of days to physiological maturity; NDTF=Number of days to 50% flowering; GFP= Grain filling period; SD=Stem diameter (cm), NDTV= Number of days of vegetative growth; LL= Leaf length (mm); LW= Leaf width (mm); PH= Plant height; HFP=High to the first pod (cm); NPPN= Number of pod per node; NPPP= Number of pods per plan; NND= Number of nodules per plant GPF= Grain production efficacy; PL= Pod length; NNWP= Number of Node with pod; NSPP= Number of seeds per pod; PW= Pod weight (gm); NNPP= Number of node per plant; SL=Seed Length; SW=Seed width; SB= Seed breadth (mm);AGBP= Above ground biomass production rate; EGR=Economic growth rate; BM=Total above ground biomass; GY= Grain yield (kg/ha); HSW=Hundred seed weight (gm); HI= Harvest index; DS= Disease Severity (%)

Contribution of morphological characters towards divergence of the genotypes and Shannon-Weaver diversity index (H) values for the 29 traits studied are presented in Table 4.6. The first 6 principal component (CPC)having eigen values above 1 with a value of 80.92% cumulative variance among the genotypes for the 29 quantitative traits of which 52.03% was contributed by the first two PCs. The high degree of variation in the first six PCs indicates a high degree of variation in these characters. Of the 6 major components, only 5 were able to demonstrate existence of morphological variability in the faba bean genotypes. The first principal component (PC1) had 9.6 of the original variables and contributed 33.1% to the total observed variability; PC2 had 5.49 and contributed 18.92% of the total variation (Figure 4.1). In PC1, there were 13 morphological traits that contributed to the variability, 8 morphological traits in PC2, 4 traits in PC3 and only 1 each in PC4 (days to physiological maturity) and PC6 (number of basal branch). The first PC contrasted and averaged 18 out of the 29 traits. The traits; grain yield, harvest index, total above ground biomass, economic growth rate, above ground biomass production rate, pod weight, number of seeds per pod, grain production efficacy, number of pod per node, plant height, leaf width, leaf length and stem diameter gave high weight and were the most important contributing traits for the first PC. Hence the first PC was mostly for yield components. The second PC, accounted for 18.92% variability and related with the most predominant characters of number of pods per plant, disease severity number of node per plant, hundred seed weight, number of nodes with pods, pod length, seed breadth and pod length. The most predominant traits for the third PC were number of days to 50% flowering, grain filling period, number of days of vegetative growth and height to the first pod. The Shannon-Weaver diversity index (H') value for the traits ranged from 3.85 - 7.15, with a mean of 4.18.

Table 4.6 Percentage and cumulative variances and eigen-vectors of the first six principal components axes (CPC) and Shannon-Weaver diversity index (H') estimates for the 29 morphological character used to classify the faba bean germplasm evaluated in 2012

Parameter	PC1	PC2	PC3	PC4	PC5	PC6	H'
Number of basal branches per plant	0.04	-0.09	-0.27	0.00	0.60	-0.66	7.15
Number of days to physiological maturity	-0.26	0.34	-0.02	-0.73	-0.26	-0.11	3.92
Number of days to 50% flowering	0.33	0.49	-0.76	-0.05	-0.02	0.06	3.93
Grain filling period	-0.46	-0.24	0.69	-0.40	-0.14	-0.12	3.92
Stem diameter	-0.68	0.01	-0.18	0.36	0.04	0.04	4.08
Number of days of vegetative growth	0.33	0.49	-0.76	-0.05	-0.02	0.06	3.93
Leaf length	-0.64	0.35	-0.32	0.02	-0.32	-0.40	4.15
Leaf width	-0.65	0.30	-0.36	0.04	-0.27	-0.41	4.44
Plant height	-0.71	-0.37	-0.25	0.19	-0.11	0.07	3.92
High to the first pod	-0.03	0.39	-0.56	-0.25	-0.10	-0.14	3.93
Number of pod per node	-0.50	-0.43	-0.48	-0.13	0.07	0.13	5.34
Number of pods per plant	-0.23	-0.77	-0.52	0.11	0.01	-0.05	3.96
Number of nodules per plant	-0.26	0.30	-0.11	-0.54	0.12	0.22	3.90
Grain production efficacy	-0.92	-0.09	0.28	-0.19	0.06	0.00	3.82
Pod length	-0.56	0.51	0.38	0.33	0.08	-0.03	4.15
Number of Node with pod	-0.44	-0.67	-0.15	0.06	0.03	0.08	4.04
Number of seeds per pod	-0.57	-0.39	0.24	0.20	0.00	-0.09	4.80
Pod weight	-0.97	0.00	-0.01	-0.10	0.10	0.09	3.85
Number of node per plant	-0.24	-0.75	-0.53	0.09	0.00	-0.07	3.96
Seed Length	0.29	-0.51	-0.28	0.09	-0.48	0.08	4.23
Seed width	-0.35	-0.20	0.11	0.37	-0.38	-0.05	4.23
Seed breath	-0.32	0.72	0.23	0.44	0.03	-0.03	4.17
Above ground biomass production rate	-0.84	0.18	-0.31	0.01	0.18	0.20	4.06
Economic growth rate	-0.95	0.00	-0.12	-0.05	0.08	0.12	3.85

Parameter	PC1	PC2	PC3	PC4	PC5	PC6	H'
Total above ground biomass	-0.84	0.19	-0.32	-0.04	0.16	0.18	3.88
Grain yield	-0.97	-0.03	0.02	-0.14	0.08	0.08	3.85
Hundred seed weight	-0.47	0.75	0.31	0.22	-0.02	0.03	3.90
Harvest index	-0.63	-0.19	0.43	-0.24	-0.17	-0.16	3.93
Disease Severity	0.19	-0.77	-0.37	0.05	0.12	-0.04	3.93
Eigen value	9.60	5.49	4.18	1.94	1.19	1.07	
%Variance	33.10	18.92	14.41	6.70	4.10	3.68	
Cumulative%	33.10	52.02	66.43	73.13	77.23	80.92	
Mean diversity index (H)							4.18

Cluster Analysis of faba bean quantitative traits

All the genotypes were grouped into three major clusters (I, II, and III). The first major cluster had two sub clusters (I-1 and I-2) and the second major cluster had 4 sub-clusters (Figure 4.1 and 4.2). The number of genotypes per cluster varied from 35 in cluster II to 4 in cluster III. All improved faba bean varieties released from HARC were grouped in cluster II. The third cluster comprised all exotic faba bean germplasm from ICARDA and one faba bean collection from Central highlands of Ethiopia (FBColl-36). The 10 traits which explained the relationships among the genotypes are presented in Table 4.7. The distribution of the genotypes by region of origin and altitude class is presented in Table 4.8. The genotypes with similar traits were grouped together irrespective of the collection region and all collections from Wollega were grouped in cluster II but separated in sub-cluster II-1. Similarly, faba bean collections from Wollo were grouped in cluster I. The majority (75%) of the landrace collections from Tigray were grouped in the first cluster. Cluster II included 100% of the genotypes collected from Arsi, Gojjam, Gonder, North Shewa, Wollega, 75% from Harar, 42.86% from Central highland, and 25% from Tigray.

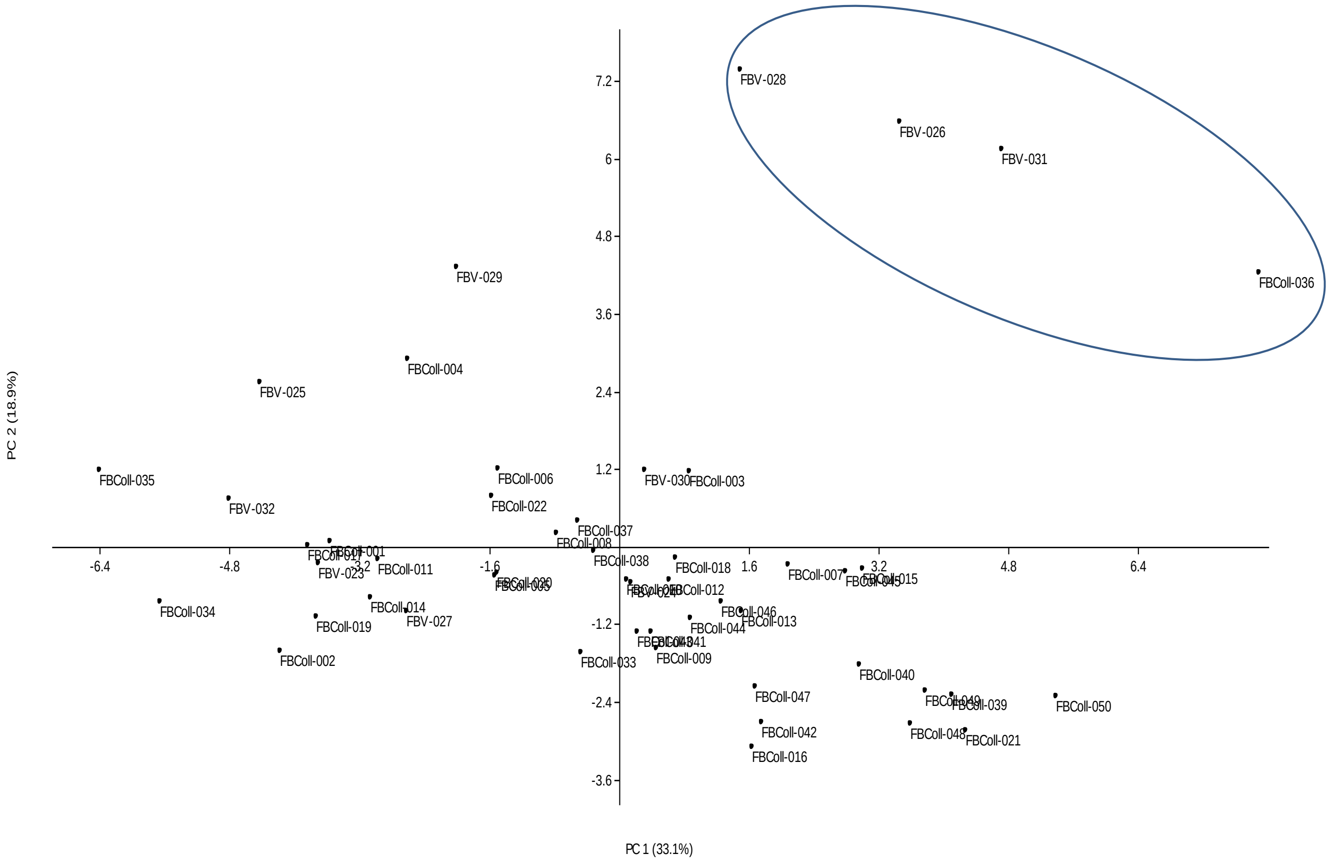

Figure 4.1 Scatter distribution of 50 faba bean genotypes based on 29 morphological characters on the first and second principal components.

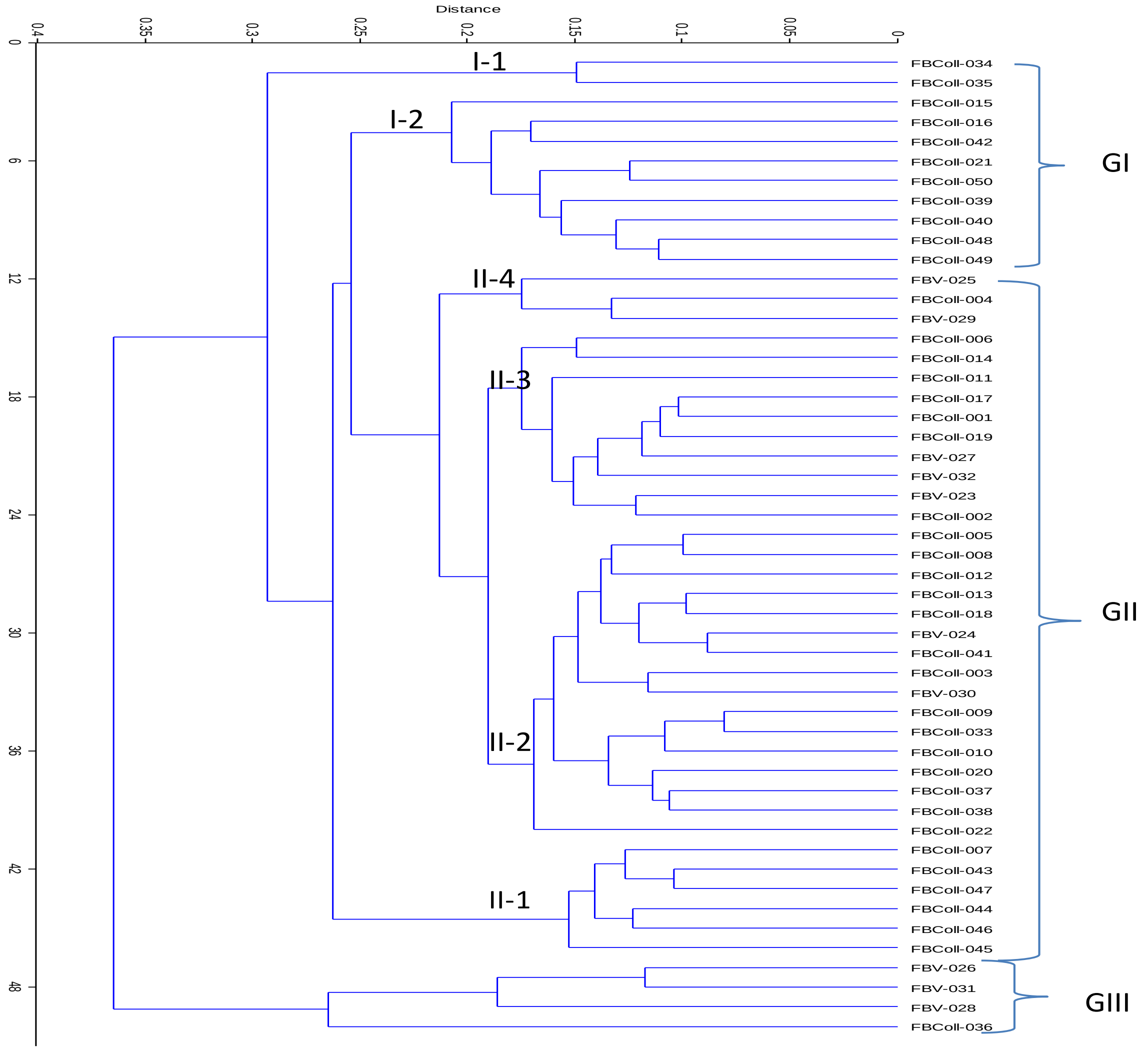

Figure 4.2 Dendrogram based on 29 phenotypic traits of 50 faba bean genotypes evaluated at HARC and KARC in 2012.

Table 4.7 Step-wise regression for important morphological traits for clustering 50 faba bean lines

Step	Trait	Partial R^2	F-value	Pr>F
1	Number of days to 50% flowering	0.744	11.06	<0.0001
2	Disease Severity (%)	0.585	5.22	<0.0001
3	Grain yield (kg/ha)	0.565	4.67	0.0003
4	Number of pods per plant	0.523	3.83	0.0015
5	Number of basal branches per plant	0.409	2.35	0.0311
6	Harvest index	0.496	3.25	0.0051
7	Number of seeds per pod	0.397	2.10	0.0542
8	Pod length	0.399	2.05	0.0612
9	Total above ground biomass	0.464	2.60	0.0212
10	Seed width	0.392	1.87	0.0922

Table 4.8 The distribution of 50 faba bean genotypes into three clusters and six sub-cluster by eleven regions and four altitude classes.

Region/Altitudes class	No of Genotypes	Number of Genotype by cluster			Number of genotypes by sub-cluster for Cluster I and II					
Region		I	II	III	I-1	I-2	II-1	II-2	II-3	II-4
Arsi	5	-	5	-	-	-	-	2	2	1
Gojjam	3	-	3	-	-	-	1	1	1	-
Gonder	3	-	3	-	-	-	-	2	1	-
Harar	5	1	4	-	-	1	-	2	1	-
North Shewa	6	2	4	-	-	2	-	3	2	-
HARC	7	-	7	-	-	-	-	2	3	2
ICARDA	3	-	-	3	2	-	-	-	-	-
Central highland	6	2	3	1	-	-	-	3	-	-
Tigray	4	3	1	-	-	3	-	1	-	-
Wollega	5	-	5	-	-	-	5	-	-	-
Wollo	3	3	-	-	-	3	-	-	-	-
Total	50	11	35	4	2	9	6	16	10	3
Altitude class										
I		1	-	-	-	1	-	-	-	-
II		3	17	-	2	1	5	7	4	1
III		6	10	1	-	6	1	6	2	1
IV		4	8	-	-	4	-	3	4	1
Total		14	35	1	2	12	6	16	10	3

4.4 Discussion

In this study significant variation was exhibited among the genotypes for 22 phenotypic traits including yield and yield components suggesting that there is genetic variability. Moreover, there were strong correlations among different traits which allows for simultaneous selection and use of the related traits interchangeably in parent material selection. These 22 descriptors were considered and would be used for further characterization of faba bean germplasm. The results are consistent with previous investigations in faba bean. Keneni et al. (2009) reported significant differences among Ethiopian faba bean accessions for most of the morphological traits. Similar phenotypic variation was observed by Al-Barri and Shataya (2013) on faba bean landraces from Palestine. The positive correlation of grain yield and hundred seed weight with other traits can be used to determine the yield potential. This was also observed in previous studies (Mohsin et al., 2009). The recorded associations among the phenotypic traits have implications for breeding of faba bean. That is, the positive or negative strongly correlated traits are possibly under the influence of the same genes or pleiotropic effects (Miko, 2008). Therefore, if two strongly correlated traits are desired, they can both be selected simultaneously basing on one of the traits (Kwon and Torrie, 1964).

In this study high morphological variation for regions and different altitude classes from where the germplasm was collected based on quantitative characters detected, suggests that the morphological variation in Ethiopian faba bean germplasm is strongly affected by environmental factors. The results are in agreement with previous studies on other crops. Depending on the elevation at which a species occurs, variability of genetic diversity due to several factors was found along altitudinal gradients (Ohsawa and Ide, 2008). Significant effects of altitude were reported on the genetic differentiation of oat (*Avena sativa* L.) landraces (Boczkowska and Tarczyk, 2013). This study suggested that to exploit the potential of genetic variation breeders can focus within an altitude range of 2000-3000 m a. s. l.

Principal component analysis (PCA) results illustrated the pattern of genetic diversity of the faba bean germplasm based on morphology. The principal component analysis confirmed the existence of diversity in faba bean germplasm since the entire variation cannot be explained in terms of few PCs. Moreover, PCs indicate the involvement of number of traits in contributing towards the overall observed diversity. In this study the PCA separated variability among the genotypes according to grain yield and 10 associated traits in Table 4.7. This suggests that the 10 traits are the most important for use in future faba bean characterization and conservation studies.

The Shannon-Weaver diversity index (H') for the characters showed high levels of diversity among the faba bean genotypes. The overall mean of H' value 4.18 confirmed the existence of high level of phenotypic diversity among faba bean genotypes (Hennink and Zeven, 1991). Clustering of the genotypes based on the morphological traits evaluated revealed some of the genotypes from the regions; Central highland, North shewa, Tigray and Harar, appeared in different clusters suggesting genotypes from these regions were relatively diverse than from the other regions. This could be partly attributed to gene flow through farmer to farmer seed exchange, which was previously reported (Alemu et al., 2010).

4.5 Conclusion

There is a broad genetic diversity of faba bean germplasm in Ethiopia. The germplasm clustered into three major groups which can be exploited by the breeding programmes that aim to improve varieties for the highland ecologies. Moreover, there were strong correlations among different traits which allows for simultaneous selection and use of the related traits interchangeably in parent material selection. This highly diversified germplasm provide opportunity for identification of parental sources in future breeding programmes to develop new faba bean varieties. The 10 traits which should be used for faba bean variety characterisation were identified.

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Chapter 5

Genetic variability of faba bean genotypes for chocolate spot (*Botrytis fabae*) resistance, yield

Abstract

Faba bean (*Vicia faba* L.) has high utility as a food and soil fertility improving crop. However, diseases such as chocolate spot (*Botrytis fabae*) compromise its productivity in the smallholder farming sector, because of limited technology options. Therefore, it was prudent to evaluate 60 faba bean landraces for chocolate spot resistance. The landraces were evaluated both in the field and in the greenhouse under natural and artificial inoculation with the selected aggressive *Botrytis fabae* isolate (Iso-016) from West Gojjam, in Ethiopia. There were highly significant differences ($p<0.001$) among the landraces for reaction to the disease and agronomic traits. Significant positive correlation was recorded between reaction of genotypes in the field and greenhouse disease data. The overall mean disease epidemics varied from 92.5 to 697.5 for the area under disease progress curve (AUDPC).The highest level of resistance was found in the ICARDA lines, ILB-4726, ILB-938 and BPL-710. At least 18 landrace collections displayed significantly lower disease reaction than the susceptible check. However the resistance was moderate. The following landraces will be recommended for use in breeding for chocolate resistance: FBColl-0030, FBColl-0036, FBColl-0003, FBColl-0001, FBColl-0012, FBColl-0024, FBColl-0055, FBColl-18, FBColl-0035, FBColl-0011, FBColl-0057, FBColl-0002, FBColl-0042, FBColl-31, FBColl-0034, FBColl-0008, FBColl-0039 and FBColl-0032. Overall, resistance was highly heritable, suggesting that phenotypic selection can be exploited to improve chocolate spot resistance in faba bean varieties.

Key words: Aggressiveness; *Botrytis fabae*; faba bean; landrace; resistance.

5.1 Introduction

Ethiopia is the leading producer of faba bean in Africa. The country accounts for 56% of the production of the crop in the continent (FAOSTAT, 2008). Faba bean is widely grown in the mid-altitude and highland areas of Ethiopia and serves as a multi-purpose crop leading the pulse category, both in area and production (CSA, 2009) in the smallholder sector. The crop provides an alternative source of protein and cash income to rural households. It is agronomically important, because it serves as a break crop in the cereal-based rotations to improve soil fertility. In this regard it has been found to reduce the need for artificial nitrogen fertilizers (Schmidtke and Rauber, 2000; Agegnehu et al., 2006), which are not readily accessible to the smallholder and resource-poor farmers in developing countries.

However, faba bean production is adversely affected by numerous fungal diseases, which vary both in incidence and severity from one region to another. Among them, the most relevant are chocolate spot (*Botrytis fabae*), rust (*Uromyces viciae-fabae*) and ascochyta blight (*Ascochyta fabae*). The chocolate spot, which is caused by *Botrytis fabae,* is one of the most yield destructive leaf diseases (Sundheim, 1973). *Botrytis fabae* can induce a total crop failure under heavy epidemic infestation. Yield losses due to chocolate spot can reach 100% especially when it occurs at the flowering stage (Stoddard et al., 2010). The disease affects all growth stages of the crop from seedling to pod filling, and all plant parts from leaf to stem and the seed. The yield is compromised through significant reduction of the photosynthetic capacity, increased transpiration and reduced accumulation of organic matter. Consequently, it reduces seed size and quality. Therefore a sustainable management strategy should be put in place.

Host plant resistance can be implemented as the core of an integrated strategy for managing chocolate spot. Previous investigators suggested integration of chemical with cultural practices, such as early planting (Sahile et al., 2008; Teshome and Tagegn, 2013) but this should be extended to include variety resistance. Although chemical control can provide good protection of varieties against the disease, it is in general difficult to sustain in the smallholder sector in developing countries. This is because it comes as an additional cost to improved seed, thus compromises profits and it is generally viewed as unfriendly to the environment (Robinson, 1996). The use of chemicals without proper training and storage facilities might also compromise safety of the farmers. Therefore, the best strategy for controlling faba bean chocolate spot is employment of resistant varieties (Bouhassan et al., 2004) However, utilisation of host plant resistance requires effective identification of potential sources of chocolate spot resistance genes in adapted materials, and use of suitable isolates of the causal organism for screening of germplasm.

Screening of the aggressiveness of *Botrytis fabae* isolates would be best conducted before their use in germplasm screening. This ensures that hypo virulent isolates are not used to screen germplasm. The aggressive isolates would discriminate the germplasm into clear resistant and susceptible categories (Reid et al., 1993). Existence of any interaction between faba bean genotypes and fungal isolates can influence the efficiency of breeding programmes. Therefore, understanding differential responses of faba bean genotypes to different *Botrytis fabae* isolates is useful for development of durable resistance and for plant breeding. This implies that breeders have to know which types of *Botrytis fabae* exist in the geographic areas they are targeting. The use of aggressive isolates in selection of germplasm increases heritability by reducing genotype by environment interactions (Carson et al., 2002). However, the lack of information on the interaction between resistance genes and pathogen populations places limitations on the effective development of resistance. The aim of this study was therefore to select an aggressive isolate of *Botrytis fabae* and evaluate the faba bean landraces for their response to the pathogen, in order to identify resistant genotypes for utilisation as sources of resistant genes in breeding programmes.

5.2 Materials and method

5.2.1 Description of study sites

Evaluation of sixty faba bean materials for their resistance to chocolate spot disease and yield potential was conducted at Holetta Agricultural Research Centre (HARC) (09º03’N, 38º30’E, and 2390 m a. s. l) and Bekoji research site (07º32’N, 039º16’E and 2803 m a. s. l) in 2012 main cropping season. The average maximum and minimum temperature at HARC in 2012 from June to December was 22.8ºC and 5.4ºC respectively and the average rainfall received was 786.8 mm. Similarly, the average maximum and minimum temperature at Bekoji was 19.9ºC and 13.5ºC, respectively, and the average rainfall received was 427.3 mm. The greenhouse experiment to screen the 60 genotypes under controlled conditions using an aggressive isolate of *Botrytis fabae* was conducted at Ambo Plant Protection Research Centre (APPRC) in Ethiopia. Laboratory isolation of the pathogen was undertaken at Holetta Agricultural Research Centre Molecular Microbiology laboratory.

5.2.2 Faba bean germplasm used

A total of 50 faba bean landraces were collected from major faba bean growing areas of Ethiopian highlands (Table 5.1). In addition, 10 improved varieties from the International Centre for Agricultural Research in the Dry Areas (ICARDA) and Holetta Agricultural Research Centre (HARC) pulse breeding program (Moti, ILB 938, NC 58, Dosha, ILB 4726, CS 20 DK, Kassa, BPL 710, Gebelcho, Bulga 70 and local variety), were included.

Table 5.1 Sources of faba bean landraces and improved varieties evaluated for chocolate spot resistance/ tolerance and yield potential at two locations in 2012.

No	Accession	Region	Woreda	Latitude	Longitude	Altitude
1	FBColl-0001	Hareri	Kulubbi	09º28’30”N	41º14’30”E	2380
2	FBColl-0002	Hareri	Arberkoti	09º03’11”N	40º54’39”E	2266
3	FBColl-0003	Hareri	Borda	Unknown	Unknown	2240
4	FBColl-0004	Hareri	Water	09º28’29”N	41º48’21”E	2136
5	FBColl-0005	Hareri	Gerawa	09º74’9”N	41º51’61”E	2288
6	FBColl-0006	Hareri	Karamile	Unknown	Unknown	Unknown
7	FBColl-0008	Amehara	Wadla	11º38’45”N	38º55’4”E	2930
8	FBColl-0009	Amehara	Wereilu	10º34’38”N	39º24’23”E	2640
9	FBColl-0010	Amehara	Jamma	10º21’30”N	39º18’30”E	2670
10	FBColl-0011	Amehara	Ambassel	12º73-91 N	55º65’44”E	2972
11	FBColl-0012	Tigray	Degua tembien	13º39' N	39º09' E	2770
12	FBColl-0013	Tigray	Sa'es'e' Tsaeda'	12º28' N	39º17' E	2547
13	FBColl-0015	Tigray	Atsiwemberta	12º28' N	39º17' E	2840
14	FBColl-0016	Tigray	Naedier	13º46' N	38º12' E	1855
15	FBColl-0017	Amehara	Debark	14º49’94”	37º73’75”	2739
16	FBColl-0018	Amehara	Wogera	14º10’98”	34º64’83”	2951
17	FBColl-0020	Amehara	Wogera	14º11’45”	34º79’31”	2961
18	FBColl-0023	Oromia	Limu Na Bilbilo	07º34-176 N	39º16’32”E	2853
19	FBColl-0024	Oromia	Arsi Robe	07º51’25”N	39º34’31”E	2410
20	FBColl-0025	Oromia	Honkolowabe	07º25’58”N	39º24’57”E	2784
21	FBColl-0026	Oromia	Limu Na Bilbilo	07º31’35”N	39º17’04”E	2989
22	FBColl-0027	Oromia	Tiyo	07º49’25”N	39º06’14”E	2476
23	FBColl-0029	Amahera	Menz Gera	10º18’38”N	39º39’31”E	3022
24	FBColl-0030	Oromia	Limu Na Bilbilo	07º26’55”N	39º15’05”E	2908
25	FBColl-0031	Oromia	Chole	08º13’49”N	39º55’03”E	3050
26	FBColl-0032	Amehara	Banja	10º38’N	36º56’E	2610
27	FBColl-0034	Amehara	HuletEju Enebsie	Unknown	Unknown	2412
28	FBColl-0035	Amehara	Yilmana Densa	11º5'N	37º25'E	2240
29	FBColl-0036	Amahera	Basona Worana	09º41’32”N	39º38’23”E	3012
30	FBColl-0037	Amahera	Tarma Bere	09º58’73”N	39º44’29”E	3058
31	FBColl-0038	Oromia	Tole	08º42’54”N	38º23’41”E	2127
32	FBColl-0039	Oromia	Dendi	09º01’24”N	38º18’48”E	2270
33	FBColl-0040	Amahera	Mortna Jiru	09º57’06”N	38º18’-48”E	2663

No	Accession	Region	Woreda	Latitude	Longitude	Altitude
34	FBColl-0041	Amahara	Menz Keya	10º30”N	39º50”E	2300
35	FBColl-0042	Amahara	Ensaro	09º46’10”N	38º54’47”E	2620
36	FBColl-0043	Amahara	Hageremariam	09º20”N	39º15”E	2250
37	FBColl-0045	Oromia	Ginbichu	08º55’35”N	39º07’25”E	2263
38	FBColl-0046	Oromia	Ejere	09º2’25”N	38º23’56”E	2280
39	FBColl-0047	Oromia	Jima Arjo	08º46’29”N	36º29’52”E	2361
40	FBColl-0049	Oromia	Tulu Bolo	08º39’43”N	38º12’51”E	2192
41	FBColl-0050	Amehara	Siya Deberna	09º15’08”N	39º15’04”E	2667
42	FBColl-0051	Oromia	Jima Arjo	08º44’51”N	36º30’18”E	2460
43	FBColl-0052	Oromia	Jima Arjo	08º47’11”N	36º28’49”E	2424
44	FBColl-0053	Oromia	Horo Guduru	09º34’11”N	37º07’42”E	2451
45	FBColl-0054	Oromia	Horo Guduru	09º34’16”N	37º07’40”E	2435
46	FBColl-0055	Oromia	Tulu Bolo	08º39’43”N	38º12’51”E	2192
47	FBColl-0057	Oromia	Horo-guduru	10º45’57”N	32º79’97”E	2296
48	FBColl-0059	Oromia	Chaliya	10º04’76”	32º13’50”E	2839
49	FBColl-0060	Oromia	Chaliya	09º91’72”E	33º30’91”E	2507
50	FB Coll-0061	Amehara	Ankober	Unknown	Unknown	3200
51	NC 58	Improved	HARC	Unknown	Unknown	2300-3000
52	Bulga -70	Improved	HARC	Unknown	Unknown	1900-2000
53	Moti	Improved	HARC	Unknown	Unknown	1800-3000
54	Dosha	Improved	HARC	Unknown	Unknown	Unknown
55	CS-20-DK	Improved	HARC	Unknown	Unknown	1900-2300
56	Kasa	Improved	HARC	Unknown	Unknown	Unknown
57	Gebelcho	Improved	HARC	Unknown	Unknown	1800-3000
58	ILB 4726	Improved	ICARDA	Unknown	Unknown	2300-3000
59	ILB 938	Improved	ICARDA	Unknown	Unknown	Unknown
60	BPL 710	Improved	ICARDA	Unknown	Unknown	1800-3000

HARC: Holetta Agricultural Research Centre; ICARDA: International Centre for Agricultural Research in the Dry Areas.

5.2.3 Isolation of Botrytis fabae procedure and inoculum preparation

Faba bean leaf samples naturally infected with *Botrytis fabae*- were collected from five major faba bean growing areas of Ethiopia: West Gojjam, East Gojjam, North Shewa, West Shewa and Arsi during the main growing season of 2011. The leaf samples were placed in a paper bag, air dried at room temperature and stored at 4ºC. Isolation was made from a small part of a necrotic leaf lesion of a leaflet from each sample. Leaves were surface sterilized with 0.5% sodium hypochlorite solution for 2 minutes and plated on autoclaved 9 cm diameter petri dishes containing faba bean dextrose agar medium [200 g faba bean seed (autoclaved in 1 litre distilled water and filtered to obtain faba infusion), 20 g dextrose and 18 g agar] incubated at 24ºC. From all the samples, 25 isolates were made and only 12 isolates from different locations were selected based on their performance and characteristics on culture media. Out of the 12, only four isolates, representing the four locations: Arsi (Isolate-011), North Shewa (Isolate-007), West Shewa (Isolate-002) and West Gojjam (Isolate-016), were randomly selected for further evaluation (Table 5.2).

Table 5.2 Description of locations for the 12 *Botrytis fabae* isolate samples collected and the incidence and severity in the field during the sampling.

No	Isolate	Region	Zone	Woreda	Altitude	Soil type	Incidence %	Severity %
1	Isolate-002	Oromia	West Shewa	Ejere	2390	Red	100	70
2	Isolate-004	Oromia	West Shewa	Wolmera	2480	Red	100	80
3	Isolate-005	Amehara	North Shewa	Chacha	2698	Black	100	80
4	Isolate-006	Amehara	North Shewa	Baso	2808	Black	100	80
5	Isolate-007	Amehara	North Shewa	Mendida	2757	Black	100	70
6	Isolate-008	Amehara	North Shewa	Deneba	2666	Black	100	30
7	Isolate-010	Oromia	West Shewa	Wolmera	2400	Red	100	80
8	Isolate-011	Oromia	Arsi	Kofelle	2675	Sandy loam	80	70
9	Isolate-012	Oromia	Arsi	Limubilbilo	2931	Black loam	40	70
10	Isolate-013	Oromia	Arsi	Limubilbilo	2629	Clay loam	60	60
11	Isolate-015	Amehara	East Gojjam	Gozamen	2750	Red	60	50
12	Isolate-016	Amehara	West Gojjam	Hodanshe	2400	Red	50	70

5.2.4 Botrytis fabae isolates screening in the greenhouse

Aggressiveness test of the selected isolates; Isolate-002, Isolate-007, Isolate-011 and Isolate-016 was made on eleven faba bean genotypes. The eleven genotypes were selected based on there different levels of resistance to chocolate spot (Moti, Gebelcho, Dosha (moderatly resistant) ILB 938, ILB 4726, BPL 710 (highly resistant) and CS 20 DK, Kassa, NC 58, Bulga 70 and local variety (susceptable)). Five seeds of faba bean from each test genotype were sown in sterilized soil in clay pots (15 x 20 cm). A randomized complete block design in a factorial arrangement (factors: pathogen isolate and genotype) was used with each treatment combination replicated three times. All genotypes were also planted without inoculation of the isolated as a control. The pots were maintained in a greenhouse (25ºC), watered as required and plants were thinned to three seedlings.

5.2.5 Inoculum preparation and inoculation

Spores of each isolates were mass produced from a single-conidium of *Botrytis fabae* on autoclaved chrysanthemum (*Chrysanthemum Sinense* Sabine) flower media (30 ml distilled water, 1 g of glucose and 4 g of dry chrysanthemum flower) (Beniwal and Gorfu, 1989). The number of spores per ml of suspension was adjusted using a haemocytometer (Bernard et al., 2006). The genotypes were artificially inoculated with a *Botrytis fabae* isolate with spore concentration of 2.5 x 10^5 spores ml^{-1} after one month of planting at the seedling stage

(Mohamammed et al., 1994). Tween-20 was mixed with the suspension to disperse the spores. Temperature and relative humidity in the greenhouse were adjusted to 24ºC and 90% respectively 2 days before inoculation. Inoculation was done using a hand sprayer late in the afternoon and the plants were covered with polyethylene plastic for 24 h to maintain high relative humidity for better infection by the pathogen (Enriquez, 1977).

5.2.6 Disease assessment

The severity of chocolate spot was recorded seven days after inoculation using Bernard et al. (2006) and Bernier et al. (1993) modified percentage leaf area infected scale as described in Table 5.3. Disease scoring was made for all genotypes three times, at seven day interval.

Table 5.3 Modified scale for scoring faba bean chocolate spot disease based on % leaf area infection on graphic paper for six different rates of infection

Infection rates	Description of the symptom
1% (Highly resistant)	no lesions or few small brown, non-sporulating specks, covering up to 1% of leaf surface
3% (Resistant)	few small, discrete, brown, circular, non-sporulating lesions (2–3mmin diameter) covering 2–3% of leaf surface
6% (Moderately resistant)	lesions common (3–5mm in diameter), some coalesced, covering 3–6% of leaf surface, with some defoliation and very poor sporulation;
12%(Moderately resistant)	Large coalesced irregular blackish sporulating lesions that cover 6–12% of leaf surface, average defoliation, flower drop, and some dead plants;
25% (Susceptible)	extensive large coalesced heavily sporulating lesions, covering 25% of the leaf surface, with severe defoliation, stem girdling, and death of great majority of plants
50% (Highly susceptible)	>25% of the leaf or pod surface area is covered by large sporulating and often coalescing lesions. Leaf tissues are generally chlorotic resulting in severe premature defoliation. Infected pods are often deformed and shriveled and contain a low number of seeds. Abundant sporulating lesions are present on stem and branches (90%)

5.2.7 Evaluation of faba bean genotypes using *Botrytis fabae* Isolate-016 in the greenhouse and in the field

Greenhouse evaluation

Five faba bean seeds per genotype were planted in plastic pots. The design was randomized complete block design (RCBD) with three replications. Spores of the aggressive isolate; Isolate-016 which was selected from section 5.2.3 was mass produced, adjusted to 2.5 x 10^5 conidia ml^{-1} and inoculated (Figure 5.1) using the same procedure described in section 5.2.5. Chocolate spot disease severity was assessed three times, seven days after inoculation using the modified percentage of leaf area infected as described in Table 5.3.

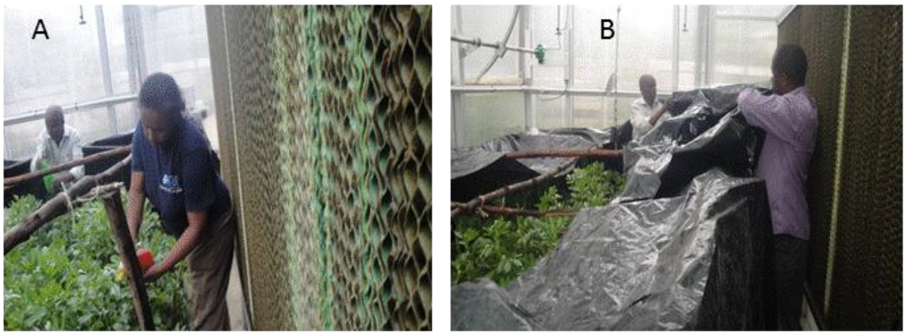
Field evaluation

Field evaluations were conducted for the 60 faba bean materials to determine their potential resistance/tolerance to chocolate spot disease and yield potential during the 2012 main cropping season. At the two sites (Holetta and Bekoji) the design was a 10 x 6 alpha lattice with three replications. Each genotype was planted on plot sizes of 2 m x 0.4 m and a common border row of the improved, '*Messay*' but less competent faba bean variety was used to avoid the inter-plot interference among the test genotypes. Diammonium phosphate (DAP) fertiliser at the rate of 100 kg ha^{-1} (Agegnehu and Fessehaie, 2006) was applied and other management practices were implemented as per recommendations at each site.

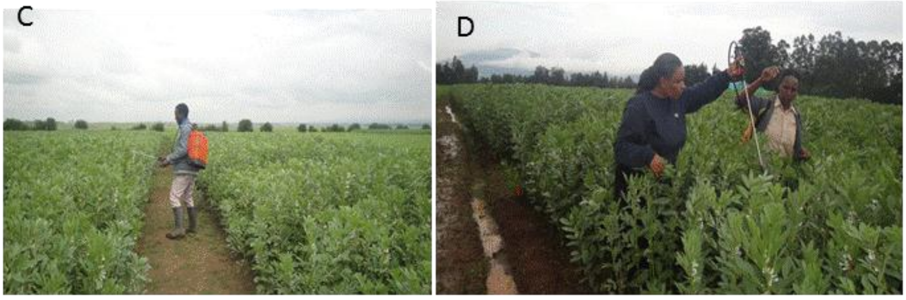
At each site in addition to the natural inoculum, the test genotypes were artificially inoculated with Isolate-016 as described in section 5.2.3. At flowering, the faba bean genotypes were sprayed with the suspension of *Botrytis fabae* using the pressurized hand sprayer at Holetta and Bekoji until runoff (Figure 5.1). Inoculation was done late in the afternoon to avoid the effects of sunlight on spore viability. Genotype reaction to *Botrytis fabae* was evaluated seven days after inoculation and disease severity scoring was taken five times at weekly intervals from the same five randomly pre-tagged faba bean plants per genotype. In addition a general disease assessment to determine the response of genotypes to chocolate spot was done from the whole plot twice; at pod initiation and grain filling stages. Severity on leaves was rated using the modified infected leaf area described in Table 5.3. These disease scores were summarised as indicated in Table 5.4.

Table 5.4 Scale of per cent disease severity for chocolate spot disease in faba bean

Per cent disease severity	Inference
0-3	Highly resistant
4-12	Resistant
13-20	Moderately resistant
21-49	Susceptible
>50	Highly susceptible



Artificial inoculation of chocolate spot inoculum in the green house



Artificial inoculation of chocolate spot inoculum in the field

Figure 5.1 Artificial inoculation of *Botrytis fabae* on faba bean in the greenhouse at APPRC (A and B) and in the field (C and D) at HARC and Bekoji

Agronomic data collected from field evaluation

Agronomic data were recorded on days to emergence, days to 50% flowering (DTF) and days to physiological maturity (DTM) for the entire plot. Fifteen plants per genotype that were randomly selected to record data on height from ground to the first pod (HFP), number of pods per node (PPN), number of pods per plant (PPP), number of nodes with pod (NWP), number of seeds per pod (SPP) and plant height (PH). Data on pod weight, total biomass (BM) at harvest, hundred seed weight (HSW) from randomly selected 100 seeds from each plot and grain yield was taken from the entire plot. Grain yield adjustment was made as seeds were weighed, oven dried and adjusted to constant moisture level of 10%. The total biomass and grain yield recorded on a plot basis were converted to t ha^{-1} for statistical analysis. Harvest index was calculated as: Harvest index (%) = (Grain yield/biological yield) x 100 (biological yield is total of grain yield and total biomass) (Reddy, 2004).

5.2.8 Data analysis

The disease severity data from the field and greenhouse experiments were subjected to statistical analysis using SAS 9.3 (SAS Institute, 2012), and the genotypes were evaluated based on their severity reaction to the disease. The data collected weekly in the field were used to calculate the area under the disease progress curve (AUDPC). The AUDPC

(Contreras-Medina et al., 2009) was calculated for each genotype using the disease severity score according to the following formula proposed by Shaner and Finney (1977)

$$AUDPC = \sum_{i=1}^{n} 1/2[(yi + 1 + yi)(xi + 1 - xi)]$$

Where: yi = the cumulative disease severity percentage of infected plants at the ith observation (day i), xi = time (days) at the ith observation, n = total number of symptom observations. AUDPC was calculated using Microsoft excel and graphs plotted for the different resistant reactions of the faba bean improved varieties. The estimate of AUDPC was normalized by dividing with the total area of the graph *(i.e.* the number of days between the first and the last readings multiplied by maximum potential AUDPC), for a better visual comparison among genotypes over location (Mohapatra et al., 2008). The normalized AUDPC was referred to as the relative area under disease progress curve (RAUDPC). All agronomic and disease data from each location were analysed using PROC GLM procedure in SAS 9.3 (SAS Institute, 2012). The data were subjected to ANOVA first by location with environment as main effect, and then a combined analysis across environments was made to analyse the effect of locations, genotypes and interaction. The correlation coefficients of selected traits were calculated using the SAS procedure PROC CORR (SAS Institute, 2012).

Variance components were calculated by equating the mean squares from the analysis of variance (ANOVA) estimates to the expectations of mean squares (EMS). Estimations were done on the combined environments using the following model (Singh et al., 1993): $Y_{ijk} = \mu + g_i + \alpha_k + \beta_{jk} + \delta_{ik} + \varepsilon_{ijk}$ where Y_{ij} is the response of i^{th} genotype grown in the j^{th} block (i = 1, 2, ….v), j^{th} block (j = 1, 2, ….b) over the k^{th} environment (k = 1, 2, ….l), μ = general mean, g_i= effect of the i^{th} genotype, α_k = effect of the k^{th} environment, δ_{ik} = interaction effect between the i^{th} genotype and the k^{th} environment and β_{jk} = effect of the j^{th} block within the k^{th} environment. The effectsg_i, δ_{ik} and ε_{ijk} are assumed to be independently and normally distributed with zero mean and variancesσ_g^2, σ_{gxe}^2 andσ_e^2.

Heritability was then estimated as $h^2 = \sigma_g^2/(\sigma_g^2 + \frac{\sigma_{\mathrm{gxe}}^2}{e} + \frac{\sigma_e^2}{re})$, where σ_g^2 is the genetic variance, σ_e^2 = environmental variance and σ_{gxe}^2 = variance of the genotype x environment interaction (Hallauer and Miranda, 1988).

5.3 Results

5.3.1 Botrytis fabae isolates selection in the greenhouse

Significant variation (P<0.001) for aggressivenes on the test genotypes was recorded among the isolates (Isolate-002, Isolate-007, Isolate-011 and Isolate-016) based on the chocolate spot disease score (Table 5.5).The highest disease score (49.6%) was recorded from infection by Isolate-016 followed by 44% disease severity by Isolate-007 (Table 5.6). There was significant difference (P<0.001) between the test genotypes and test genotype x isolate interaction.

Table 5.5 ANOVA for the evaluation of the aggresivnes of four *Botrytis fabae* isolate on eleven genotypes with different levels of resistance to chocolate spot disease

Source of variation	df	Chocolate spot mean squares	
		DI	DS
Block	4	21178.13***	50690.1***
Rep	2	561.30**	40.2 0
Isolate	4	21178.13***	9399.55***
Genotype	10	7130.87***	6271.31***
Block*Genotype	40	693.57***	604.15***
Isolate*Genotype	40	669.04***	604.78***
Error	105	179.69	147.01
Corrected total	161		
CV%		31.38	46.17
Mean		42.72	26.26
LSD		9.71	8.78
R^2		0.91	0.89

Note:;*** significant at p<0.001, ** significant at 5%, DI=disease incidence; DS=Disease severity.

Table 5.6 Average disease severity score of the four isolates and control

Test Isolate	**Mean**	
	DI (%)	DS (%)
Isolate-016	65.3_{a}	49.6_{a}
Isolate-007	61.7_{a}	44_{a}
Isolate-011	47.6_{b}	30.1_{b}
Isolate-002	44.5_{b}	21.7_{c}
Control	0_{c}	0_{d}

*Means with the same letter are not significantly different. DI=disease incidence, DS= disease severity.

Disease severity and incidence score values varied considerably among the test genotypes and statistically significant (P ≤ 0.001) genotypic differences were recorded (Table 5.7). The resistant varieties ILB-4726, ILB-938 and BPL-710 had mean diseases severity of < 3%, while the suceptable varieties NC-58, CS-20-DK, Kasa and local variety had mean disease score of >40%.

Table 5.7 Duncan's grouping of the eleven genotypes used for the aggressiveness test of the selected isolates

Test genotype	Mean DI (%)	Mean DS (%)	Inference
Moti	48.00_{cd}	18.13_{d}	MR
ILB 938	14.64_{f}	1.57_{e}	HR
NC58	66.67_{a}	55.00_{a}	HS
Dosha	41.33_{de}	18.73_{d}	MR
ILB 4726	5.33_{f}	0.47_{e}	HR
CS 20 DK	66.00_{a}	49.33_{ab}	S
Kasa	62.67_{ab}	46.67_{abc}	S
BPL 710	12.69_{f}	2.39_{e}	HR
Gebelcho	35.33_{e}	12.40_{d}	MR
Bulga 70	56.67_{abc}	38.00_{c}	S
Local	54.67_{bc}	41.33_{bc}	S

*Where HR=highly resistant, R= resistant, MR=moderately resistant, S=susceptible, DI=Disease incidence; DS=Disease severity.*Means with the same letter are not significantly different

There was variation on the mean disease severity score among isolate x test genotype combination for the chocolate spot disease. Isolate-016 caused considerable disease severity on most of the test genotypes. Highest (83%) disease severity score was recorded from the combination of Isolate-016 with suscseptible test genotype NC-58 (Table 5.8).

5.3.2 Evaluation of faba bean genotypes using Isolate-16 in the greenhouse and field

Greenhouse Evaluation

Analysis of variance for the disease severity of chocolate spot and area under disease progress curve are presented in Table 5.9. Highly significant variations ($P \leq 0.001$) were recorded among the faba bean genotypes.

Mean disease scores of chocolate spot ranged between 1.22% on ILB 4726 to 37.44% on faba bean landrace collection FBColl-0015 (Appendix 5.1). In this evaluation 10% of the genotypes had highly resistant to resistant reaction, 35% moderately resistant and 55% were susceptible for the disease (Figure 5.2). The AUDPC-GH value ranged from 16.33 (ILB 4726) to 539 (FCOll-0015) (Appendix 5.1).

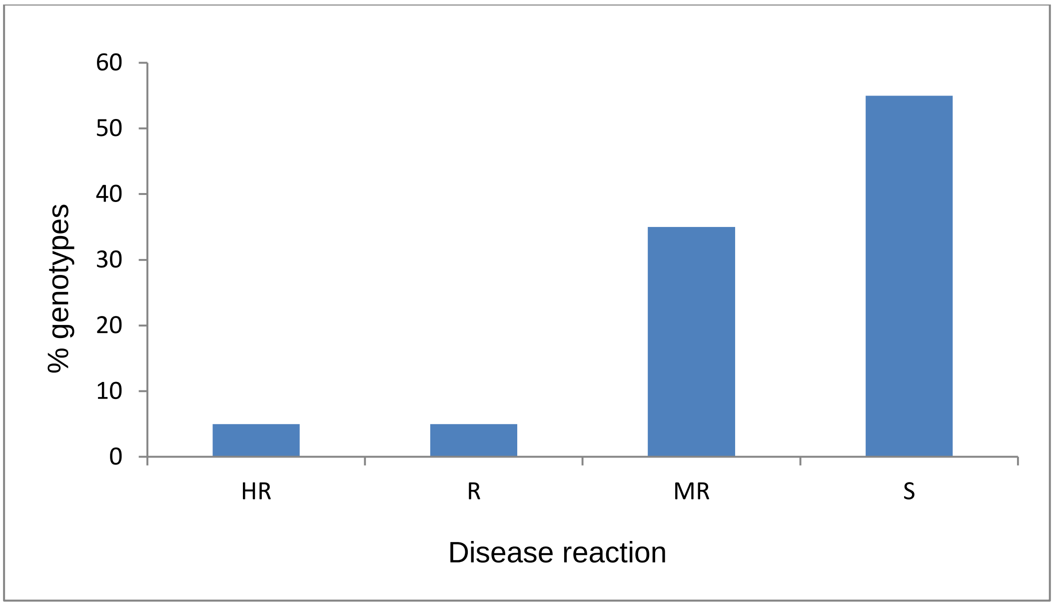
Table 5.8 Mean of disease severity of Isolate x test genotype interaction for *Botrytis fabae* isolates evaluated in the greenhouse 2012

Isolate	Test genotype	Mean disease severity (%)	Isolate	Test genotype	Mean disease severity (%)
Isolate-002	Moti	15.0	Isolate-011	Moti	23.3
Isolate-002	ILB 938	0.5	Isolate-011	ILB 938	1.0
Isolate-002	NC58	50.0	Isolate-011	NC58	60.0
Isolate-002	Dosha	13.7	Isolate-011	Dosha	20.3
Isolate-002	ILB 4726	0.0	Isolate-011	ILB 4726	0.3
Isolate-002	CS 20 DK	50.0	Isolate-011	CS 20 DK	53.3
Isolate-002	Kasa	23.3	Isolate-011	Kasa	66.7
Isolate-002	BPL 710	1.5	Isolate-011	BPL 710	1.0
Isolate-002	Gebelcho	13.3	Isolate-011	Gebelcho	13.3
Isolate-002	Bulga 70	23.3	Isolate-011	Bulga 70	36.7
Isolate-002	Local	26.7	Isolate-011	Local	46.7
Isolate -007	Moti	19.0	Isolate -016	Moti	33.3
Isolate -007	ILB 938	3.0	Isolate -016	ILB 938	3.0
Isolate -007	NC58	81.7	Isolate -016	NC58	83.3
Isolate -007	Dosha	28.0	Isolate -016	Dosha	31.7
Isolate -007	ILB 4726	1.0	Isolate -016	ILB 4726	1.0
Isolate -007	CS 20 DK	63.3	Isolate -016	CS 20 DK	80.0
Isolate -007	Kasa	65.0	Isolate -016	Kasa	78.3
Isolate -007	BPL 710	5.0	Isolate -016	BPL 710	5.0
Isolate -007	Gebelcho	12.0	Isolate -016	Gebelcho	23.3
Isolate -007	Bulga 70	60.0	Isolate -016	Bulga 70	70.0
Isolate -007	Local	70.0	Isolate -016	Local	63.3

Table 5.9 Analysis of variance of DS and AUDPC-GH for chocolate spot disease in the greenhouse at APPRC in 2012

Source of variation	df	Mean Squares Chocolate spot disease	
		DS	AUDPC-GH
Rep	2	417.96174***	106944.95***
Genotype	59	309.0927***	63123.049***
Error	118	37.16119	9917.821
Corrected total	179		
Cv%		25.92822	30.29825
Mean		23.51106	326.8417
LSD		9.8565	161.02
R^2		0.813065	0.770909

***Significant at p≤0.001, DS= disease severity score (%); AUDPC-GH=Area under diseases progress curve in the greenhouse



Where: HR: highly resistant, R: resistant, MR: moderately resistant, S: susceptible

Figure 5.2 Disease severity reactions of the 60 faba bean genotypes in the greenhouse at A PPRC.

The progress of the disease in the greenhouse for selected faba bean genotypes representing the three disease reaction types is shown in Figure 5.3. Disease progress for the resistant varieties remained constant throughout the season, while there was a sudden increase in disease observed on moderately resistant and susceptible varieties 14 days after inoculation. Pearson correlation coefficient between AUDPC-GH values and disease severity score were highly significant ($p \leq 0.001$) and positive ($r=0.974$).

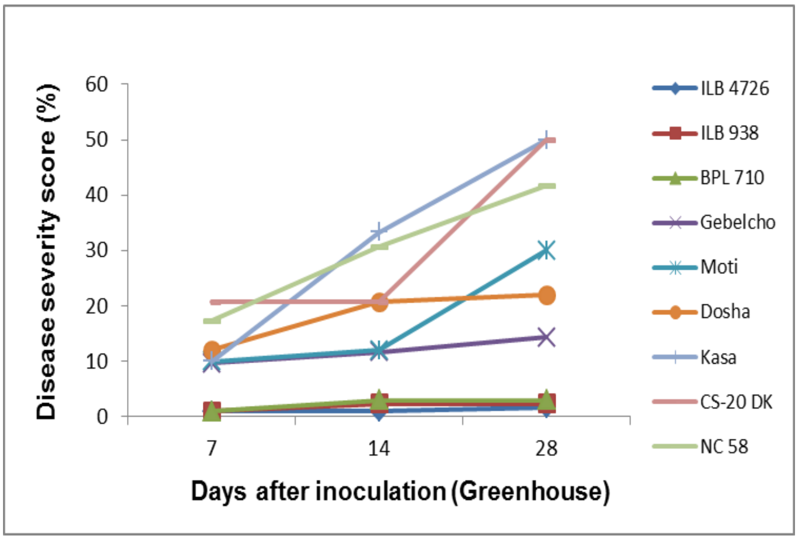


Figure 5.3 Disease progress of chocolate spot based on severity scores of selected representative faba bean varieties in the greenhouse at APPRC.

Field Evaluation

Mean squares for the genotypes were highly significant ($p<0.001$) for all traits measured as indicated in Table 5.10. Genotype x environment interaction was siginificant for all traites except DTF and GDS. Environmental variance was also significant for all the trait except PPP and HSW.

Response of the faba bean germplasm to *Botrytis fabae* in the field

There were significant ($P<0.001$) variation among the genotypes for the disease severity across the two locations (Appendix 5.2). The mean disease severity ranged from 1.47% for an improved variety ILB-4726 to 39.29% for a landrace collection FBColl-0061 across the two locations. The overall mean disease severity was higher at HARC (22.29%) than at Bekoji (20.17%). The resistant and exotic varieties ILB-4726, ILB-938 and BPL-710 had disease severity of < 3% in all locations except BPL-710 which had a disease severity score of 4.99% at HARC.

The resistant checks ILB-4726, ILB-938 and BPL-710 had mean disease severity score of 2.67%, the moderatly resistant improved varieties Gebelcho, Dosha and Moti had mean disease severity of 9.38% and the susceptable chakes; CS-20-DK, Kasa, NC-58 and Bulga-70 had mean disease severity of 31.13% (Appendix 5.2). All the local landrace collections had different disease scores with mean disease severity of 22.26%. Of the sixty genotypes evaluated; 35% had disease severity reaction of highly resistant to resistant reaction, 10% moderately resistant, while the majority (55%) of the genotypes were susceptible across the two locations (Figure 5.4)

Table 5.10 Analysis of variance for selected agronomic traits and chocolate spot disease severity score

Source of variation	df	Mean Squares												
		DTF	DTM	HFP	PPN	NWP	PPP	NPP	SPP	PWt	HSW	BM	GY	GDS
Environment	1	124.84***	42401.80***	18774.89***	3.45***	113.56***	68.12^{ns}	12201	5.20***	245.41***	0.02^{ns}	1339.69***	87.36***	402.09***
Rep (Env)	2	21.22^{ns}	132.54***	164.33^{ns}	0.17^{ns}	22.23***	210.95***	108.6***	0.13^{ns}	5.33**	0.01^{ns}	211.835***	4.77***	137.04***
Genotype	59	49.56***	166.13***	105.65***	0.25***	7.52***	49.19***	6.18*	0.19***	5.24***	0.13***	15.30***	4.273***	523.28***
Genotype x Env	59	17.84^{ns}	17.96***	74.22***	0.17**	6.32***	44.04***	4.46***	0.11***	7.03***	0.04***	19.0***	3.22***	14.03^{ns}
Error	196	15.89	7.79	45.46	0.12	3.29	23.39	4.89	0.05	1.68	0.02	8.23	0.8248	12.34
Corrected total	359													
CV%		6.35	2.12	15.66	20.06	19.76	29.83	8.35	8.39	26.85	20.47	33.25	25.86	16.55
Mean		62.81	131.85	43.07	1.7	9.19	16.21	26.47	2.82	4.83	0.74	8.63	3.61	21.23
LSD		4.661	3.35	8.26	0.39	2.191	5.933	2.623	0.295	1.599	0.179	1.868	1.1	4.189
R^2		0.63	0.97	0.79	0.61	0.66	0.63	0.93	0.72	0.79	0.74	0.76	0.77	0.95

Where:*, **, *** stands for significant at p≤0.01, p≤0.05, and p≤0.001, respectively, ns indicates non-significant (p>0.05); DTF=Days to 50% flowering, DTM=Days to physiological maturity, HFP=Hight for the first pod from the ground; PPN= number of pod per node; NWP=Number of node with pod; PPP= number of pod per plant; NPP=number of node per plant; SPP= number of seed per pod; PWt= Pod weight; HSW= hundred seed weight; BM= Total biomass; GY= Grain yield; GDS= General disease severity score for chocolate spot.

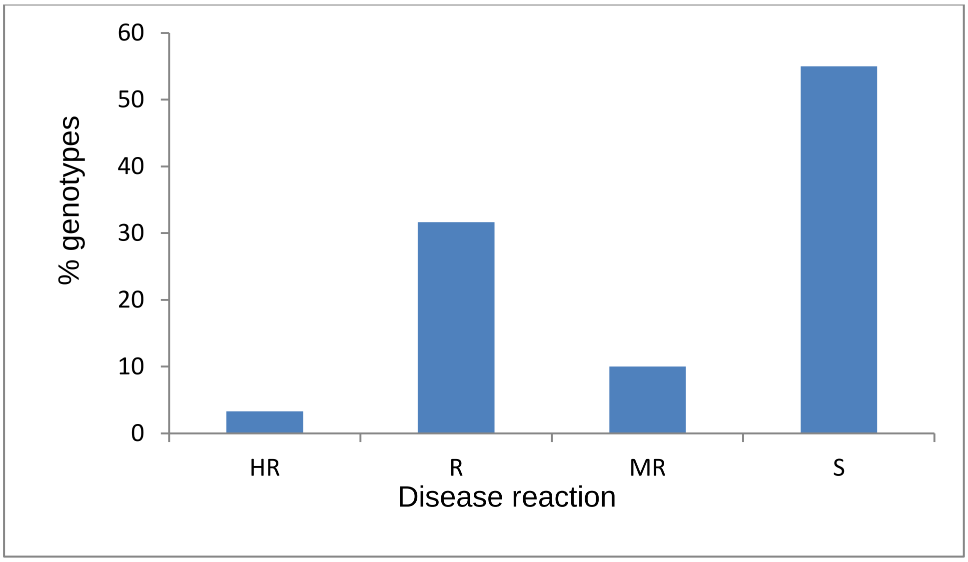


Figure 5.4 Disease severity reactions of the 60 faba bean genotypes across the two locations

Correlation between chocolate spot and selected agronomic traits

The correlation between the mean general disease severity scores (GDS) and area under disease progress curve value (AUDPC) for all genotypes was strong positive and highly significant (r= 0.83; p< 0.001) (Table 5.11). Grain yield had a highly significant but weak negative correlation (r=-0.18; p<0.001) with general disease severity score (GDS) and (-0.22; p<0.001) AUDPC. Similarly, there was highly significant but moderately weak negative correlation between the general disease severity score (GDS) and hundred seed weight (HSW) (r=-0.34; p<0.001) and between HSW and AUDPC (r=-0.32; p<0.001). Highly significant positive and moderately weak correlation observed for grain yield with SPP (r=0.43, p<0.001), PPP(r=0.35; p<0.001) and NWP(r=0.33; p<0.001). DTF had weak significant, negative correlation (r=-0.32, p<0.001) with GDS and (r-0.33; p<0.001) AUDPC.

Table 5.11 Pearson correlation coeficients among selected traits of 60 faba bean germplasms evaluated over two locations

HARC	DTF	DTM	HFP	NPP	NWP	PPP	SPP	HSW	GY	GDS	AUDPC
DTF	-	0.5768***	0.0387^{ns}	-0.048^{ns}	-0.189**	-0.161*	-0.053^{ns}	0.226***	-0.027^{ns}	-0.319***	-0.320***
DTM		-	0.0761^{ns}	0.008^{ns}	-0.134^{ns}	-0.119^{ns}	-0.222***	0.301***	-0.082^{ns}	-0.491***	-0.454***
HFP			-	0.229***	-0.294***	-0.134^{ns}	0.002^{ns}	0.208***	-0.099^{ns}	-0.072^{ns}	-0.194**
NPP				-	0.444***	0.377***	0.119^{ns}	-0.226***	0.209***	0.156^{ns}	0.094^{ns}
NWP					-	0.651***	0.022^{ns}	-0.528***	0.485***	0.112^{ns}	0.108^{ns}
PPP						-	0.149*	-0.506***	0.419***	0.178^{ns}	0.138^{ns}
SPP							-	0.022^{ns}	0.212***	0.197**	0.192**
HSW								-	-0.104^{ns}	-0.291***	-0.261***
GY										-0.016^{ns}	-0.003 ^{ns}
GDSF										-	0.825***
AUDPC											-

Bekoji	**DTF**	**DTM**	**HFP**	**NPP**	**NWP**	**PPP**	**SPP**	**HSW**	**GY**	**GDS**	**AUDPC**
DTF	-	0.646***	0.118 ^{ns}	-0.078 ^{ns}	-0.412***	-0.329***	-0.438***	0.283***	-0.240***	-0.323***	-0.319***
DTM		-	0.064 ^{ns}	-0.190*	-0.432***	-0.363***	-0.516***	0.410***	-0.302***	-0.341***	-0.287***
HFP			-	0.213***	-0.419***	-0.054^{ns}	-0.067 ^{ns}	0.069^{ns}	-0.115 ^{ns}	-0.026 ^{ns}	-0.084 ^{ns}
NPP				-	0.221***	0.234***	0.112 ^{ns}	-0.194**	0.188**	0.036ns	0.028 ^{ns}
NWP					-	0.700***	0.411***	-0.467***	0.466***	0.218***	0.207**
PPP						-	0.338***	-0.548***	0.316***	0.275***	0.187**
SPP							-	-0.209***	0.403***	0.255***	0.178**
HSW								-	0.006 ^{ns}	-0.394***	-0.40***
GY									-	-0.236***	-0.241***
GDSF										-	0.825***
AUDPC											-

Combined	**DTF**	**DTM**	**HFP**	**NPP**	**NWP**	**PPP**	**SPP**	**HSW**	**GY**	**GDS**	**AUDPC**
DTF	-	0.411^{***}	-0.027^{ns}	-0.134^{**}	-0.296^{***}	-0.220^{***}	-0.176^{***}	0.240^{***}	-0.083^{ns}	-0.322^{***}	-0.330^{***}
DTM		-	-0.529^{***}	-0.812^{***}	-0.335^{***}	-0.053^{ns}	0.141^{**}	0.147^{**}	0.200^{***}	-0.294^{***}	-0.373^{***}
HFP			-	0.660^{***}	-0.098^{ns}	-0.119^{*}	-0.261^{***}	0.130^{**}	-0.294^{***}	0.023^{ns}	0.038^{ns}
NPP				-	0.332^{***}	0.037^{ns}	-0.296^{***}	-0.043^{ns}	-0.245^{***}	0.115^{*}	0.220^{***}
NWP					-	0.633^{***}	0.128^{**}	-0.472^{***}	0.330^{***}	0.181^{***}	0.201^{***}
PPP						-	0.273^{***}	-0.530^{***}	0.348^{***}	0.223^{***}	0.145^{**}
SPP							-	-0.124^{**}	0.429^{***}	0.179^{***}	0.085^{ns}
HSW								-	-0.041^{ns}	-0.342^{***}	-0.321^{***}
GY									-	-0.177^{***}	-0.217^{***}
GDSF										-	0.822^{***}
AUDPC											-

Where: DTF=Days to 50% flowering, DTM=Days to physiological maturity, HFP=Hight for the first pod from the ground; NPP= number of node per plant; NWP=Number of node with pod; PPP= number of pod per plant; SPP= number of seed per pod; HSW= hundred seed weight; GY= Grain yield; GDS= General disease severity score for chocolate spot; AUDPC=Area under disease proguse curve value

Area under disease progress curves for chocolate spot disease severity score in the field.

Analysis of variance for relative area under disease progress curves (RAUDPC) indicated highly significant ($p<0.001$) variation among the genotypes, environment and genotype x environment interaction (Table 5.12). The overall mean value of AUDPC at HARC was higher (493.9) than Bekoji (406.9) as the disease pressure was more severe at HARC than Bekoji in 2012 cropping season (Appendix 5.3).

Table 5.12 Analysis of variance of AUDPC for chocolate spot disease scores over two locations in 2012

		Across locations			HARC	Bekoji
Source of variation	df	AUDPC	RAUDPC	df		
Environment	1	679424.14***	0.3466***			
Rep (Env)	2	431139.08***	0.2199***	2	78724.7***	514272.9***
Genotype	59	153976***	0.0618***	59	57653.61***	71631.1***
Genotype x Env	59	11176.86	0.0057			
Error	196	10224	0.0051	76	7957.72	8535.02
Corrected total	359			179		
CV%		22.15	22.45		18.06	22.7
Mean		450.38	0.3217		493.83	406.94
LSD		122.4	0.015			164.6
R^2		0.8633	0.8633		0.9	0.91

*, **, *** stands for significant at $p\leq0.05$, $p\leq0.01$ and $p\leq0.001$, respectively, AUDPC: area under disease progress curve; RAUDPC: relative area under disease progress curve value

The resistant variety ILB-4726 had mean RAUDPC value of 0.08 (95.2) across locations and the highest RAUDPC value 0.49 (697.5) was recorded on the faba bean landrace FBColl-0061. In general, higher area under disease progress curve values were recorded on most of the faba bean local landrace and susceptible varieties than resistant varieties (Appendix 5.3).

The progress of chocolate spot disease severity with time on resistant (ILB4726, ILB938 and BPL710), moderately resistant (Gebelcho, Moti and Dosha) and susceptible varieties (NC58, CS-20-DK) is presented in Figure 5.5 and 5.6. Progress of disease in the resistant, moderately resistant and susceptible varieties was similar in pattern at the two sites. However, the resistant varieties had lower AUDPC values. The progress of the disease was fast in the second week after inoculation on the susceptible faba bean genotypes at HARC and Bekoji. However, slow disease development was observed on the moderately resistant and resistant faba bean varieties. Disease scores >40% were recorded on some faba bean genotypes by the fifth week of disease assessment at HARC.

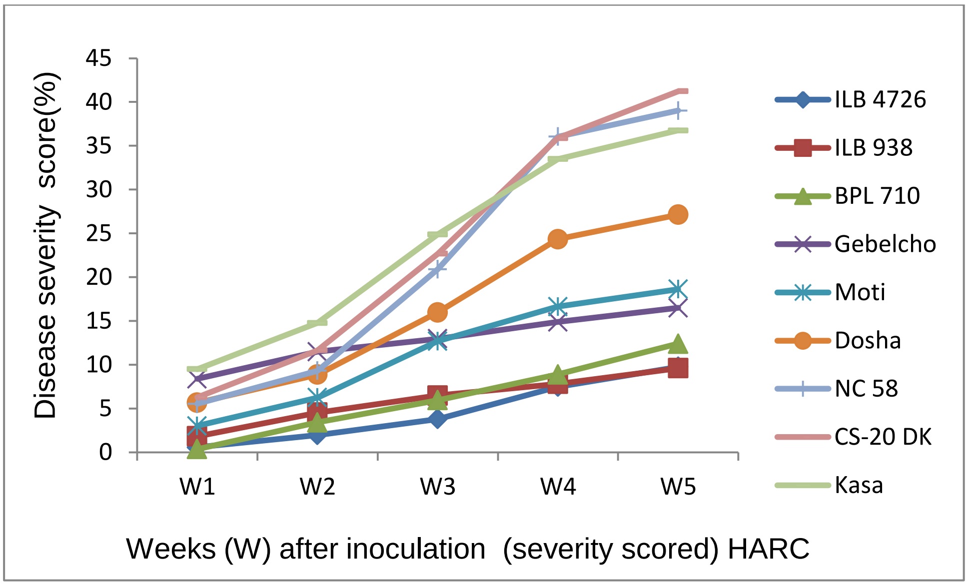


Figure 5.5 Disease progress based on selected representative faba bean varieties for disease severity score over time in the field at HARC in 2012.

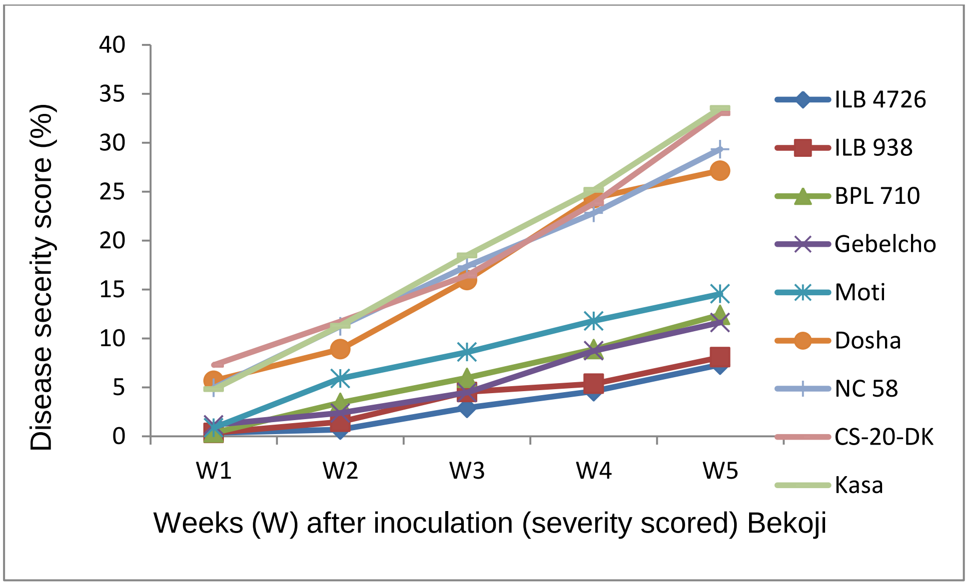


Figure 5.6 Disease progress based on selected representative faba bean varieties for disease severity score over time in the field at Bekoji in 2012.

Correlation between the greenhouse and field evaluation

Pearson correlation analysis for the association between the field and greenhouse evaluation for the 60 genotypes is presented in Table 5.13. There was significant strong and positive (r=0.89; p<0.001) correlation between the disease severity in the greenhouse and in the field. Similarly, there was a significant strong positive ($r=0.92$; $p<0.001$) correlation between the area under disease progress values in the greenhouse and field.

Table 5.13 Pearson correlation coefficients between the field and greenhouse disease assessment.

Pearson Correlation Coefficients				
	GDSF	RAUDPCF	DSGH	AUDPCGH
GDSF	1	0.90^{***}	0.89^{***}	0.89^{***}
AUDPCF		1	0.93^{***}	0.92^{***}
DSGH			1	0.99^{***}
AUDPCGH				1

Where: ***=significant at $p\leq0.001$; GDSF= general disease severity score in the field; RAUDPCF= relative area under disease progress curve value in the field; DSGH=disease severity score in the greenhouse; AUDPCGH=area under disease progress curve value in the greenhouse

Variance components and heritability estimates

The variance components and heritability estimates based on chocolate spot disease severity and area under disease progress curve value are presented in Table 5.14. Heritability estimates were high for chocolate spot disease scores (97%) and RAUDPC (92%). The variance ratio was higher for general disease severity score than RAUDPC.

Table 5.14 Variance components and heritability estimates for chocolate spot disease resistance

Component		GDS	RAUDPC
	$\boldsymbol{\sigma_g^2}$	184.875	23800
	$\boldsymbol{\sigma_{gxe}^2}$	0.282	158.81
	$\boldsymbol{\sigma_e^2}$	2.057	1704
	$\boldsymbol{\sigma_p^2}$	87.214	25662.8
Heritability (%)		0.9732	0.9274
	$\boldsymbol{\frac{\sigma_g^2}{\sigma_{gxe}^2}}$	300.98	149.86

5.4 Discussion

The four local *Botrytis fabae* isolates (Isolate-002, Isolate-007, Isolate-011 and Isolate-016) tested against the eleven varieties of faba bean showed variation in virulence, with Isolate-016 from West Gojjam being the most virulent followed by Isolate-007 from North Shewa. In a similar study of variability of nine *Botrytis fabae* isolates from Ethiopia, Gorfu (1996) reported an isolate from Inewari North Shewa to be the most virulent against the faba bean genotypes. In this study, significant difference among the test genotype x isolate combination indicates that the genotypes have different resistant alleles resulting in genetic differences among isolates or different alleles for aggressiveness. Differences in the virulence and the existence of race variability in *Botrytis fabae* isolates was reported by other researchers (Hutson and Mansfield, 1980; Hanounik and Maliha, 1986)

Overall, there was highly significant correlation between the disease severity score and area under disease progress curve value for the greenhouse and field evaluations. This is in agreement with the findings of Villegas-Fernández et al. (2011), who also reported a high correlation coefficient between laboratory and field experiment in the screening of faba bean for chocolate spot resistance. This indicates that controlled environment conditions could be used successfully to identify resistance to *Botrytis fabae*. However, the performance of some genotypes depended upon the conditions under which they were screened. The greenhouse evaluation resulted in the same ranking as the field for highly resistant and susceptible genotypes, but for the intermediate there was no clear agreement. For example, the moderately resistant variety Moti had very low levels of chocolate spot disease in the field, but had higher disease severity score in the greenhouse. Similar results were reported by Bouhassan, et al. (2004). This implies that genotypes with high resistance under field condition still need to be confirmed under controlled environment.

Under both greenhouse and field conditions at HARC and Bekoji, the tested genotypes differed significantly in their diseases severity score. Bernard et al. (2006) also reported significant differences among faba bean genotypes for this disease in the field. The cumulative progress of disease estimate using AUDPC also varied greatly among the susceptible, moderately resistant and resistant groups. In general, genotypes within the resistant and moderately resistant groups resulted in a lower AUDPC value than the susceptible group. This indicates that the resistant genotypes respond by slowing down the disease development, thus delay the progress during the season which was also observed in various host-pathogen interaction studies (Phama et al., 2010; Daisy et al., 2013).

Chocolate spot disease severity and RAUDPC values were significantly different across the two locations. This could be attributed to variations in the weather conditions which affected the development of the *Botrytis fabae* and /or the virulence and aggressiveness of the isolate-016 varied in space and time. *B. fabae* is favored by temperature between 20 to 23°C (Harrison, 1988). The aggressive stage of chocolate spot pathogen is favored by high-rainfall and acid soils (Parry, 1990; Sahile et al., 2008). At HARC during the flowering stage to grain filling of the crop (August to October), the average maximum temperature ranged between 20.4 - 26.2°C and rainfall was 436.9 mm. At Bekoji, on the other hand, the average maximum temperature ranged between 16.7-19.5°C with rainfall 264.4 mm during the flowering to pod filling growth stage at which the disease progress was peak in severity (Stoddard et al., 2010). In addition, the soil was very acidic at HARC with pH of 4.63 compared to Bekoji with soil pH of 5.01. Hence, the environmental conditions at HARC were more ideal for disease infection than Bekoji. This could explain the difference in the disease severity and RAUDPC values of chocolate spot at the two locations. The non-significant genotype x environment interaction for disease severity means that the genotypes were ranked similarly at the two locations, despite the differences in disease pressure.

The significant variation in disease severity and AUDPC values among the faba bean genotypes evaluated indicate the availability of adequate genetic variability. Genotypes from ICARDA (ILB 4726, ILB 938 and BPL 710) consistently showed the lowest levels of disease severity and AUDPC values in both greenhouse and field conditions. These materials were previously reported as highly resistant to the disease (Villegas-Fernа́ndez et al., 2009). In contrast, the susceptible checks which are improved varieties; Kasa, NC 58, CS-20-DK and Bulga-70 from HARC showed a susceptible reaction to the disease under both conditions. Most of the local landraces were susceptible to the disease in both the greenhouse and field. However, faba bean landrace collections FBColl-0030, FBColl-0036, FBColl-0003, FBColl-0001, FBColl-0012, FBColl-0024, FBColl-0055, FBColl-18, FBColl-0035, FBColl-0011, FBColl-0057, FBColl-0002, FBColl-0042, FBColl-31, FBColl-0034, FBColl-0008, FBColl-0039 and FBColl-0032 were resistant to moderately resistant to chocolate spot disease. These results corroborate findings by Bouhassan et al. (2004) who reported nine faba bean genotypes from the Maghreb to be highly resistant to chocolate spot. In this current study, the genotypes varied in reaction to the disease, but while some genotypes showed high levels of resistance, none were immune, suggesting partial resistance which could be quantitatively inherited. This is in agreement with the suggestion of the presence of quantitative resistance in the genotypes (Parlevliet, 1979). Sillero et al. (2010) also reported absence of complete resistance in faba bean genotypes for this disease.

Although the local cultivars are susceptible to the chocolate spot disease (Mussa et al., 2008), farmers still prefer them for their taste, moderate yield, early maturity and adaptation to the local environment (Beyene, unpublished). The resistant genotypes ILB 4726, ILB 938 and BPL 710 had a lower mean yield as compared to the intermediate resistant and the susceptible genotypes. This could have been attributed to the fact that the resistant genotypes are exotic resistant checks and are poorly adapted to the Ethiopian conditions, hence low yield. In addition, these cultivars would not be accepted by the farmers because of low yield and late maturity. Therefore, the farmers' preferred local landraces, with relatively lower disease severity and high yield, can be used as sources of resistance in breeding programmes or evaluated over locations as resistant/tolerant genotypes. These landraces also need to be improved using different breeding strategies.

There was high positive correlation between AUDPC and the mean general disease severity scores (GDS) in the field as well as controlled condition. This suggests that either characteristic could be used in identifying resistance in genotypes. However, the variance ratio was higher for general disease severity score than AUDPC implying that genotypic differentiation was better using the general disease score. Significant and negative correlation was observed between days to 50% flowering and disease severity as well as area under disease progress curve. Genotypes with late flowering had low disease severity scores and low AUDPC value. On the other hand genotypes with lower days to 50% flowering i.e. early maturing genotypes developed more disease and higher AUDPC value. Thus, late maturing faba bean genotypes can appear to be resistant but in reality are more likely to be disease escapes. This could be due to reduced amount of disease per unit of inoculum and unfavourable conditions for better infection setting in around time of flowering for late genotypes (Arraianoa et al., 2009). Although, weak negative correlation was observed between general disease severity and seed yield. This still indicate that the variability in yield was explained by the degree of the disease to some extent. Similar result was reported in the evaluation of faba bean germplasm for chocolate spot (El-Sayed et al., 2011). Similar results were reported in the study of fungicidal management of chocolate spot of faba bean (El-Sayed et al., 2011).

Heritability estimates were high for chocolate spot disease severity (0.97) indicating that genetic variance highly controlled the trait and phenotypic selection would be effective for improving chocolate spot resistance. El-Badawy et al. (2012) also found higher broad sense heritability (0.97) for disease severity score when they evaluated faba bean for chocolate spot disease. The higher magnitude of heritability indicates that these traits could be improved by direct selection.

5.5 Conclusion

In conclusion, this study has demonstrated that landraces are an important source of genetic diversity as they are adapted to local conditions and have farmer preferred traits. In addition, considerable level of resistance is available in locally adapted germplasm. Genotypes FBColl-0030, FBColl-0036, FBColl-0003, FBColl-0001, FBColl-0012 and FBColl-0024 were identified with low (<10%) chocolate spot severity and had good yield. These could be additional sources of resistance to chocolate spot breeding programmes. The resistance in these genotypes would be very valuable and useful in stabilising faba bean production in the Ethiopian highlands where chocolate spot is very serious. Nevertheless, the genotypes that exhibited high levels of resistance are recommended for intensified multi-location evaluation.

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Appendices

Appendix 5.1 Chocolate spot disease reaction of 60 faba bean genotypes evaluated in the greenhouse at APPRC 2012

Genotype	DS (%)	AUDPC-GH	Disease reaction	Genotype	DS (%)	AUDPC-GH	Disease reaction
ILB 4726	1.22	16.33	HR	FBColl-0051	27.67	378	S
ILB 938	1.89	28	HR	FBColl-0010	29	406	S
BPL 710	2.33	35	HR	NC 58	29.89	421.17	S
Gebelcho	11.89	165.67	R	FBColl-0040	30.33	420	S
FBColl-0031	12	168	R	CS-20 DK	30.44	392	S
FBColl-0002	12	168	R	FBColl-0047	30.44	421.17	S
FBColl-0042	12.11	169.17	MR	FBColl-0027	30.45	436.33	S
FBColl-0003	12.11	169.17	MR	FBColl-0060	31.11	443.33	S
FBColl-0011	12.34	173.83	MR	Kasa	31.11	443.33	S
FBColl-0030	12.44	172.67	MR	FBColl-0041	31.78	435.17	S
FBColl-0018	12.44	173.83	MR	FBColl-0050	31.78	435.17	S
FBColl-0036	12.56	175	MR	FBColl-0016	31.78	435.17	S
FBColl-0035	12.67	179.67	MR	FBColl-0038	31.78	435.17	S
FBColl-0055	13.44	183.17	MR	FBColl-0061	31.78	450.33	S
FBColl-0025	14.22	191.33	MR	FBColl-0006	31.89	451.5	S
FBColl-0001	14.33	192.5	MR	FBColl-0037	32.55	451.5	S
FBColl-0039	14.89	198.33	MR	FBColl-0005	33.11	453.83	S
FBColl-0024	14.89	198.33	MR	Bulga -70	33.22	465.5	S
Dosha	15.67	221.67	MR	FBColl-0023	33.22	465.5	S
FBColl-0034	16.22	212.33	MR	FBColl-0013	33.22	465.5	S
FBColl-0008	16.33	213.5	MR	FBColl-0009	34.11	474.83	S
FBColl-0012	16.33	213.5	MR	FBColl-0026	34.44	478.33	S
FBColl-0049	16.44	229.83	MR	FBColl-0004	34.67	480.67	S
Moti	17.33	224	MR	FBColl-0020	34.67	480.67	S
FBColl-0057	19.22	274.17	MR	FBColl-0059	34.67	509.83	S
FBColl-0032	19.89	266	MR	FBColl-0052	34.67	509.83	S
FBColl-0053	21.22	264.83	MR	FBColl-0045	34.67	509.83	S
FBColl-0054	23.33	302.17	S	FBColl-0043	36	508.67	S
FBColl-0017	24.78	332.5	S	FBColl-0029	36.11	525	S
FBColl-0046	26.11	375.67	S	FBColl-0015	37.45	539	S
Mean					23.511	326.842	
CV (%)					25.928	30.4699	
LSD					9.8565	161.02	
Pr > F					<.0001	<.0001	
R^2					0.8131	0.77091	

Where: DS=Diseases severity score; AUDPC-GH= Area under disease progress curve- greenhouse; HR=highly resistant, R= resistant, MR=moderately resistant, S=susceptible.

Appendix 5.2 Faba bean chocolate spot disease reaction and grain yield of 60 faba bean landrace and improved varieties at HARC and Bekoji in 2012.

Location	HARC			Bekoji			Across location		
Faba bean accession/ variety	GDS (%)	Reaction type	Yield	GDS (%)	Reaction type	Yield	GDS (%)	Reaction type	Yield
ILB 4726	1.13	HR	1.82	1.81	HR	1.39	1.47	HR	1.60
ILB 938	2.72	HR	3.57	2.61	HR	2.55	2.67	HR	3.06
BPL 710	2.75	HR	2.60	4.99	R	2.03	3.87	R	2.31
Gebelcho	10.54	R	2.97	5.34	R	3.55	7.94	R	3.26
FBColl-0030	10.98	R	3.08	7.25	R	3.99	9.12	R	3.53
Moti	12.32	MR	2.91	6.29	R	5.63	9.31	R	4.27
FBColl-0036	11.96	MR	3.32	7.30	R	5.15	9.63	R	4.23
FBColl-0003	10.45	R	2.98	8.90	R	5.25	9.68	R	4.11
FBColl-0001	10.76	R	3.24	8.62	R	4.07	9.69	R	3.66
FBColl-0012	11.88	R	3.37	8.09	R	4.97	9.99	R	4.17
FBColl-0024	12.08	MR	2.53	8.00	R	5.06	10.04	R	3.79
Dosha	14.29	MR	3.74	7.52	R	5.50	10.9	R	4.62
FBColl-0055	11.76	R	4.04	10.07	R	7.15	10.91	R	5.60
FBColl-0018	13.76	MR	3.77	8.11	R	6.71	10.93	R	5.24
FBColl-0035	13.90	MR	3.86	8.04	R	5.65	10.97	R	4.76
FBColl-0011	12.11	MR	3.04	10.02	R	6.15	11.07	R	4.60
FBColl-0057	10.81	R	4.10	11.39	R	4.47	11.1	R	4.29
FBColl-0002	13.07	MR	2.35	9.73	R	6.09	11.4	R	4.22
FBColl-0042	12.58	MR	3.34	10.45	R	5.17	11.51	R	4.26
FBColl-0031	15.80	MR	3.38	7.95	R	6.03	11.88	R	4.71
FBColl-0025	14.96	MR	3.43	9.36	R	5.10	12.16	R	4.27
FBColl-0034	14.44	MR	3.30	10.46	R	5.55	12.45	MR	4.43
FBColl-0008	11.58	R	2.44	13.79	MR	4.08	12.69	MR	3.26
FBColl-0039	13.92	MR	3.20	12.42	MR	5.00	13.17	MR	4.10
FBColl-0032	19.59	MR	3.29	8.49	R	3.01	14.04	MR	3.15
FBColl-0049	16.33	MR	2.92	14.43	MR	3.46	15.38	MR	3.19
FBColl-0004	19.49	MR	2.55	18.19	MR	3.44	18.84	MR	2.99
FBColl-0037	20.66	S	3.12	21.21	S	4.37	20.93	S	3.75
FBColl-0005	21.66	S	2.28	20.77	S	3.89	21.22	S	3.08
FBColl-0054	23.10	S	3.04	19.82	MR	2.68	21.46	S	2.86
FBColl-0053	23.23	S	3.08	23.12	S	2.08	23.18	S	2.58
FBColl-0041	23.23	S	3.73	24.27	S	5.75	23.75	S	4.74
Bulga -70	25.97	S	3.07	22.01	S	2.77	23.99	S	2.92
FBColl-0051	25.52	S	3.39	22.47	S	2.20	23.99	S	2.80
FBColl-0009	24.92	S	2.65	25.11	S	3.51	25.01	S	3.08
FBColl-0015	25.42	S	3.44	25.86	S	3.04	25.64	S	3.24
FBColl-0013	28.03	S	2.60	24.47	S	2.50	26.25	S	2.55

Location	HARC			Bekoji			Across location		
FBColl-0023	27.49	S	2.55	25.14	S	4.30	26.32	S	3.43
FBColl-0017	27.19	S	3.09	28.48	S	4.11	27.84	S	3.60
FBColl-0050	31.42	S	2.82	26.17	S	4.00	28.8	S	3.41
FBColl-0045	29.54	S	4.60	29.07	S	6.86	29.3	S	5.73
FBColl-0052	31.00	S	2.73	27.99	S	2.20	29.5	S	2.46
FBColl-0027	31.90	S	3.52	27.38	S	6.78	29.64	S	5.15
NC 58	30.88	S	3.57	28.47	S	2.13	29.67	S	2.85
FBColl-0046	31.86	S	2.69	28.30	S	6.69	30.08	S	4.69
FBColl-0010	29.39	S	2.60	31.05	S	3.49	30.22	S	3.05
FBColl-0020	29.98	S	2.84	34.23	S	3.29	32.11	S	3.06
FBColl-0029	31.84	S	3.68	33.18	S	5.88	32.51	S	4.78
FBColl-0040	35.22	S	2.77	31.72	S	3.30	33.47	S	3.04
FBColl-0006	35.56	S	2.70	31.84	S	3.13	33.7	S	2.91
FBColl-0043	33.15	S	3.17	34.45	S	3.47	33.8	S	3.32
CS-20 DK	34.68	S	3.18	33.47	S	4.03	34.08	S	3.60
FBColl-0016	31.61	S	3.59	36.66	S	1.91	34.13	S	2.75
FBColl-0059	37.14	S	3.74	32.73	S	2.50	34.93	S	3.12
FBColl-0026	36.36	S	2.48	34.74	S	4.16	35.55	S	3.32
Kasa	34.82	S	3.40	38.75	S	1.92	36.78	S	2.66
FBColl-0038	38.49	S	3.09	36.40	S	3.35	37.44	S	3.22
FBColl-0060	37.80	S	3.64	37.16	S	3.16	37.48	S	3.40
FBColl-0047	42.31	S	1.86	35.41	S	2.48	38.86	S	2.17
FB Coll-0061	39.77	S	2.89	38.76	S	3.75	39.26	S	3.32
Mean	22.29		3.11	20.17		4.09	21.23		3.61
CV (%)	13.92		27.17	16.64		23.12	16.55		25.86
LSD	5.35		1.33	6.05		1.56	4.149		1.1
Pr > F	<.0001		0.097	<.0001		<.0001	<.0001		<.0001
R 2	0.96		0.68	0.97		0.86	0.95		0.77

Where: HARC= Holetta Agricultural Research Centre; GDS= General disease severity score HR=highly resistant, R= resistant, MR=moderately resistant, S=susceptible

Appendix 5.3 Chocolate spot AUDPC value of 60 faba bean genotypes evaluated in the field at HARC and Bekoji 12012

		AUDPC value		Combined	
No	Faba bean accession/ variety	HARC	Bekoji	AUDPC	RAUDPC
1	FBColl-0011	341.3	290.4	315.8	0.1916
2	FBColl-0031	492.2	207.5	349.8	0.2519
3	FBColl-0015	683.8	699.5	691.6	0.4774
4	FBColl-0041	683.6	564	623.8	0.4307
5	FBColl-0050	640.3	602.8	621.5	0.4534
6	CS-20 DK	656.8	499.1	577.9	0.3965
7	FBColl-0016	618.4	665.9	642.2	0.4417
8	FB Coll-0061	722	673.1	697.5	0.4920
9	FBColl-0017	679.7	656.6	668.1	0.4875
10	FBColl-0060	653.6	570.5	612.1	0.4317
11	FBColl-0053	632.3	544	588.1	0.4149
12	FBColl-0034	385.1	290.7	337.9	0.2202
13	FBColl-0039	340	295.6	317.8	0.1868
14	FBColl-0001	318.1	265.8	292	0.1732
15	FBColl-0005	628.1	549.5	588.8	0.4043
16	FBColl-0042	354.6	243.5	299	0.1914
17	FBColl-0004	587.7	477.7	532.7	0.4018
18	FBColl-0003	256.1	207.9	232	0.1463
19	FBColl-0026	658.2	628	643.1	0.4954
20	FBColl-0020	568.5	418.8	493.7	0.3601
21	FBColl-0008	271.5	354.4	312.9	0.2426
22	FBColl-0059	625.9	559.1	592.5	0.4568
23	FBColl-0006	624.7	544.7	584.7	0.4376
24	FBColl-0037	592.7	545.5	569.1	0.4097
25	FBColl-0057	250.6	329.6	290.1	0.2109
26	FBColl-0038	566.8	523.1	544.9	0.4094
27	Kasa	633	501	567	0.4332
28	FBColl-0054	594.9	494.4	544.7	0.3935
29	Gebelcho	375.3	180.2	277.7	0.2259
30	ILB 4726	89.8	95.2	92.5	0.0767
31	FBColl-0002	317.8	207.7	262.8	0.1616
32	FBColl-0024	267	235	251	0.1729
33	FBColl-0055	333.1	285.8	309.4	0.2203
34	FBColl-0040	606.1	459.8	532.9	0.3483
35	ILB 938	203.8	96.6	150.2	0.0974
36	FBColl-0009	667.9	554.7	611.3	0.4097
37	NC 58	610.7	514.1	562.4	0.3863
38	FBColl-0036	263.3	143.2	203.3	0.1464
39	FBColl-0025	357.5	184.8	271.2	0.1962
40	Bulga -70	480.1	495.7	487.9	0.3544

		AUDPC value		Combined	
41	BPL 710	202.5	220.4	211.5	0.1691
42	FBColl-0052	603.9	534.9	569.4	0.4069
43	FBColl-0047	575.6	517.4	546.5	0.3879
44	FBColl-0043	635.5	537.9	586.7	0.4257
45	FBColl-0023	597.9	530	563.9	0.4144
46	FBColl-0032	607.4	229.3	418.4	0.3266
47	FBColl-0049	389.5	348.6	369.1	0.2598
48	Moti	352.1	260.9	306.5	0.2089
49	FBColl-0018	344.8	151.8	248.3	0.1802
50	FBColl-0013	692.9	564.5	628.7	0.4526
51	Dosha	444.9	133.1	289	0.2505
52	FBColl-0030	287	168.5	227.7	0.1655
53	FBColl-0010	620.5	558.3	589.4	0.4477
54	FBColl-0012	323.8	217.4	270.6	0.2226
55	FBColl-0035	308.5	223.5	266	0.1984
56	FBColl-0027	610.2	477.7	544	0.3987
57	FBColl-0046	635.8	443.5	539.6	0.3772
58	FBColl-0029	637.2	566	601.6	0.4167
59	FBColl-0045	670.5	509.2	589.8	0.3983
60	FBColl-0051	456.4	567.8	512.1	0.3556
Mean		493.9	406.9	450.3831	0.3217
CV (%)		17.97	22.7	22.14754	22.45
LSD		152.9	164.6	122.4	0.015
Pr > F		<.0001	<.0001	<.0001	<.0001
R^2		0.9	0.91	0.863	0.863

AUDPC: Area under disease progress curve, RAUDPC: relative area under disease progress

Chapter 6

Gene action determining grain yield and chocolate spot (*Botrytis fabae*) resistance in a faba bean

Abstract

Production of faba bean (*Vicia faba* L.) is hampered by chocolate spot disease caused by *Botrytis fabae.* To devise a viable strategy for breeding for chocolate spot resistance and yield, crucial information on the mode of inheritance of the traits would be required. Therefore, a 10 x 10 full diallel was evaluated at three sites in the Ethiopian highlands, with two replications under natural conditions and supplemented with artificial inoculation of the pathogen. The yield and disease resistance data were analysed using the Diallel SAS05 macros in SAS. There was significant variation for chocolate spot disease resistance and yield among the genotypes ($P \leq 0.001$). Further, chocolate spot disease severity assessment at 88 days after planting had better precision for discriminating the genotypes. General combining ability (GCA) and specific combining ability (SCA) effects were highly significant ($P \leq 0.001$) for chocolate spot resistance. However, the GCA was predominant (84.5%) suggesting that additive gene effects were more important than non-additive effects (8.5%) and that selection would be effective to enhance disease resistance. In sharp contrast, the SCA effects were predominant (89.3%) for grain yield suggesting that the non-additive gene action was in control and that the hybridization strategy would be effective to improve yield. Reciprocal effects were generally negligible (<10%) for both yield and disease resistance. The genotypes x environment and SCA x environment effects were highly significant for yield ($P \leq 0.001$) underlining the need to perform multi-environment trials before varieties can be recommended for the target production areas. The line ILB-4726, which combined good disease resistance with high grain yield potential would be recommended for programmes that emphasise chocolate spot resistance in faba bean.

Keywords: Chocolate spot, diallel analysis, faba bean, gene action, legume breeding.

6.1 Introduction

Faba bean (*Vicia faba* L.) is a leading staple legume in many countries, spanning from Africa to the Middle East. However, there is a reduction in production of faba bean in many countries (Torres et al., 2006; Pe´rez-de-Luque et al., 2010) which can worsen the gap between production and consumption. Adequate production is threatened by chocolate spot disease among other constraints of faba bean. Chocolate spot (*Botrytis fabae* Sardina) compromises yield by damaging the foliage and limiting photosynthetic activity. The disease affects crop productivity worldwide, but especially in the north east and southern Africa (Akem and Bellar, 1999; Bouhassan et al., 2004; Tivoli et al., 2006). Severe epidemics of chocolate spot can be devastating resulting in over 90% yield reduction. For example, extensive damage has been reported in Australia (90%), United Kingdom (59%), China (>50%) and in Ethiopia (>61%) (Dereje and Yaynu, 2001; Elad et al., 2004). Therefore, breeding for chocolate spot resistance should be emphasised.

An integration of cultural methods with host plant resistance would be effective to manage the disease, especially in developing countries. Integrating disease control options, such as early sowing, sprays of mancozeb, chlorothalonil fungicide, use of moderately resistant cultivars, and cereal intercropping can reduce the disease epidemics (Gorfu, 2000; Sahile et al., 2008). However, smallholder farmers who dominate faba bean production in developing countries cannot afford to buy fungicides. Additionally, completely resistant cultivars for chocolate spot have not been developed. The use of resistant cultivars and incorporating favorable resistance genes into the locally adapted susceptible varieties is the most practical way of controlling chocolate spot disease (Sillero et al., 2010). The current study, therefore, seeks to initiate the process of breeding resistant cultivars but crucial genetic information is required to optimize the breeding strategy.

It is certainly necessary to understand the mode of inheritance and gene action for important traits in faba bean (Lithourgidisa et al., 2005). However, this information is still scarce. The information is fundamental to devise viable chocolate spot resistance breeding strategies. A survey of the literature indicates that there are limited studies, which have been conducted on the genetic mechanisms for chocolate spot resistance and yield in the faba bean germplasm being used in the highlands of Ethiopia. Therefore, the objective of this study was to determine the mode of inheritance and type of gene action which govern chocolate spot resistance and yield in faba bean and to determine the time of disease rating on the precision of discriminating crosses for chocolate spot resistance.

6.2 Materials and methods

6.2.1 Experimental sites

The experiments were conducted at three locations in Ethiopia during the 2013 season. The sites included Holetta Agricultural Research Centre (HARC) (09º03’N, 38º30’E, and 2390 meter above sea level, i.e., m a. s. l). The average maximum and minimum temperature at HARC in 2013 from June to December was 22.5ºC and 8.5ºC, respectively, and the average rainfall received was 533.6 mm, and soil pH of 4.62. The second site was the Kulumsa Agricultural Research Centre (KARC) (08º00’N; 39º09’E; 2211 m a. s. l). The average maximum and minimum temperature at KARC in 2013 from June to December was 22.5ºC and 10.9ºC, respectively. The average rainfall received was 584.1 mm and the soil pH of 5.2. The third site was the Kofele (07º04’N; 38º48’E; 2620 m a. s. l.). The average maximum and minimum temperature in 2013 from June to December was 18.3ºC and 2.3ºC, respectively. The average rainfall received was 1077.4 mm and the soil pH of 4.37.

6.2.2 Faba bean germplasm

The characteristics and sources of the 10 faba bean parental lines used for the 10 x 10 full diallel mating with reciprocal crosses are indicated in Table 6.1. These lines are genetically different and form three genetic clusters (Figure 6.1).

Table 6.1 Characteristic and source of the 10 faba bean parental lines used in a full 10x10 diallel cross

Faba bean parent lines	Pedigree	Seed size	Resistance status	Maturity period	Source of seed[a]
ILB-4726	ICARDA	Large	Resistant	Late	ICARDA
ILB-938	ICARDA	Large	Resistant	Late	ICARDA
BPL-710	ICARDA	Large	Resistant	Late	ICARDA
Moti	ILB-4432 x kuse-2-27-33	Medium	Moderately resistant	Medium	HARC
Dosha	Landrace collection	Medium	Moderately resistant	Medium	HARC
Gebelcho	Tesfa x ILB-4726	Medium	Moderately resistant	Medium	HARC
Kasa	Landrace collection	Small	Susceptible	Early	HARC
NC58	National collection	Small	Susceptible	Early	HARC
CS-20-DK	National collection	Small	Susceptible	Early	HARC
Bulga-70	Landrace collection	Small	Susceptible	Early	HARC

[a] ICARDA: International Center for Agricultural Research in the Dry Area; HARC; Holetta Agricultural Research Center

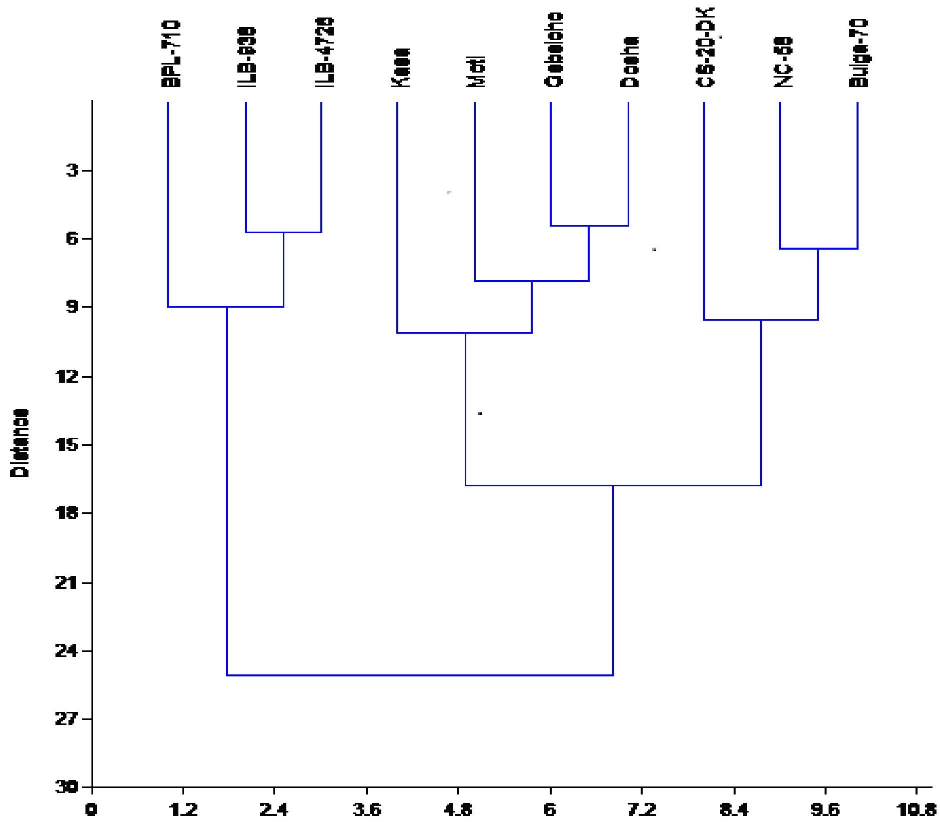


Figure 6.1 Dendrogam of the ten genotypes used for the full diallel cross based on 30 SSR markers. Source: (Beyene, unpublished data, 2012)

6.2.3 Experimental design and management

The 100 entries consisting of 90 F_1 reciprocal progenies (i.e., 45 progenies and their reciprocals) developed by the 10 x 10 full diallel mating design and 10 parental lines were planted in a 10 x 10 α- lattice design in single row plots of 3 m at 40 cm spacing between the rows and 6 cm between plants. There were 51 plants per plot. To minimize inter-plot interference each test genotype was bordered by a common susceptible faba bean variety NC 58, which also acted as a disease spreader. Fertilizer was applied at 100 kg ha $^{-1}$ of Diammonium phosphate (DAP) at planting. The trials were kept weed-free by hand weeding. In addition, trials were artificially inoculated with *Botrytis fabae* isolate with a spore concentration of 5 x 10^5 ml^{-1} at 60 days from planting, which coincided with the flowering stage at all sites. This practice was recommended by the previous researchers (Bernard et al., 2006). Inoculation was made during the late afternoon to avoid the effect of sunlight on the spore viability.

Data collection

Genotype reaction to Botrytis fabae was evaluated seven days after inoculation and disease severity scoring was taken six times weekly (7-days interval) from the same five randomly pre-tagged plants per genotype. In addition, general disease assessment of the genotypes for disease severity of chocolate spot was recorded from the whole plot once at 88 days after planting. The severity of chocolate spot was recorded as percentage of leaf area infected using the following scale: 1%-no disease symptoms or very small specks (highly resistant); 3%-few small disease lesions (highly resistant); 6%-small coalesced lesions with some defoliation (resistant); 12%-large coalesced sporulating lesions, 20%-defoliation (moderately resistant); 25%-large coalesced sporulating lesions, 50% defoliation and some dead plants (susceptible); and 50% extensive, heavy sporulation, stem girdling, blackening and death of more than 80% of the plants (highly susceptible). The scale was used by previous researchers (Bernier et al., 1993; Bernard et al., 2006). The grain yield t ha-1 data was taken from the entire plot. Grain yield was adjusted to 10% moisture content following the oven drying method. The total grain yield recorded on a plot basis was converted to t ha-1 for statistical analysis.

6.2.4 Data analysis

The analyses of variance (ANOVA) was performed using the PROC GLM in SAS (SAS Institute, 2012). The disease data severity score collected weekly in the field was used to calculate the area under the disease progress curve (AUDPC). The AUDPC was calculated for each genotype using the disease severity scores according to the following formula proposed by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n} 1/2[(yi + 1 + yi)(xi + 1 - xi)]$$

Where: yi = the cumulative disease severity percentage of infected plants at the ith observation (day i), xi = time (days) at the ith observation, n = total number of symptom observations. AUDPC was calculated using Microsoft excel. The estimate of AUDPC was normalized by dividing with the total area of the graph (i.e. the number of days between the first and the last readings multiplied by maximum potential AUDPC), for a better visual comparison among genotypes over location (Mohapatra et al., 2008). The normalized AUDPC was referred to as the relative area under disease progress curve (RAUDPC).

GCA effects of the parents and SCA of the crosses, as well as their mean squares in each environment were estimated in the 10 x 10 full diallel crosses following Griffing’s method 1, model 1 (Griffing, 1956). In addition, the role of maternal effects of chocolate spot in faba bean resistance was assessed from the reciprocal effects. This was done using DIALLEL-SAS05 program developed by Zhang et al. (2005) in SAS software version 9.3 (SAS

Institute, 2012). The relative sum of squares was calculated for each element of the crosses: GCA, SCA and reciprocal effects as a percentage of the entry sum of squares.

Genetic variance ($\hat{\sigma}^2\mathrm{GCA}$ $\hat{\sigma}^2\mathrm{SCA}$ and $\hat{\sigma}^2\mathrm{REC}$) were estimated by using Diallel SAS Griffing's random-effect model method 1 diallel analysis using DIALLEL-SAS05 (Zhang et al., 2005). The variances were then used to estimate the following parameters: the general pridicted ratio (GPR) Baker's (1978) $\frac{2\sigma^2\mathrm{GCA}}{2\sigma^2\mathrm{GCA}+\sigma^2\mathrm{SCA}}$; and broad-sense heritability $(\mathrm{h}_\mathrm{b}^2)$: $\frac{\hat{\sigma}^2\mathrm{GCA}+\hat{\sigma}^2\mathrm{SCA}+\hat{\sigma}^2\mathrm{REC}}{\hat{\sigma}^2\mathrm{phenotypic}}$; narrow-sense heritability $(\mathrm{h}_\mathrm{n}^2)$: $\frac{\hat{\sigma}^2\mathrm{GCA}}{\hat{\sigma}^2\mathrm{phenotypic}}$; Coefficient of genetic variation (CVg) was calculated as: $\frac{\sqrt{\sigma^2}gca+\sigma^2 sca+\sigma^2 REC}{\mu}$ where μ is the mean value while coefficient of error variation (Kao and Mcvetty 1987) was calculated as $\frac{\sqrt{\sigma^2}e}{\mu}$. (Habarurema et al., 2012; Vieira et al., 2012).

6.3 Results

6.3.1 Genetic variation

There was significant (P ≤ 0.001) variation among the genotypes for chocolate spot general disease scores, area under disease progress curve values and grain yield (Table 6.2). Genotype by environment interaction was significant (P<0.05) for general disease score and grain yield (P ≤ 0.001).

Table 6.2 Combined analysis of variance for chocolate spot general disease score (GDS), relative area under disease progress curve value (RAUDPC) and grain yield (GY t ha-1) from 10 x 10 full diallel cross over three environments

		Mean squares		
Source of variation	**d.f**	**General disease severity score (%)**	**Relative Area under disease progress curve value**	**Grain yield (t ha^{-1})**
Rep	1	72.17*	403112***	17.97***
Row	9	153.23***	175219***	8.19***
Col	9	180.79***	53702***	12.37***
Genotype	99	99.89***	47710***	11.16***
Environment	2	3675.02***	2453637***	257.74***
Genotype x environment	198	14.98*	15102	1.92***
Error	281	11.34	14662	1.26
Total	599	44.19	32060	4.27
CV		21.8	23.8	32.00
LSD		0.66	23.84	0.22

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

6.3.2 Performance of the parents and F_1 progenies of faba bean for chocolate spot disease

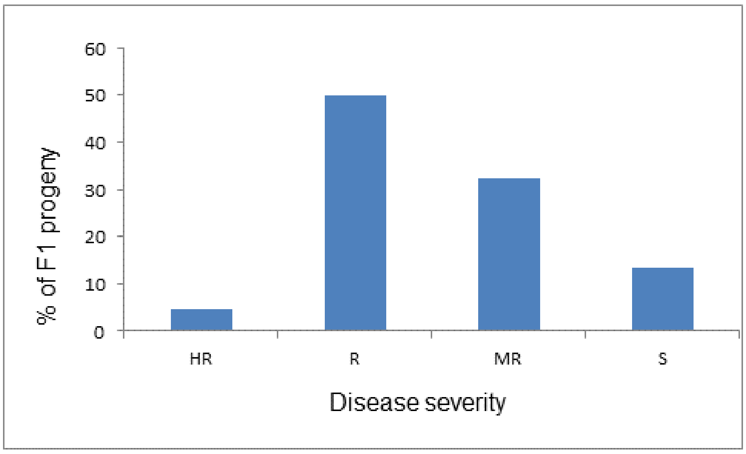
The top 25 and bottom 10 F_1 faba bean progenies and parental lines are presented in Table 6.3. The chocolate spot general severity score for the parental lines ranged from 3.8% (ILB-4726) for the resistant variety to 32.2% for the susceptible (Kasa) faba bean variety. The disease severity score for the F_1 progenies ranged from 2.2% to 25.7%. All F_1 progenies among the resistance parent lines, R x R, (ILB 4726, ILB 938 and BPL 710) had the lowest disease severity from 2.2% to 4.1% and the lowest RAUDPC values, ranging from 0.20 (346.4) to 0.28 (491.7). Similarly, the R x MR crosses of ILB-4726 (R) x Gebelcho (MR) recorded lowest general disease severity of 9.4% across the three environments. On the contrary, the F_1 progenies between the susceptible parents (S x S) NC58, Kasa, CS-20-DK and Bulga-70 had the highest disease severity scores of 21.1 to 25.7% and high RAUDPC values 0.35 (606.7) to 0.40 (693.2). The F_1 progeny derived from Kasa x NC58 had the highest diseases severity of 25.7% and NC58 x Kasa recorded the highest RAUDPC value of 0.40 (693.2). In general, the majority (86.6%) of F_1 progenies exhibited highly resistant to moderately resistant reactions to chocolate spot disease severity scores (Figure 6.2). On the other hand, 13.3% of F_1 progenies displayed a susceptible disease reaction to the chocolate spot disease. The 10 inbred lines showed significant differences ($P \leq 0.001$) in reaction to the chocolate spot disease and yield (Table 6.3).

Table 6.3 Performance of the top 25 F_1 and bottom 10 F_1 progenies (based on disease severity) and parental lines for chocolate spot disease response and grain yield across three environments

Genotype	GDS (%)	Reaction	RAUDPC (AUDPC)	Grain yield (t ha-1)	Rank based on GDS
Top Crosses					
ILB-938 x BPL-710 (R x R)	2.184	R	0.28 (491.7)	1.11	1
BPL-710 x ILB-4726 (R x R)	2.782	R	0.20 (346.4)	1.31	2
BPL-710 x ILB-938 (R x R)	2.934	R	0.25 (443.0)	1.35	3
ILB-938 xILB-4726 (R x R)	3.035	R	0.21 (363.6)	1.92	4
ILB-4726 x ILB-938 (R x R)	4.129	R	0.24 (421.5)	1.62	7
ILB-4726 x Gebelcho (R x MR)	9.429	MR	0.22 (389.3)	1.81	9
ILB-938 x Gebelcho (R x MR)	10.140	MR	0.28 (491.7)	2.09	10
ILB-4726 x CS-20-DK (R x S)	10.189	MR	0.19 (338.3)	5.66	11
ILB-4726 x Bulga-70 (R x S)	10.339	MR	0.20 (351.8)	6.90	12
ILB-938 x Dosha (R x MR)	10.861	MR	0.30 (520.2)	3.27	13
Dosha x ILB-4726 (MR x R)	10.888	MR	0.23 (407.4)	4.35	14
BPL-710 x Gebelcho(R x MR)	11.004	MR	0.26 (452.5)	1.62	15
BPL-710 x CS-20-DK (R x S)	11.087	MR	0.24 (417.1)	5.46	16
ILB-4726 x Dosha (R x MR)	11.417	MR	0.27 (470.2)	3.66	17
Dosha x BPL-710(MR x R)	11.475	MR	0.28 (481.7)	3.34	18
Gebelcho x ILB-938(MR x R)	11.517	MR	0.24 (416.9)	3.05	29
Bulga-70 x ILB-4726 (S x R)	11.626	MR	0.26 (459.9)	5.63	20
Dosha x Gebelcho (MR x MR)	11.747	MR	0.23 (407.4)	4.38	21
CS-20-DK x Gebelcho (S x MR)	11.984	MR	0.26 (448.7)	4.21	22
NC58 x BPL-710 (S x R)	11.987	MR	0.23 (401.9)	6.22	23
Moti x ILB-4726 (MR x R)	12.205	MR	0.26 (461.6)	4.58	24
NC58 x ILB-4726 (S x R)	12.280	MR	0.25 (434.8)	5.95	25
CS-20-DK x ILB-938(S x R)	12.308	MR	0.30 (528.8)	6.11	26
ILB-4726 x Moti (R x MR)	12.420	MR	0.27 (466.9)	5.91	27
Bulga-70 x BPL-710(S x R)	12.451	MR	0.26 (458.9)	5.04	28
Bottom 10 crosses					
Moti x Kasa (MR x S)	21.170	MS	0.33 (583.0)	1.51	87
CS-20-DK x NC58 (S x S)	21.182	MS	0.35 (606.6)	2.51	88
Kasa x Dosha (S x MR)	21.448	MS	0.36 (621.6)	3.33	89
NC58 x Moti (S x MR)	21.534	MS	0.33 (575.8)	2.70	90
Moti x Bulga-70 (MR x S)	22.749	MS	0.36 (627.7)	1.95	91
Kasa x Bulga-70 (S x S)	22.856	MS	0.36 (632.9)	1.54	92
CS-20-DK x Kasa (S x S)	23.156	S	0.39 (689.7)	1.59	93
NC58 x Kasa (S x S)	24.123	S	0.40 (693.2)	2.35	95
Kasa x CS-20-DK (S x S)	24.673	S	0.39 (680.9)	1.73	98
Kasa x NC58 (S x S)	25.650	S	0.39 (682.4)	2.03	99
Parent lines					
ILB-4726	3.817	R	0.07 (122.7)	2.03	5
ILB-938	4.012	R	0.08 (142.1)	2.29	6
BPL-710	5.427	R	0.12 (211.1)	2.35	8

Genotype	**GDS (%)**	**Reaction**	**RAUDPC (AUDPC)**	**Grain yield (t ha-1)**	**Rank based on GDS**
Gebelcho	14.848	MR	0.30 (516.6)	3.99	47
Moti	16.493	MR	0.36 (629.5)	4.69	57
Dosha	17.707	MR	0.33 (584.3)	4.09	66
CS-20-DK	23.468	S	0.39 (680.9)	2.68	94
NC58	24.554	S	0.38 (662.5)	2.59	96
Bulga-70	24.557	S	0.37 (649.8)	3.42	97
Kasa	32.251	S	0.40 (698.9)	2.66	100
Trial Mean	15.402			3.43	

Descriptions: R= resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible. RAUDPC: relative area under disease progress curve value, number in bracket AUDPC, GDS: general disease severity score



R=resistant (<9.5% disease severity); MR=moderately resistant (10 to 20% disease severity score) MS=moderately susceptible (20 to 23%), and S=susceptible (>23% disease severity score)

Figure 6.2 Distribution of the 90 F_1 progenies for chocolate spot disease severity scores over three environments

6.3.3 Effect of rating date on precision of experiment

The ANOVA for chocolate spot disease scores (%) at the different dates as measured by the number of weeks after disease inoculation is presented in Table 6.4. There was significant variation (P<0.001) among the genotypes for each of the six weeks for disease severity scores. The trial statistics also varied for the R^2, percentage of coefficient of variation (CV %) and the broad sense heritability (h^2b) which generally tended to improve from the first rating to the fourth and then declined. The best R^2, CV and h^2b were computed from disease severity score at the 4thweek after inoculation which was at 88 days after planting. The dates of disease rating affected the efficiency of detecting the genetic effects in the diallel cross. The general combining ability (GCA) was significant (P<0.001) for the disease score at all dates of rating, but the specific combining ability (SCA) data started showing significant differences from the 3rd week onwards. Similarly, the reciprocal, maternal effect and non-maternal effects were only significant from the 3rd week and 4th week rating. The value of contribution of GCA sums of squares for crosses was higher than the contribution of SCA sums of squares for crosses computed at all the rating dates for diseases severity score except at the 6th week where the % GCA (SS) contribution was lower than the % SCA (SS) contribution (Table 6.4).

Table 6.4 Mean squares and trial statistics for the 10 x 10 full diallel cross evaluation for chocolate spot disease on a weekly basis over three locations in 2013 in Ethiopia

Source of variation	df	Rating date (Days after planting)					
		67	74	81	88	95	102
		1stWeek	2nd Week	3rdWeek	4thWeek	5th Week	6th Week
ENV	2	5.74***	1431.65***	5718.36***	5073.05***	2603.0***	3220.39***
REP(ENV)	3	3.19**	373.95***	917.63***	503.16***	624.44***	605.23***
ENTRY	99	1.46***	28.09***	73.39***	79.76***	95.88***	104.09***
ENV*ENTRY	198	0.61	22.66**	26.075*	23.20*	24.69	23.82
GCA	9	9.36***	196.77***	418.35***	440.2***	438.6***	377.78***
SCA	45	0.77	11.76	51.22***	54.64***	82.78***	111.65***
REC	45	0.56	10.69	26.57	32.80**	40.43**	41.81**
MAT	9	1.00	11.78	33.15	42.04*	49.39*	44.33
NMAT	36	0.44	10.43	24.92	30.49**	38.18*	41.18**
GCA*ENV	18	0.84	66.96***	71.11***	63.94***	56.91**	37.64
SCA*ENV	90	0.5	13.94	20.49	16.781	18.63	20.69
REC*ENV	90	0.67	22.53*	22.64	21.481	24.3	24.19
MAT*ENV	18	0.85	23.64	16.23	14.045	12.33	16.96
NMAT*ENV	72	0.63	22.25	24.24	23.34	27.29	25.99
MSE		0.67	16.47	19.95	18.03	23.34	24.23
Trial Statistics:							
CV (%)		64.04	74.01	31.49	23.98	22.1	19.19
R^2		59.00	69.00	81.79	82.85	75.56	76.39
H^2b		61.34	43.42	86.55	84.67	83.83	85.09
Mean		1.28	5.48	14.18	17.71	21.86	25.63
% GCA (SS) contribution		58.40	63.67	51.82	50.17	41.59	32.99
% SCA (SS) contribution		24.15	19.03	31.72	31.13	39.25	48.75
% REC (SS) contribution		17.45	17.30	16.45	18.69	19.17	18.26

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; 1stWeek to 6th Week: week disease score recorded after inoculation; the first score was at 67 days after planting.

The trend for the trial statistics was also observed for the RAUDPC data (Table 6.5). The best R^2, CV (%) and h^2b were obtained when the RAUDPC included all the data from the 1st week to 6th week after inoculation. Even though there was variation in the values of the % GCA (SS) contribution and % SCA (SS) contribution, the % GCA (SS) contribution was higher than% SCA (SS) contribution in all combinations. The RAUDPC data which was computed from all the observations (from 1st week to 6th week rating) was adopted for further analysis of the diallel cross.

Table 6.5 Mean squares and trial statistics for the 10 x 10 full diallel cross analysis for chocolate spot disease area under disease progress curve for the different weekly data sets over three locations in 2013

Source	**DF**	**RAUDPC Week 1-4†**	**RAUDPC Week 1-6**	**RAUDPC Week 2-5**	**RAUDPC Week 3-6**
ENV	2	847790.5***	2453637.3***	1525075.96***	1247070.18***
REP(ENV)	3	178190.0***	536797.3***	254312.79***	253710.49***
ENTRY	99	14377.6***	61210.3***	27365.39***	36984.28***
ENV*ENTRY	198	4904.6***	15102.3*	7511.11**	8808.74
GCA	9	932144.67***	369381.7***	167952.56***	186424.91***
SCA	45	306602.11***	40287.9***	17291.29***	29709.94***
REC	45	184630.73	20498.5**	9322.05**	14370.49***
MAT	9	56101.65*	27229.6*	12703.87*	17503.97*
NMAT	36	128529.09	18815.7*	8476.60*	13587.12
GCA*ENV	18	316545.29***	49213.2***	25137.89***	23120.12***
SCA*ENV	90	240338.14	9001.02	4760.95	6267.08
REC*ENV	90	414217.79*	14381.38	6735.90	8488.12
MAT*ENV	18	84431	10514.02	5146.00	4840.26
NMAT*ENV	72	329786.79*	15348.22	7133.38	9400.08
MSE		3337.39	12244.04	5655.313	7911.41
Trial Statistics:					
CV (%)		28.3	21.7	23	21.4
R^2		82.35	81.1	82.66	78.66
H_2b		83.04	86.35	85.79	85.81
Mean		0.12(204.1)	0.29(508.8)	(0.18)318.9	0.24(416.3)
% GCA (SS) contribution		65.49	54.86	55.79	45.82
% SCA (SS) contribution		21.54	29.92	28.72	36.51
% REC (SS) contribution		12.97	15.22	15.48	17.66

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively;

†RAUDPC14: relative area under disease progress curve value taken from disease score of 1st Week to 4th Week; RAUDPC16: relative area under disease progress curve value taken from disease score of 1st Week to 6th Week; RAUDPC25: relative area under disease progress curve value taken from disease score of 2nd Week to 5th Week; RAUDPC36: relative area under disease progress curve value taken from disease score of 3rd Week to 6th Week. Number in bracket= AUDPC mean value.

6.3.4 General and specific combining ability

The disease score data for the 4th week rating and the RAUDPC for all the rating dates (week 1-6) were used for the diallel cross evaluation based on the findings from section 6.3.3. The combining ability data is presented in Table 6.6. The GCA and SCA effects were highly significant ($P<0.001$) for resistance to chocolate spot based on the two disease assessments. Reciprocal effects (REC) were also significant for GDS ($p<0.05$) and for the RAUDPC ($p<0.01$). The GCA effects contributed most of (84.9% GDS) the sums of squares and (54.9% RAUDPC value) for the crosses. The SCA contributed 8.7% for the GDS and 29.9% for the RAUDPC. The REC effects were small and contributed only 4.9% for the GDS and 15.2% for the RAUDPC. There was significant interaction for GCA x Environment ($p<0.001$) for both disease assessments. However, contribution of GCA x Environment was low 24% and 25.9% for GDS and RAUDPC, respectively. Significant ($P<0.05$) REC x Environment effects were observed for the GDS and its contribution to the variation of chocolate spot in crosses accounted for 49.7% and 43.3% based on GDS and RAUDPC, respectively.

The GCA and SCA effects were highly significant ($P<0.001$) for grain yield but the reciprocal effects (REC) were not significant (Table 6.6). Although the GCA effects were highly significant, the % GCA (SS) contribution was only 5.84% while the SCA effect contributed most of (89.3%) the sums of square for grain yield. The SCA x Env effect was highly significant ($P<0.001$) for grain yield. The GCA x Env accounted for 6.8%; the REC accounted 29.3% while SCA x Env accounted 64% of the G x E.

6.3.5 Genetic parameters

The variance components, namely GCA ($\hat{\sigma}^2\mathrm{GCA}$), SCA ($\hat{\sigma}^2\mathrm{SCA}$) and REC ($\hat{\sigma}^2\mathrm{REC}$) for chocolate spot disease, exhibited a similar trend for both the GDS and RAUDPC (Table 6.7). The GCA variance was predominant over the SCA variance and the REC variance for both disease assessments. The general pridicted ratio Baker's (1978) $\frac{2\hat{\sigma}^2\mathrm{GCA}}{2\hat{\sigma}^2\mathrm{GCA}+\hat{\sigma}^2\mathrm{SCA}}$ was greater than 0.5 (0.99 for GDS and 0.75 for RAUDPC). Heritability in the broad sense and narrow sense was high for the GDS (93.4% and 82.6) and for RAUDPC (86.4% and 48.9%). However, the narrow sense heritability of chocolate spot was lower for the RAUDPC than the GDS. Moreover, the ratio of the coefficient of genetic variation to coefficient of error variation was greater than one in both assessments. The SCA variance were predominant than GCA variance and the REC variance for grain yield implies non-additive genetic effects were predominant (Table 6.7). In addition, general pridicted ratio Baker's (1978) was less than 0.5

(0.21). Heritability in the broad sense was high (94.95%), but heritability in the narrow sense was low (9.1%).

Table 6.6 Mean squares and trial statistics for the 10 x 10 full diallel cross analysis for chocolate spot disease severity and grain yield over three locations in 2013.

Source of variation	MS	DF	Grain yield (t ha^{-1})	General disease score (GDS)	RAUDPC
ENV	257.74	2	257.74***	7350.05***	2453637.3***
REP(ENV)	11.99	3	11.99***	630.41***	536797.3***
ENTRY	12.88	99	12.88***	12186.59***	61210.3***
ENV*ENTRY	1.92	198	1.92***	2965.39*	15102.3*
GCA	8.28	9	8.28***	1149.67***	369381.7***
SCA	25.29	45	25.29***	23.54***	40287.9***
REC	1.38	45	1.38	17.34*	20498.5**
MAT	0.8074	9	0.81	11.90	27229.6*
NMAT	1.5257	36	1.53	18.70*	18815.7*
GCA*ENV	1.4094	18	1.41	40.35***	49213.2***
SCA*ENV	2.705	90	2.705***	8.52	9001.0
REC*ENV	1.2395	90	1.24	16.36*	14381.4
MAT*ENV	1.2127	18	1.21	25.32**	10514.0
NMAT*ENV	1.2462	72	1.25	14.12	15348.22
Error			1.18	11.24	12244.04
Trial Statistics					
CV (%)			31.45	21.75	21.70
R^2			86.25	87.39	81.10
Mean			3.46	15.41	508.83
% GCA (SS)GCA*ENV			6.67	24.49	29.62
% SCA (SS)SCA*ENV			64.00	25.85	27.09
% RCA (SS)REC*ENV			29.33	49.66	43.28
% GCA (SS) contribution			5.84	84.90	54.86
% SCA (SS) contribution			89.28	8.69	29.92
% REC (SS) contribution			4.88	6.40	15.22

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Table 6.7 Estimates of genetic parameters for resistance to chocolate spot over three environments with general disease score, RAUDPC value and yield

Genetic parameters	GDS	RAUDPC	GY
$\hat{\sigma}^2\mathrm{GCA}$	70.38	20568.36	1.06
$\hat{\sigma}^2\mathrm{SCA}$	6.15	14021.95	12.06
$\hat{\sigma}^2\mathrm{REC}$	3.05	4127.21	0.10
$\hat{\sigma}^2\mathrm{error}$	5.62	6122.02	0.59
$\hat{\sigma}^2\mathrm{phenotypic}$	85.21	44839.54	11.69
$\frac{2\hat{\sigma}^2\mathrm{GCA}}{2\hat{\sigma}^2\mathrm{GCA}+\hat{\sigma}^2\mathrm{SCA}}$	0.99	0.75	0.21
$h_b^2(\%)$	93.41	86.35	94.95
$h_n^2(\%)$	82.60	45.87	9.10
CV g	57.89	38.67	96.20
CV e	15.38	15.38	22.20
CV g/CV e	3.76	2.51	4.34

GDS: general disease score; RAUDPC: relative area under disease progress curve value GY: Grain yield

6.3.6 Combining ability and specific combining ability effects

The GCA effects of the lines for chocolate spot disease scores, RAUDPC and grain yield are presented in Table 6.8. The GCA effects for chocolate spot were highly significant ($P \leq 0.001$) across the three environments. Based on the disease scale used, the negative GCA effects were desirable for chocolate spot resistance. Negative and significant ($P \leq 0.001$) GCA effects for disease resistance were recorded for parents ILB-4726, ILB-938 and BPL-710 in GDS and RAUDPC. On the other hand, the lines Kasa, Bulga-70, NC58, CS-20-DK, and Moti displayed significant ($P \leq 0.001$) and positive GCA effects for chocolate spot across location. For grain yield, the desirable positive GCA effects were significant ($P\leq0.001$) for the parental line ILB-4726, while the line Gebelcho exhibited significant ($P\leq0.05$) negative GCA effects for grain yield potential.

The SCA effects of crosses for the chocolate spot for GDS and RAUDPC are presented in Tables 6.9 and 6.10. Significant ($P\leq0.05$) and positive SCA effects for chocolate spot were observed for the crosses Kasa x CS-20-DK, CS-20-DK x Bulga-70, ILB-938 x Bulga 70 and Kasa x BPL 710 (Table 6.9 and 6.10).

Table 6.8 General combining ability (GCA) effects for chocolate spot general disease severity score (GDS), relative area under disease progress curve value (RAUDPC) and grain yield for 10 faba bean lines over three sites

Parent	GDS[a]	RAUDPC	GY (t ha^{-1})
Moti	1.89***	45.08***	0.21
ILB-938	-3.65***	-49.54***	0.16
NC58	2.74***	28.69*	-0.09
Dosha	0.42	19.38	-0.20
ILB-4726	-5.06***	-117.07***	0.59***
CS-20-DK	1.65***	30.33*	0.01
Kasa	3.50***	49.11***	-0.25
BPL-710	-3.47***	-44.94***	-0.01
Gebelcho	-0.88*	-13.69	-0.26*
Bulga-70	2.85***	52.67***	-0.17

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively. GDS: general disease score; RAUDPC: Area under disease progress curve value; GY: grain yield (t ha^{-1}); [a]Negative GCA effects were desirable for Chocolate spot resistance

Table 6.9 Specific combining ability (SCA) effects of the 90 F_1 progenies for chocolate spot disease scores

Parent	Moti	ILB-938	NC58	Dosha	ILB-4726	CS-20-DK	Kasa	BPL-710	Gebelcho	Bulga-70
Moti		1.54	-0.62	0.72	0.07	-1.29	-0.53	0.84	-0.66	1.35
ILB-938	-0.60		-1.01	0.22	1.78	-0.35	-0.77	1.45	-0.75	6.4*
NC58	-2.11	0.31		-0.43	-0.64	0.62	1.23	-0.80	0.01	-3.66
Dosha	0.38	-1.54	0.77		0.39	0.46	-0.04	-0.35	-2.46	-1.43
ILB-4726	-0.11	-0.46	-0.17	-0.26		-0.06	-1.11	1.38	1.68	-0.95
CS-20-DK	0.54	0.76	-0.76	0.78	-1.75		-0.65	-0.57	-2.36	5.31*
Kasa	0.89	-0.18	1.24	-2.16	-0.07	3.24*		1.56	1.12	-0.49
BPL-710	0.83	0.40	-1.90	-0.53	1.19	1.94	0.71		0.80	1.77
Gebelcho	-0.45	-1.40	0.88	-0.74	-1.71	-1.83	-0.26	-0.85		0.22
Bulga-70	1.95	0.26	-0.09	0.96	-0.64	1.11	1.74	1.07	-1.57	

*, Significant at 0.05 probability levels

Table 6.10 Specific combining ability (SCA) effects of the 90 F_1 progenies for chocolate spot disease RAUDPC

Parent	Moti	ILB-938	NC58	Dosha	ILB-4726	CS-20-DK	Kasa	BPL-710	Gebelcho	Bulga-70
Moti		49.70	-75.01	22.32	27.40	-38.04	-8.88	60.86	-61.06	-38.29
ILB-938	-33.93		-10.43	58.06	50.32	23.95	-0.27	52.98	-44.26	355.3***
NC58	-68.21	-38.14		-19.90	-19.27	-3.88	16.18	-26.65	40.72	-94.44
Dosha	14.60	-16.53	50.99		27.68	-16.96	-30.11	-4.67	-75.49	-34.36
ILB-4726	-2.61	-28.94	33.65	-31.40		-25.09	-11.75	21.80	79.51	73.42
CS-20-DK	-24.38	-15.24	-42.64	-7.49	-58.65		-2.99	9.17	-32.34	-136.68
Kasa	-11.14	-29.63	20.38	-74.33	-27.08	104.4*		102.4*	11.16	-19.48
BPL-710	16.83	24.35	-64.03	3.12	22.27	86.26	-12.51		18.36	181.48
Gebelcho	-8.61	-15.54	-2.74	-31.60	-68.29	-44.39	11.06	-16.03		-6.99
Bulga-70	28.93	5.66	-16.14	-3.29	-54.04	22.77	69.99	31.20	-83.55	

*, ***, Significant at 0.05 and 0.001 probability levels, respectively

Significant (P≤0.001) SCA effects were observed for grain yield (Table 6.11). Fifteen hybrids had significant and positive SCA effects for grain yield.

Table 6.11 Specific combining ability (SCA) effects of the 90 F_1 progenies for grain yield (t ha-1)

Parent	Moti	ILB-938	NC58	Dosha	ILB-4726	CS-20-DK	Kasa	BPL-710	Gebelcho	Bulga-70
Moti		1.36***	0.09	-0.59	0.99*	-0.63	-0.51	-0.13	-0.57	-1.62*
ILB-938	-0.07		1.33***	-0.07	-2.44***	2.19***	0.87*	-2.39***	-0.78*	2.91***
NC58	0.35	0.02		-1.03*	2.09***	-1.90***	-1.47***	-2.38***	0.80*	-0.91
Dosha	-0.23	-0.08	-0.08		0.16	-0.08	-0.05	0.13	1.02*	-1.56*
ILB-4726	-0.67	0.15	-0.10	0.34		1.67***	2.05***	-2.69***	-1.61***	4.99***
CS-20-DK	0.73	-0.29	-0.02	-0.72	-0.08		-1.28**	1.91***	0.65	-0.93
Kasa	0.16	-0.13	-0.07	0.45	0.30	-0.40		1.29***	0.65	-0.97
BPL-710	0.05	-0.12	0.47	-0.05	0.05	-0.07	-0.47		-1.23**	2.95***
Gebelcho	0.54	-0.48	0.83	0.37	-0.36	0.34	0.11	-0.34		-1.03
Bulga-70	0.01	0.04	-0.09	0.19	0.64	0.06	0.17	0.09	0.27	

, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

6.3.7 Association between grain yield and disease resistance

A simple regression analysis of grain yield on chocolate spot general disease score was significant ($P \leq 0.001$) across the three environments. The linear regression model of grain yield (t ha^{-1}) on chocolate spot disease severity score was found with negative slope as Yield = 5.12 - 0.11. There was a significant ($P \leq 0.001$) and negative correlation (r = - 0.32) between grain yield and chocolate spot general disease severity score. Pearson correlation coefficients between the GCA effects and trait means of the lines, and between the SCA effects and trait means of the crosses are presented in Table 6.12.

Table 6.12 Pearson's phenotypic correlation coefficients between the mean and GCA effects of the parents (below diagonal) and between specific combining ability and mean of the F_1 progenies (above diagonal) for resistance to chocolate spot and grain yield

	GDS	RAUDPC	GY	GDS effect	RAUDPC effect	GY effect
GDS	1	0.85***	-0.32***	0.0005	0.09	-0.16
RAUDPC	0.93***	1	-0.30***	0.09	0.004	-0.17
GY	0.25	0.52	1	-0.05	0.10	0.64***
GDS effect	0.96***	0.97***	0.43	1	0.82***	0.002
RAUDPC effect	0.87***	0.93***	0.54	0.96***	1	0.28**
GY effect	-0.65*	-0.64*	-0.35	-0.62*	-0.69*	1

GDS: mean general disease severity score; RAUDPC: mean area under disease progress curve value; GY mean grain yield (t ha^{-1}),

6.4 Discussion

6.4.1 Disease assessment

The general disease score and RAUDPC had similar capacity for discriminating the crosses hence any of the assessment methods could be used for evaluating genotypes for chocolate spot resistance. Clearly the correlation coefficient of the GDS and RAUDPC was strong and positive. The study also indicates that disease severity rating at 88 days after planting would be more reliable for discriminating the genotypes. It is also revealed that the RAUDPC which is based on the data collected over the entire season would be most effective for evaluating of genotypes for chocolate spot resistance. However, a single rating at the fourth week after inoculation will be adequate when the resources are limiting. The results were in contrast with a previous study which found that a single rating at about 3 weeks after inoculation was sufficient to rank the faba bean genotypes for the reaction to the disease (Hanounik and Robertson, 1988; Bouhassan et al., 2004). In this current study, the disease pressure provided good genetic discrimination for chocolate spot disease among the inbred parents and their hybrids. The results are in agreement with the study made on genetic analysis of resistance to leaf spot in groundnuts (Janila et al., 2013) and inheritance of maize for grey leaf spot assessment using RAUDPC (Munthali et al., 2003). Therefore, a single assessment of faba bean chocolate spot disease severity on average at 88 days after planting can reduce time and cost for the breeding programmes.

6.4.2 Performance of parents and F_1 progenies of faba bean for chocolate spot disease

The parental lines ILB-4726, ILB 938 and BPL 710 showed consistently resistant reaction for the chocolate spot disease across the three environments. These genotypes were reported as resistant in a study to identify resistance *Vicia faba* to *Botrytis fabae* (Villegas-Fernańdez et al., 2009). This suggested that the four lines; ILB-4726, ILB 938, BPL 710 and Gebelcho would be best sources for chocolate spot resistance. There was no cross between two susceptible lines that resulted in a progeny with better resistant reaction indicating the absence of transgressive segregants. In general, resistance of the crosses was greater when both parents were resistant which is consistent with additive gene effects.

6.4.3 Gene action

Highly significant GCA and SCA effects implied that both additive and non-additive gene effects were important in determining resistance to chocolate spot disease of faba bean. However, the contribution of GCA to the crosses sums of squares was 84.9% for chocolate spot. This suggested predominance of additive effects expressed by principal of GCA to crosses sums of squares. The higher values of GCA variance ($\hat{\sigma}^2\mathrm{GCA}$) compared with the SCA ($\hat{\sigma}^2\mathrm{SCA}$) and REC ($\hat{\sigma}^2 REC$) variance indicated the greater relative importance of genes with additive effects. Further, general pridicted ratio Baker's (1978) were close to unity signifying a predominant role of additive gene action. This indicated that additive gene action played a more significant role in the inheritance of chocolate spot disease than non-additive gene action. Previous studies reported that resistance was quantitative (Hartwell et al., 2008), which is consistent with observation of large additive effects in the current study. Reciprocal effects (REC) were also observed yet the REC contributed only <15% of the sums squares for crosses for chocolate spot. Therefore, the low magnitude of reciprocal effect indicates that maternal effects or cytoplasmic inheritance were less influential (Kearsey and Pooni, 1996) for chocolate spot. The estimates of broad sense heritability for chocolate spot disease was high suggesting reasonable progress can be achieved in selection for disease resistance to faba bean. Significant effects of GCA x Environment were observed for chocolate spot disease, indicating that selection progress might be compromised by the G x E.

There were highly significant differences among the parents and the F_1 progenie for grain yield. The importance of both GCA and SCA effects was evident with predominance of the latter, which is sufficiently reflected from higher magnitude (89.3%) of its contribution to the crosses sums of squares for grain yield. This implies the importance of non-additive gene action for grain yield. Further, the general pridicted ratio of 0.096 showed predominance of non-additive gene action in the inheritance of grain yield. Moreover, the predominance of SCA variance ($\hat{\sigma}^2\mathrm{SCA}$) indicated the importance of non-additive gene action. This suggests that breeding gain can be realized through hybridization and selection at advanced generation would be effective for substantial genetic gain in grain yield of faba bean. Since GCA effects are the manifestation of additive properties of genes, parents selected based on GCA effects will be useful for developing breeding lines with high grain yield. The parental line ILB-4726 with significant and positive GCA effects could be used in the breeding programmes which emphasises chocolate spot resistance. The large heritability estimates obtained in the current study are in agreement with large broad sense heritability from 90 to 99% which was reported in the previous study of faba bean for chocolate spot disease (El-Badawy et al., 2012). Toker (2004) reported large broad sense heritability of 97% for grain yield, which is comparable to the 94.95% in the current study. However, the estimate of narrow sense heritability was low (9.1%) suggesting the significant role of environmental effects and large SCA effects in modifying this trait. The SCA x Env effects were highly significant with high values of variance components across the environments indicating the genotypes responded differently in different environments. Consequently, multi-location evaluation of genotypes for yield could be essential.

6.4.4 Combining ability and specific combining ability estimates

Significant estimates of GCA effects for chocolate spot were observed in this study. The parental lines ILB-4726, ILB-938 and BPL-710 had negative estimates for chocolate spot. Therefore, these lines were decreasing the overall chocolate spot mean of the crosses by contributing auspicious alleles for chocolate spot resistance in their crosses. Based on their combining ability effects these lines may be used in intra-population breeding with focus on chocolate spot resistance. Parental line Gebelcho also exhibited a negative and significant estimate for chocolate spot and this could be due to its pedigree from ILB-4726. In general, based on GCA effects parental line ILB-4726 was found to be a good combiner for improving chocolate spot disease and grain yield. Non-significant negative estimates of SCA effect were observed for the disease. However, significant SCA effects would not be appreciated since in the short-term faba bean breeding programmes do not aim at producing F_1 progenies.

6.4.5 Regression analysis and correlations among genotypic means and genetic effects

The linear regression model of grain yield on chocolate spot disease severity score across the three locations showed a negative slope and indicated that grain yield of faba bean was decreased by 0.11 t ha^{-1} per percent unit increase of chocolate spot disease severity. However, the regression model accounted for less than 20% of the total variation, implying that chocolate spot was not the only factor affecting the grain yield. In general, there was negative correlation between the mean of chocolate spot disease score and grain yield and GCA effect of the disease and GCA effect of grain yield.

6.5 Conclusion

The parental lines ILB-4726, ILB-938, BPL-710 and Gebelcho had negative GCA effects indicating good combining ability for chocolate spot resistance. These lines would be exploited as sources of resistance to chocolate spot disease in breeding program. Genes with additive effects were predominant over the non–additive gene effects for chocolate spot resistance. The predominance of additive effect indicates that resistance to faba bean chocolate spot can be improved through selection. On the contrary highly significant non-additive gene effects were predominant over additive gene effects for grain yield, indicating that development of hybrids and selection would enhance productivity. The reciprocal effects were negligible (<20%), indicating that cytoplasmic gene effects did not play a major role in modifying the resistance of the diallel cross to chocolate spot disease.

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Chapter 7

A diallel cross analysis of yield components in faba bean

Abstract

Secondary traits which are highly associated with yield can be targeted for improving grain yield, especially under challenging environments. However, there is limited genetic information for these traits in faba bean. Therefore, a 10 x 10 diallel cross of faba bean was evaluated to determine the general combining ability (GCA), specific combining ability (SCA) and reciprocal effects for eleven yield component traits. The diallel was evaluated across three sites and two replications in the Ethiopian highlands. The GCA and SCA effects differed significantly ($P \leq 0.001$) for all of the characters except plant height. This indicated that both additive and non-additive genetic effects played a significant role in the inheritance of these traits. The reciprocal effects were significant ($P \leq 0.01$) for the number of days to 50% flowering and days to physiological maturity. However, its contribution was negligible for all traits. This indicated that maternal or cytoplasmic effects played a minor role in the governance of the yield component traits. The GCA mean squares were larger than those of SCA for only two traits while the SCA effects were predominant for nine traits, suggesting that genes with non-additive effects were preponderant for the yield components and that hybridisation would be effective to improve the varieties. However, heritability ranging between 70% and 95%, was large in a broad sense and moderate to large (40% - 76%) for the narrow sense, indicating that selection strategies would also be effective. Crosses and lines with outstanding combining ability would be recommended for use in breeding programmes.

Keywords: Faba bean; heritability; non-additive gene action; specific and general combining ability; yield components.

7.1 Introduction

Low productivity is one of the major constraints of faba bean production in Ethiopia (Mussa et al., 2001; Gemechu et al., 2006). Therefore, the crop requires improvement through breeding of productive varieties. In order to break the barriers of production and to increase the yield potential of the faba bean, information on the genetic mechanisms of inheritance is of major importance. Moreover, the choice of selection methods for the improvement of target traits will depend on the type of gene action involved (Esmail, 2007).

An effective breeding strategy can be devised after understanding the nature of gene action and inheritance of quantitative characters that impact the yield. Estimates of heritability and genetic variances help to predict responses from selection (Holland et al., 2003). Genetic correlations of traits are also helpful in determining the desirability of direct or indirect selection of traits (Rebetzke et al., 2002). The choice of promising genotypes from genetic base and their subsequent utilization for hybridization is one of the strategies for improving the productivity of crops including faba bean. Thus this information could prove to be essential to faba bean breeders in the screening of better parental combinations for further enhancement.

Breeders are able to gather information on general combining ability (GCA), specific combining ability of parents in crosses (SCA), effects due to reciprocal and maternal effects from diallel analysis for different crops and yield and other traits including, chickpea (*Cicer arietinum* L) (Kumar et al., 2001), soya bean (*Glycine max* L.) (Gwata et al., 2005), common bean (*Phaseolus vulgaris* L.) (Franco et al., 2001) and maize (*Zea mays* L.) (Derera et al., 2008). The role of cytoplasmic or maternal effects has not been established in faba bean yet it can impact on effective selection. Significant reciprocal effects can actually inflate both GCA and SCA if it is of additive and non-additive nature (additive maternal and non-maternal interaction effects, respectively).

The yield of food legume is usually expressed as a function of number of pods per plant, number of seeds per pod and average seed weight (Salehi et al., 2008). These traits have often been considered in a breeding programme of beans aiming to enhance yield since they contribute to yield and show consistent correlation with yield (Sinha, 1977; Alghamdi, 2007). Yield components are relatively easy to measure and grain yield is the result of the development and growth of yield components during the life of the crop (Slafer, 1994). Therefore, grain yield can be improved through selection for the components which are highly heritable and genetically correlated with yield. Therefore, the present study aims to establish the nature of gene action and the relative magnitude of genetic variance for the yield components in faba bean, and combining ability estimates of yield components and to deliver information for a successful hybridization programme.

7.2 Materials and methods

7.2.1 Germplasm, experimental sites, design and management

Description of the faba bean lines used in the 10x10 full diallel mating are presented in Chapter 6. The experimental sites, experimental layout and management, have all been described in chapter 6 section 6.2.1.

7.2.2 Data collection

Data for the following agronomic traits were recorded on a plot basis at all locations; days to 50% flowering (DTF) and days to physiological maturity (DTM) for the entire plot. Fifteen plants per genotype were randomly selected to record the data on the number of primary branches (BB), height from ground to the first pod (HFP), number of pod per node (NPPN), number of pods per plant (NPPP), number of nodes with pod which have seed (NWP), number of nodes per plant (NNP), number of seeds per pod (NSPP) and plant height (PH). Data on total biomass (BM) at harvest, hundred seed weight (HSW) from randomly selected 100 seeds from each plot and grain yield were taken from the entire plot. Grain yield adjustments were made as seeds were weighed, oven dried and adjusted to constant moisture level of 10%. The total biomass and grain yield recorded on plot basis were converted to t ha^{-1} for statistical analysis.

7.2.3 Data analysis

Analyses of variance (ANOVA) to detect differences among the 100 entries (90 F_1 and 10 parents) for each trait in the study were separately performed on the data collected across environment with PROC GLM in SAS (SAS Institute, 2012). Correlation coefficient values were computed for the mean and SCA effects among the yield and yield component using the PROC CORR procedure in SAS. GCA effects of the parents and SCA of the crosses, as well as their mean squares in each environment were estimated in the 10 x 10 full diallel crosses following Griffings method 1, model 1 (Griffing, 1956). This was done using DIALLEL-SAS05 program developed by Zhang et al. (2005) adapted to the SAS software version 9.3 (SAS Institute, 2012).

Genetic variance ($\hat{\sigma}^2\mathrm{GCA}$ $\hat{\sigma}^2\mathrm{SCA}$ and $\hat{\sigma}^2\mathrm{REC}$) were estimated by using Diallel SAS Griffing's random-effect model method 1 diallel analysis using DIALLEL-SAS05 (Zhang et al., 2005). The general pridicted ratio Baker's (1978) $\frac{2\sigma^2\mathrm{GCA}}{2\sigma^2\mathrm{GCA}+\sigma^2\mathrm{SCA}}$; and (vii) broad-sense heritability $\left(\mathrm{h_b^2}\right)$: $\frac{\hat{\sigma}^2\mathrm{GCA}+\hat{\sigma}^2\mathrm{SCA}+\hat{\sigma}^2\mathrm{REC}}{\hat{\sigma}^2\mathrm{phenotypic}}$ (viii) narrow-sense heritability $\left(\mathrm{h_n^2}\right)$: $\frac{\hat{\sigma}^2\mathrm{GCA}}{\hat{\sigma}^2\mathrm{phenotypic}}$., (Link et al., 1995) Coefficient of genetic variation (CVg) was calculated as $\frac{\sqrt{\sigma^2}gca+\sigma^2sca+\ \sigma^2REC}{\mu}$ where μ is the mean value while coefficient of error variation (Kao and Mcvetty, 1987) was computed as $\frac{\sqrt{\sigma^2}e}{\mu}$. (Habarurema et al., 2012; Vieira et al., 2012).

7.3 Results

7.3.1 Gene action

The combined analysis of variance (ANOVA) for the diallel crosses to estimate the amount of variability for the yield component characteristics among the parents, F_1 progenies and their reciprocals are presented in Table 7.1. There were highly significant ($P \leq 0.001$) effects for environments, entry x environment and entry for all measured traits with the exception of plant height (PH) (Table 7.1). Partitioning of the crosses into the mean squares due to general combining ability (GCA), specific combining ability (SCA) were significant for all characters. However, significant ($P \leq 0.05$) mean squares due to reciprocal (REC) was observed only for days to 50% flowering (DTF), days to maturity (DTM) and total biomass (BM). While both the GCA and SCA effects were significant, predominant percentage of sum of squares due to SCA was observed for most of the characters, which is confirmed by the Bakers ratios (Table 7.1). The GCA was only predominant (73.4%) for the number of days to 50% flowering (DTF), (78.3%) for days to physiological maturity (DTM) and (70.7%) for height to the first pod from ground (HFP). The interaction effects of GCA and SCA variances with environment were significant ($P \leq 0.001$) with the exception for days to maturity and SCA x environment for height to the first pod from ground and plant height (Table 7.1). The coefficient of genetic variation to coefficient of error variation ratio (CV_g/ CV_e) was greater than one for all characters indicating that phenotypic differences from genotype were superior to experimental errors. The broad sense heritability was high for all traits ranging from 71.4 to 93.9% (Table 7.1). However, the narrow sense heritability estimates were low for the majority of traits except for the DTF (67.8%), DTM (76.5%), and HFP (75.8%) which had high estimates.

Table 7.1 A 10 x 10 diallel cross analysis for grain yield component over three environments

Source of variation	df	DTF	DTM	BB	HFP	NPPN	NPPP	NSPP	NNP	NWP	PH	HSW	BM
ENV	2	6956.5***	49163***	11.6***	41890***	8.5***	1719.5***	13.1***	112.5***	410.8***	90887***	20.9	24.2***
REP(ENV)	3	85.7*	68.1**	3.8***	571.3***	0.6*	113.3***	3.7***	158.4***	141.6***	4398.1***	145.2***	13.5***
ENTRY	99	241.9***	115.4***	1.9***	288.5***	1.8***	94.2***	2.2***	114.8***	63.4***	174.6	210.4***	5.5***
ENV*ENTRY	198	49.0***	20.1	0.9***	100.6	0.5***	23.0*	0.8***	32.7***	13.1***	153.2	40.6***	0.***
GCA	9	1953.0***	994***	3.9***	2242.5***	1.9***	66.6***	1.3***	96.0***	53.3***	212.7	568.8***	15.9***
SCA	45	86.6***	24.8*	3.0***	107.3	3.2***	177.8***	4.3***	213.7***	120.0***	218.4*	325.2***	7.9***
REC	45	55**	30.2**	0.6	78.8	0.3	16.1	0.3	19.8	8.8	123.3	23.9	0.8*
MAT	9	135.2***	61.2***	0.6	136.9	0.3	15.5	0.3	14.9	10.8	131	29.3	0.9
NMAT	36	34.9	22.5	0.6	64.3	0.3	16.3	0.3	20.9	8.4	121.3	22.6	0.8
GCA*ENV	18	78.6***	27.6	1.6***	257.8***	0.81***	32.1*	1.3***	29.3**	24.7***	244.2*	71.1***	1.7***
SCA*ENV	90	46.6**	18.3	1.5***	83.5	0.7***	28.6**	1.3***	49.9***	17.3***	156.2	58.4***	1.1***
REC*ENV	90	45.5**	20.3	0.3	86.2	0.24	15.6	0.2	16.1	6.6	131.9	16.6	0.5
MAT*ENV	18	102.5***	46.3***	0.4	81.9	0.21	16.1	0.3	17.3	3.8	190.1	9.9	0.8
NMAT*ENV	72	31.3	13.9	0.3	87.2	0.26	15.4	0.2	15.8	7.3	117.4	18.3	0.4
Error		30.8	17.7	0.6	100.7	0.29	18.3	0.4	14.7	8.7	142.1	27.1	0.6
% GCA (SS) contribution		73.4	78.3	17.8	70.7	9.7	6.4	5.3	7.6	7.6	11.1	24.6	26.6
% SCA (SS) contribution		16.3	9.8	69.4	16.9	83.6	85.8	88.2	84.6	86	56.8	70.3	66.6
% REC (SS) contribution		10.3	11.9	12.8	12.4	6.6	7.8	6.5	7.8	6.3	32.1	5.2	6.8
GCA*ENV (%)		14.6	12.5	15.2	23.3	14.2	12.7	14.3	8.2	17.1	14.5	15.9	17.8
SCA*ENV (%)		43.2	41.4	70.4	37.7	64.3	56.8	72.9	69.5	59.9	46.4	65.5	56.8
REC * ENV (%)		42.2	46.1	14.3	38.9	21.6	30.7	12.7	22.4	22.9	39.2	18.6	25.5
Bakers ratio		0.89	0.97	0.08	0.99	0.13	0.21	0.24	0.17	0.18	0.02	0.16	0.21
CV g/ CV e		3.2	2.82	2.2	1.58	3.16	2.81	3.74	3.59	3.45	1.29	1.13	3.98
Mean		61.3	148.3	1.1	57.9	1.28	10.9	1.9	18.6	8.53	135.9	594.5	15.1
H^2b		91	88.8	79.2	71.4	90.3	88.7	89.3	92.8	92.2	28.4	92.3	93.9
H^2n		67.81	76.5	3.5	75.8	5.6	8.6	9.8	7.21	7.46	0.4	8.64	10.77
CV (%)		9.1	2.8	24	17	18	19	14	20	17	4.4	22	20
R^{2}		83.9	95.6	76.9	81.8	79.7	78	80	80.9	80	85.5	78.5	82.9

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; R^{2}:coefficient of determination; H^2b : heritability in the broad sense; H^2n heritability in the narrow sense; general pridicted ratio: $\frac{2\hat{\sigma}^2\mathrm{GCA}}{2\hat{\sigma}^2\mathrm{GCA}+\hat{\sigma}^2\mathrm{SCA}}$; CV g/ CV e: Coefficient of genetic variation: coefficient of error variation; DTF: days to 50% flowering; DTM: days to physiological maturity; BB: number of primary branches; HFP: height from ground to the first pod; NPPN: number of pod per node; NPPP: number of pods per plant; NSPP: number of seeds per pod; NNP: number of node per plant; NWP: number of node with pod which have seed; PH: plant height; HSW: hundred seed weight; BM: total biomass.

7.3.2 Performance of selected crosses for their yield component characters

The mean performance of the selected hybrids with positive SCA effects for majority of the characters is presented in Table 7.2. Highly significant variation ($p<0.001$) was observed among the crosses for all characters. Overall, the days to 50% flowering was recorded from 51.6 days for cross Dosha x Cs-20-DK to 80 days for the cross ILB-4726 x ILB-938. From the selected crosses, days to 50% flowering range from 56.3 days (NC58 x BPL-710) to 75.6 days (ILB-4726 x CS-20-DK). The three crosses NC58 x BPL-710, Moti x ILB-938, and NC58 x Gebelcho had less days to 50% flowering values (Table 7.2). Similarly, days to physiological maturity ranged from 141.3 days for the cross Bulga-70 x Dosha to 157 days for the cross BPL-710 x ILB-4726. Crosses NC58 x Gebelcho, Kasa x Gebelcho and NC58 x BPL-710 had less days to physiological maturity. The number of pods per plant ranged from 9.7 for Moti x ILB-4726 to 21.5 for cross ILB-4726 x CS-20-Dk. The crosses BPL-710 x Bulga -70 and NC58 x ILB-4726 had 19.2 and 18.6 number of pods per plant, respectively. Highest number of nodes with pods which had seed was recorded from the crosses ILB-4726 x Kasa (15.4), NC58 x ILB-4726 (15.1) and 14.7 for ILB-4726 x CS-20-DK (Table 7.2).

Table 7.2 Mean performance of parents and 21 selected faba bean specific cross combinations on the basis of their positive effect for the traits in the diallel crosses over three environments

Genotype	DTF	DTM	BB	HFP	NPPN	NPPP	NSPP	NNP	NWP	PH	HSW	BM	GY
Moti x ILB-938	56.67	151.67	2.19	59.80	2.44	15.00	2.54	21.59	12.61	138.42	1098.13	24.20	5.12
Moti x ILB-4726	62.17	150.17	1.07	65.53	1.19	9.65	2.11	20.86	8.18	137.07	811.65	19.55	4.58
ILB-938 x NC58	62.17	151.50	1.31	62.33	1.61	12.02	2.54	19.45	10.07	135.71	652.70	21.45	4.88
ILB-938 x CS-20-DK	57.17	153.50	1.86	64.33	1.77	13.63	2.55	25.36	11.67	146.78	880.18	26.61	5.53
ILB-938 x Kasa	60.33	150.83	1.39	60.08	1.59	13.58	2.36	19.36	12.10	134.28	630.83	20.12	4.10
ILB-938 x Bulga-70	63.50	151.83	1.50	61.78	1.71	16.06	2.57	21.69	13.39	141.88	774.12	22.11	4.91
NC58 x ILB-4726	60.67	149.33	1.72	57.75	1.85	18.61	2.71	23.50	15.05	135.10	935.35	25.51	5.95
NC58 x BPL-710	56.33	146.00	2.78	57.27	2.48	16.61	2.55	24.65	14.20	144.11	1110.26	24.08	6.22
NC58 x Gebelcho	56.67	144.83	1.40	54.58	1.49	13.41	2.40	17.96	10.13	137.72	591.70	15.74	4.74
Dosha x Gebelcho	61.33	148.50	1.38	62.20	1.38	13.23	1.96	16.54	9.64	142.51	711.75	17.95	4.38
ILB-4726 x CS-20-DK	75.67	154.83	2.29	67.77	2.45	21.45	2.47	21.68	14.71	143.98	1243.40	34.69	5.66
ILB-4726 x Kasa	63.83	155.33	1.52	57.15	1.90	16.49	2.63	24.10	15.35	133.93	1026.45	27.87	6.14
ILB-47 26 x Bulga-70	73.83	153.17	1.84	59.08	2.03	16.37	2.63	24.17	13.03	131.85	1207.81	34.11	6.90
CS-20-DK x ILB-4726	65.00	148.17	1.67	65.87	2.13	16.70	2.66	22.79	14.46	140.38	1148.16	25.17	5.81
CS-20-DK x BPL-710	63.00	150.83	1.55	60.87	1.67	14.04	2.51	24.27	11.76	137.03	764.99	24.28	5.31
CS-20-DK x Gebelcho	62.00	148.50	1.08	55.72	1.35	13.63	2.30	20.68	10.11	133.22	545.42	13.44	4.21
Kasa x ILB-4726	59.67	151.17	1.43	60.45	1.71	16.58	2.83	24.06	13.03	126.95	875.90	22.19	5.54
Kasa x BPL-710	60.00	147.67	2.21	50.48	2.29	15.68	2.35	21.81	11.28	139.63	987.36	33.05	4.03
Kasa x Gebelcho	56.83	144.83	1.13	56.98	1.43	11.08	2.06	21.69	9.19	140.47	652.01	12.91	3.71
BPL-710 x Bulga-70	64.17	148.50	1.79	61.57	2.02	19.15	2.84	24.39	13.26	142.26	856.59	23.66	5.22
Bulga-70 x ILB-4726	63.17	153.17	1.13	59.32	1.39	15.71	2.35	21.68	9.23	138.70	788.42	20.27	5.63
Parent													
Moti	56.00	144.17	2.13	48.98	2.00	16.24	3.70	29.29	12.20	136.03	732.57	21.72	4.69
ILB-938	69.50	152.33	2.05	72.97	1.97	12.65	2.80	22.91	10.61	135.88	1427.51	19.47	2.29
NC58	55.83	144.50	1.35	54.27	1.57	12.61	3.23	27.64	9.49	126.62	430.00	11.59	2.59
Dosha	57.17	145.83	1.66	50.55	2.03	17.08	3.47	30.08	13.67	126.61	1011.93	18.86	4.09
ILB-4726	73.17	155.17	1.35	78.40	1.22	8.86	2.08	23.63	8.02	122.67	802.42	19.28	2.03
CS-20-DK	60.17	142.83	0.97	53.15	1.23	9.57	2.37	19.28	7.80	127.85	460.45	10.18	2.68
Kasa	55.67	142.67	1.19	51.98	1.16	9.84	1.93	19.65	7.40	133.43	380.03	10.29	2.66
BPL-710	65.00	152.17	2.98	60.75	1.44	12.02	3.13	19.43	7.81	129.1	1520.36	29.60	2.35
Gebelcho	60.33	143.67	1.51	58.48	1.62	12.98	2.52	25.89	10.04	134.67	623.77	15.50	3.99
Bulga-70	55.67	144.17	1.48	47.13	2.06	12.96	2.80	23.57	11.44	126.81	758.37	17.27	3.42
Experimental mean	61.30	148.3	1.15	57.90	1.3	10.90	1.95	18.60	8.53	135.92	594.5	15.10	3.46
LSD	1.33	0.83	0.16	1.95	0.11	0.84	0.13	0.78	0.62	2.67	80.09	1.68	0.22
CV %	9.1	2.8	24	17	18	19	14	20	17	8.7	22	20	31

Experimental mean and least square difference are based on over 100 entries.

7.3.4 General combining ability estimates

Six varieties had GCA effects which were significant (P≤0.001) and negative while three had significant (P≤0.001), positive values for days to 50% flowering (Table 7.3). Negative and lower GCA effects are desirable in selecting superior parents for days to 50% flowering, days to physiological maturity and height to the first pod from ground. Moti had the highest negative, significant value for days to 50% flowering followed by Kasa, and NC58. NC58 and Kasa showed significant negative effects for days to physiological maturity (P≤0.05) and height to the first pod from ground (P≤0.001). However, Kasa and NC58 showed negative effects for most of the yield component traits. ILB-4726 had the highest significant positive effects for number of pods per plant, number of nodes per plant, number of nodes with pods which had seed, hundred seed weight and total biomass, while this parent had significant (P ≤ 0.001) positive effects for days to 50% flowering, days to physiological maturity and height to the first pod from ground. ILB -938 and BPL-710 also had significant, positive effects for most of the yield component traits. The parent materials ILB 4726 followed by ILB-938 and BPL-710 had good combining ability for most of the yield component traits.

Table 7.3 The general combining ability (GCA) effects of parents for yield component traits

Parents	DTF	DTM	BB	HFP	NPPN	NPPP	NSPP	NNP	NWP	PH	HSW	BM
Moti	-4.13***	-1.72	0.11	-2.90*	0.07	0.21	0.14*	0.57	0.29	1.67	46.31	0.02
ILB-938	5.07***	3.68**	0.19*	5.60***	0.19***	0.69	0.11	1.34**	0.85***	0.48	115.02***	3.35***
NC58	-3.78***	-2.43*	-0.02	-4.47***	0.02	-0.16	0.03	-0.48	-0.05	-2.04	-84.55*	-1.65*
Dosha	-3.71***	-0.83	-0.19*	-1.30	-0.17**	-0.86*	-0.05	-0.54	-0.64*	1.84	-47.11	-2.18**
ILB-4726	9.51***	4.91***	0.13	6.94***	0.09	0.94*	0.07	1.28***	0.91***	-1.64	212.92***	5.30***
CS-20-DK	-2.28***	-1.82	-0.11	-2.85*	-0.07	-0.19	-0.07	-0.73	-0.33	1.26	-81.84*	-1.65*
Kasa	-3.88***	-2.34	-0.08	-4.37***	-0.04	0.04	-0.1	-0.41	-0.02	-0.92	-104.41***	-1.55*
BPL-710	5.52***	3.55**	0.32***	3.81***	0.15**	1.08*	0.11	0.71	0.58	0.01	125.72***	3.77***
Gebelcho	0.95	-0.65	-0.18*	2.66	-0.17**	-1.24**	-0.15*	-1.19**	-1.14***	-0.46	-96.52***	-3.41***
Bulga-70	-2.51***	-2.35	-0.17*	-3.19*	-0.08	-0.45	-0.08	-0.55	-0.46	-0.19	-85.54*	-2.02*

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; DTF: days to 50% flowering; DTM: days to physiological maturity; BB: number of primary branches; HFP: height from ground to the first pod; NPPN: number of pod per node; NPPP: number of pods per plant; NSPP: number of seeds per pod; NNP: number of node per plant; NWP: number of node with pod which have seed; PH: plant height; HSW: hundred seed weight; BM: total biomass.

7.3.5 Specific combining ability estimates for yield components

Specific combining ability (SCA) effects for all the characters studied are presented in Table 7.4. The negative SCA effects were desirable for days to 50% flowering, days to physiological maturity and height to the first pod from ground. The specific combination of Moti x ILB-938 had significant ($P \leq 0.01$) and negative estimates of SCA for days to 50% flowering. Crosses ILB -4726 x Bulga-70, ILB -4726 x Kasa, ILB -4726 x CS-20-DK and NC 58 x ILB-4726 recorded highly significant ($P < 0.001$) positive SCA effect for number of pods per node, number of pods per plant, number of seeds per pod, number of nodes per plant, number of nodes with pods which had seed, hundred seed weight, and total biomass. Moreover, NC58 x BPL-710, Kasa x BPL-710, CS-20-DK x BPL-710 and BPL x Bulga-70 recorded significant and positive SCA effects for number of pod per node, number of node per plant, number of node per plant, and number of nodes with pods which had seed.

Table 7.4 Estimates of SCA effects for faba bean for selected crosses that had significant effect for yield components from a 10 x 10 diallel cross over three environments

Genotype	DTF	DTM	BB	HFP	NPPN	NPPP	NSPP	NNP	NWP	PH	HSW	BM
Moti x ILB-938	-5.83**	0.67	0.74**	3.84	0.89***	3.67**	0.31	2.35	3.51***	1.80	397.75***	9.55***
Moti x ILB-4726	-1.12	0.94	0.67**	3.66	0.26	0.15	0.10	1.33	0.80	1.54	394.28***	7.77**
ILB-938 x NC58	-1.09	-0.12	-0.05	0.34	0.16	1.31	0.32	1.86	1.17	-0.86	11.58	2.54
ILB-938 x CS-20-DK	-3.98	1.77	0.45*	-1.33	0.37*	3.37*	0.41*	5.27***	2.76**	8.38	153.57	7.24**
ILB-938 x Kasa	-0.83	2.04	0.34	1.81	0.41*	2.62*	0.34	1.33	2.94**	-1.95	69.15	5.77*
ILB-938 x Bulga-70	-0.62	3.95	-0.35	-4.72	-0.08	3.62	-0.14	2.41	2.91	3.20	-524.23*	5.92
NC58 x ILB-4726	-2.13	-0.84	0.31	-1.17	0.37*	5.06***	0.67***	2.76*	4.61***	0.05	286.66*	6.00*
NC58 x BPL-710	-3.69	-0.90	1.08***	2.58	1.16***	4.37***	0.37	4.03**	4.77***	8.83	438.47***	12.77***
NC58 x Gebelcho	-0.41	-1.03	0.26	-1.19	0.15	1.66	0.25	-0.55	1.37	0.04	130.31	2.40
Dosha x Gebelcho	2.73	0.78	0.43	4.16	0.37*	3.08*	0.20	-0.31	2.18*	4.11	215.95*	5.04*
ILB-4726 x CS-20-DK	2.90	0.13	0.81***	4.84	0.98***	7.41***	0.61**	3.13*	5.48***	6.65	470.19***	11.13***
ILB-4726 x Kasa	-3.12	2.40	0.28	-1.65	0.47***	4.64***	0.80***	4.65***	4.77***	-2.92	248.17*	6.14**
ILB-47 26 x Bulga-70	5.18	5.26	0.43	-9.14	0.66*	8.56***	0.56	1.12	4.48**	11.16	494.16*	15.24***
CS-20-DK x ILB-4726	5.33*	3.33	0.31	0.95	0.16	2.38	-0.10	-0.56	0.12	1.80	47.62	4.76
CS-20-DK x BPL-710	-0.83	1.40	0.23	0.20	0.34*	2.78*	0.49*	4.97***	3.47***	-1.53	152.78	4.29
CS-20-DK x Gebelcho	1.71	1.85	0.08	-0.93	0.15	3.20*	0.26	1.99	1.64	-3.18	52.57	2.29
Kasa x ILB-4726	2.08	2.08	0.05	-1.65	0.09	-0.04	-0.10	0.02	1.16	3.49	75.28	2.84
Kasa x BPL-710	-2.26	0.34	0.36	-4.51	0.59***	4.71***	0.33	3.25*	3.07***	6.01	199.59	9.94***
Kasa x Gebelcho	-1.64	-0.38	0.25	1.99	0.34*	1.32	0.41*	3.16*	1.45	4.98	208.04	2.27
BPL-710 x Bulga-70	4.32	2.48	-0.86*	7.31	0.65*	6.50*	-0.49	5.04*	5.44**	11.96	-516.86*	-1.66
Bulga-70 x ILB-4726	5.33*	0.00	0.02	-0.12	0.32	0.33	0.14	1.25	1.90	-3.43	209.70	6.92**

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; DTF: days to 50% flowering; DTM: days to physiological maturity; BB: number of primary branches; HFP: height from ground to the first pod; NPPN: number of pod per node; NPPP: number of pods per plant; NSPP: number of seeds per pod; NNP: number of node per plant; NWP: number of nodes with pods which had seed; PH: plant height; HSW: hundred seed weight; BM: total biomass.

7.3.6 Correlation of GCA and SCA estimates and mean of the yield components

There was significant (P ≤ 0.05) positive correlation between GCA of number of pods per node (r=0.62), number of nodes per plant (r = 0.69) (Table 7.5). There was no significant correlation between *per se* performance of the parents and their GCA effects for most of the yield component traits. However, a positive and significant (P≤0.001) correlation between estimate of GCA effects and mean performance of the parents was observed for days to 50% flowering (r = 0.96). There was also significant (P≤ 0.05) correlation of mean and estimate of GCA effects for hundred seed weight (r=0.66) and total biomass (r=0.61) (Table 7.5).

Correlation among SCA effects estimated across environment indicated that there was highly significant (P ≤ 0.001) and positive correlation among all characters except height to the first pod from ground and plant height (Table 7.5). The SCA effects for days to 50% flowering were significantly (P ≤ 0.05) and negatively correlated with primary branch (r= -0.26) and hundred seed weight (r = -0.23). Highly significant (P ≤ 0.001) correlation was observed between estimate of SCA effect and per se performance for all yield component characteristics (Table 7.5).

Table 7.5 Correlation coefficient of the estimate of SCA effects and mean of the F_1 progenies (above diagonal) and GCA effect of and mean of the parent (below diagonal) for yield components in the 10 x 10 diallel cross of faba bean across three environments

	DTF	NPPN	NPPP	NSPP	NNP	NWP	HSW	BM	DTF E	NPPN E	NPPP E	NSPP E	NNP E	NWP E	HSW E	BM E
DTF	1	0	0.02	-0.06	0.06	-0.01	0.16	0.23*	0.33***	-0.03	0.02	0.02	0.06	-0.01	-0.11	-0.04
NPPN	-0.26	1	0.92***	0.89***	0.86***	0.95***	0.91***	0.92***	-0.15	0.57***	0.58***	0.46***	0.46***	0.58***	0.42***	0.51***
NPPP	-0.45	0.85***	1	0.91***	0.87***	0.96***	0.83***	0.85***	-0.09	0.59***	0.61***	0.49***	0.48***	0.64***	0.42***	0.56***
NSPP	-0.35	0.73*	0.88***	1	0.91***	0.94***	0.84***	0.82***	-0.09	0.54***	0.58***	0.45***	0.47***	0.61***	0.38***	0.52***
NNP	-0.32	0.65*	0.80**	0.68*	1	0.91***	0.83***	0.83***	-0.06	0.55***	0.55***	0.49***	0.52***	0.58***	0.41***	0.53***
NWP	-0.35	0.93***	0.92***	0.72*	0.82**	1	0.88***	0.88***	-0.13	0.56***	0.60***	0.49***	0.48***	0.61***	0.40***	0.54***
HSW	0.56	0.36	0.25	0.34	-0.09	0.18	1	0.94***	-0.08	0.51***	0.54***	0.44***	0.45***	0.52***	0.37***	0.47***
BM	0.41	0.32	0.34	0.47	0.06	0.2	0.85**	1	-0.07	0.50***	0.55***	0.45***	0.46***	0.54***	0.37***	0.47***
DTFE	0.96***	-0.27	-0.45	-0.34	-0.35	-0.39	0.59	0.52	1	-0.18	-0.11	-0.17	-0.15	-0.18	-0.23*	-0.19
NPPN E	0.61	-0.09	-0.24	0.07	-0.28	-0.31	0.55	0.5	0.59	1	0.92***	0.84***	0.84***	0.95***	0.82***	0.90***
NPPP E	0.65*	-0.3	-0.41	-0.1	-0.44	-0.46	0.5	0.5	0.64*	0.93***	1	0.82***	0.84***	0.97***	0.70***	0.85***
NSPP E	0.44	0.18	0.15	0.46	0.12	0.05	0.56	0.64*	0.41	0.88***	0.81***	1	0.91***	0.85***	0.76***	0.77***
NNP E	0.75*	0.02	-0.13	0.05	-0.12	-0.11	0.64*	0.6	0.70*	0.90***	0.91***	0.86***	1	0.86***	0.62***	0.72***
NWP E	0.67*	-0.18	-0.32	-0.05	-0.28	-0.33	0.48	0.45	0.62*	0.95***	0.97***	0.84***	0.95***	1	0.73***	0.86***
HSW E	0.85**	-0.07	-0.16	0.02	-0.1	-0.15	0.66*	0.71*	0.84***	0.79***	0.83***	0.79***	0.94***	0.85***	1	0.85***
BM E	0.86***	-0.2	-0.33	-0.11	-0.3	-0.33	0.64*	0.61*	0.85***	0.86***	0.92***	0.76**	0.94***	0.92***	0.97***	1

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; DTF: days to 50% flowering; NPPN: number of pod per node; NPPP: number of pods per plant; NSPP: number of seeds per pod; NNP: number of node per plant; NWP: number of nodes with pods which had seed; HSW: hundred seed weight; BM: total biomass; E=stands GCA or SCA effect for each traits.

7.4 Discussion

7.4.1 Gene action

The significant variations among the genotypes indicate considerable genetic variation among the parents and their respective progenies for the yield component traits. This study confirmed the previous findings by El-Bramawy and Osman (2012), El- Hady et al. (2007) and Attia and Salem (2006). The significant GCA mean squares for all traits indicated variability of GCA among the parents and this suggests the importance of additive gene effects and that genetic gain could be achievable through selection. The significant GCA and SCA mean square for all traits showed the importance of both additive and non-additive gene effects, respectively, meaning that breeding strategies which exploit both additive and non-additive variance should be emphasized.

The SCA effects were predominant as indicated by the percentage of sum of squares due to SCA which was greater than for the GCA and reciprocal effects for most of the characters. This indicates that non-additive effects were the most influential for the yield components. Similar results were reported by Al-Layla (2014) in the study of mechanism of genetic control of some quantitative traits in faba bean. In the present study, although reciprocal effects were significant for days to 50% flowering, days to physiological maturity and total biomass, its proportional contribution to variation among the genotypes was negligible (<20% of the sum of squares for the crosses). This suggests that the maternal effects were not important in the inheritance of the yield components. The role of reciprocal effects or maternal or cytoplasmic effects has not been reported in the literature for faba bean yield components. In this regard, results from the current study become the baseline. However, the findings of gene action are consistent with previous investigations that supported the preponderance of non-additive gene action for the yield components in faba bean. Similar results indicating the predominance of non-additive gene action for these traits was reported in the study of heterosis and nature of gene action for yield and its components in faba bean (Obiadalla-Ali et al., 2013). The role of non-additive gene effect for plant height, number of branches per plant, number of seed per pod and grain yield was reported by Alghamdi (2009). The small values of general pridicted ratio Baker’s (1978) for these characters also underscore the predominant role of non-additive gene action. Therefore, the breeding strategy of hybridisation would be more appealing than selection to improve most of the yield components of faba bean.

In contrast the GCA was only predominant for a few traits such as the number of days to 50% flowering, days to maturity and height to the first pod from ground suggesting that additive gene action was most important in controlling flowering and maturity traits. Furthermore, the high general pridicted ratio Baker's (1978) for these characters signified a predominant role of additive gene action. This indicated that direct selection could be useful for improving these traits. Similar results were reported for the number of days to 50% flowering in previous studies of F_1 progenies (Farag and Afiah, 2012). The significance of additive gene effects suggests that the best progeny might be derived from the crosses with genotypes having the greatest positive GCA effects.

Due to the preponderance of SCA effects heritability values in broad sense were high for all traits except for the plant height; while heritability in narrow sense were generally low for most of the traits. This confirms previous studies on secondary traits (Alghamdi, 2007; (Obiadalla-Ali et al., 2013). In general, the significant interaction of effects of GCA and SCA variances with environment revealed that the alleles controlling the GCA and SCA behaved differently in the environments tested. Significant genotype x environment has been reported for faba bean yield components (AL-Aysh, 2013). Therefore, genotypes should be evaluated at many locations to estimate the genotype x environment interaction effects.

7.4.2 General and specific combining ability effects for yield components

Result showed that parental line Moti, Kasa and NC58 were the best combiners for days to 50% flowering. In addition, NC58 and Kasa were the best combiners for days to maturity and height to the first pod from ground. However, Kasa and NC58 showed negative effect for yield and most of the yield component traits. Hence, line NC58 and Kasa could be suggested as a source of favorable genes for reducing days to flowering, days to maturity and improving height to the first pod from ground. The line ILB-4726 was the best general combiner and its good combining ability for yield was associated with good combining ability for most of the desirable yield components. Similarly, the parents ILB-938 and BPL-710 were good combiners for yield and most of yield component traits.

The result also revealed that GCA effects for some traits were related to desirable SCA effect of their corresponding crosses. Assessing the performance of parents on the basis of GCA, it was observed that the crosses; Moti x ILB-938, ILB-4726 x Kasa, NC58 x ILB-4726 and NC58 x BPL-710 showed desirable negative SCA effects for earliness to days to 50% flowering. Similarly specific cross combination; ILB-4726 x KasaNC58 x ILB-4726 and NC58 x BPL-710 had desirable negative SCA effect for days to maturity associated with desirable positive SCA effect for yield and most of the yield component traits. In such combinations it can be suggested that additive and non-additive genetic systems present in the crosses are acting in the same direction to maximize the character. This also suggests that to increase the concentration of favourable alleles which is desired by breeder crosses should be done where at least one parent has high GCA effect.This is in agreement with the results of Obiadalla- Ali et al. (2013).

The specific combination of ILB-938 x NC58, NC58 x ILB-4726, NC58 x BPL-710, NC58 x Gebecho and Kasa x Gebelcho had desirable negative SCA effects for days to maturity implying that they could be used to produce early maturing pure lines. The crosses ILB -4726 x Bulga-70, ILB -4726 x Kasa, ILB -4726 x CS-20-DK and NC 58 x ILB-4726, showed significant, desirable SCA effects for yield components indicating a true to type relationship among the crosses for yield component traits (Srivastava et al., 2012). The two crosses NC58 x ILB-4726 and ILB-4726 x Kasa had desirable SCA effects for earliness to flowering with associated desirable SCA effects for yield components. These promising crosses could be used for breeding programmes to produce pure lines with all desirable traits.

7.4.3 Correlation of GCA and SCA estimates and mean of yield components

In this study, significant correlation between estimates of GCA effects and *per se* mean performance of the parents was observed for days to 50% flowering. This suggested that selection for early flowering can be done on the basis of parental performance. Parents which gave the best *per se* performance for this trait were also the best general combiners indicating a positive association between the two parameters. This is in agreement with previous studies on soybean positive correlation between parental rust resistance and GCA estimates suggested that selection of parents could be based on parent performance (Maphosa et al., 2012). Similarly, there were significant correlations between estimates of GCA effects and mean performance of the parents for hundred seed weight and total biomass. These correlations between GCA and corresponding parental effects suggest that parent *per se* evaluations would be reliable indicators of hybrid performance for hundred seed weight and total biomass. Therefore, parental genotypes with both high GCA effects and mean performance could be suggested as good general combiners in the hybridization programmes of faba bean. In contrast, in this study there was no significant correlation between mean performance of the parents and their GCA effects for most of the yield component traits.

Correlation coefficients between the SCA effects and the mean values were worked out to establish the association between these two and judge whether selection of cross combinations based on performance will be effective. In this study, the correlation between SCA effect and *per se* performance was significant for all yield component traits. Therefore, *per se* performance with the SCA effects should be considered for evaluating the superiority of a cross. Although the *per se* performance may be more important where development of F_1 progenies will be the ultimate objective (Choudhary and Didel, 2014).

7.5 Conclusion

The present study indicated that the GCA and SCA effects were significant for most characters. This indicated the importance of both additive and non-additive gene effects for governing the yield components in faba bean, which confirms previous findings. However, the GCA effects were important for three, while the non-additive gene action was important for nine traits, showing that the non-additive gene effects were preponderant. Therefore, the hybridization strategy would be effective to improve the yield component characters of faba bean. The reciprocal effects were negligible (<20%) hence they would not cause much complication in selection. The role of cytoplasmic effects for the control of yield components in faba bean has been scarcely reported in the literature. The parents and crosses with superior combining ability and exhibited high utility for pedigree crosses would be recommended for exploitation in the faba bean programmes.

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Chapter 8

Heterosis and path analysis for grain yield and chocolate spot disease resistance in faba bean

Abstract

A study was undertaken with the objective to estimate better parent and mid-parent heterosis for yield and chocolate spot resistance and to study the direct and indirect effects of yield components to yield in faba bean (*Vicia faba* L.) in Ethiopia. Ten genetically diverse inbred lines were crossed in a full diallel to produce 90 F_1 progenies. The parents and their 90 F_1 progenies in the diallel crosses were evaluated in a 10 x 10 alpha lattice design with two replications at three locations. Data were analysed using the Gardner and Eberhart's analysis II and PATHSAS using SAS program. The maximum heterosis for grain yield (t ha^{-1}) was 162.3% for mid-parent and 133.9% over the better-parent. The crosses NC58 x ILB-4726, ILB-4726 x Kasa, NC58 x BPL-710, ILB-938 x CS-20-DK, ILB-938 x CS-20-DK and CS-20-DK x BPL-710 are recommended for faba bean breeding for grain yield. Similarly, crosses ILB-4726 x kasa, ILB-4726 x Bulga-70, CS-20-DK x Gebelcho, NC58 x ILB-4726, Kasa x BPL-710 and ILB-938 x Kasa are recommended for chocolate spot resistance breeding. The three crosses ILB-4726 x Kasa, ILB-4726 x Bulga-70, NC58 x ILB-4726 suggested for faba bean breeding aiming for grain yield and chocolate spot disease resistant development. The path coefficients from path analysis indicated that the number of nodes with pods which had seed and total biomass, had positive direct effect on the grain yield and hundred seed weight, primary branch, number of pods per plant, number of seeds per pod and number of node per plant. Selection based on number of nodes with pods which had seed and total biomass are recommended in selection of high yielding varieties in faba bean breeding programmes.

Key words: Chocolate spot; faba bean; heterosis; path analysis; yield.

8.1 Introduction

Faba bean (*Vicia faba* L.) is a good source of protein, starch, cellulose and minerals (Haciseferogullari et al., 2003). It constitutes the main dish particularly for the low income groups in the Middle East and most parts of the Mediterranean, China and Ethiopia (Bond et al., 1985; Ferris and Kaganzi, 2008). Faba bean is also a good break crop in cereal production systems because considerable amounts of atmospheric nitrogen can be fixed through symbiosis thus reducing the need for farmers to use artificial fertilisers (Lindemann and Glover, 2003). Despite its importance, the higher productivity of faba bean is 1666 kg ha^{-1} and the global area under faba bean cultivation has declined over the last five decades (FAOSTAT, 2014). Low productivity of faba bean is related to several constraints including lack of high yielding varieties and chocolate spot diseases (Gnanasambandam et al., 2012). Therefore, to increase production of faba bean in Ethiopia, research should focus on identification of high-yielding and resistant genotypes adapted to the local production conditions.

Heterosis (hybrid vigour) is a quantitative measure of the superiority of a hybrid compared with their parents (Fehr, 1987; Fu et al., 2014). It has been widely used in breeding programmes for identification of genetically divergent populations as base for development of inbred lines, to develop crosses with improved characters of interest in faba bean (El-Hady et al., 2006; Alghamdi, 2009), common bean (Tiruneh et al., 2013), wheat (Jatoi et al., 2014), maize (Munaro et al., 2011) and other field crops. Heterosis is described by the trait-specific performance of hybrids relative to the average of its two parents, termed mid-parent heterosis (MPH) or relative to the parent having the best value for the trait, termed best-parent heterosis (BPH) (Duvick, 1999). In faba bean, heterosis for yield has been demonstrated and hybrid varieties have been proposed (Link et al., 1996). Heterotic effects over mid and better-parents were reported in faba bean crosses (Alghamdi, 2009). Zeid et al. (2004) also reported a stable superiority of the hybrids in faba bean over their inbred parents with marked and varying amount of heterosis.

Faba bean is a self - pollinating crop though significant partially allogamous with the rate of 10 to 80% out-crossing with an average of 35% was reported depending on the environment and genotypes (Bond, 1987; Suso and Moreno, 1999). Thus, populations are highly heterogeneous and considerable potential exists to select within populations for specific traits as practiced for disease resistance (Hanounik and Robertson, 1989). Since faba bean is a predominately self-pollinated crop, commercial products of F_1 seed are not currently feasible. However, heterosis provides the basis of genetic diversity, guide for choice of desirable parents for developing superior F_1 progenies to exploit hybrid vigour and building gene pools to be employed in breeding programmes. Moreover, the study of magnitude of heterosis has a direct effect on the breeding methodology to be followed in varietal improvement.

Component characters may influence yield either jointly or singly and either directly or indirectly through other related characters (Sincik and Goksoy, 2014). Thus, selection on the basis of *per se* performance without considering the yield component character may not be effective. Subsequently, understanding of the association of yield component characters with grain yield and among themselves is essential for formulating selection indices. This can be determined by path analysis, an efficient statistical technique specially designed to quantify the interrelationship of different components and their direct and indirect effects on yield (Dewey and Lu, 1959).

Path co-efficient value measures the magnitude of direct and indirect effects of characters on complex dependent characters such as yield, and thus enables the breeders to judge the important component during selection. In a study of path analysis between traits and yield selection based on 1000-seed weights and crude protein ratios were recommended due to their significant direct effect to yield in sunflower breeding programme (Sincik and Goksoy, 2014). Path analysis of yield and its components were examined by different authors on different crops such as *Eruca sativa* L. (Jat and Jakhar, 2014), soybean (Garud et al., 2014), mung bean (Dhuppe et al., 2005) and maize (Amini et al., 2013). Significant and direct correlation of plant height with yield was reported in the study of path analysis of faba bean yield and its components (Azarpour et al., 2012).

Grain yield is a complex quantitative character influenced by environmental conditions. Therefore it is necessary to understand the relationships existing between grain yield and other metric traits of the crop. Such information is scarce for faba bean breeding in Ethiopian condition. Therefore, the objective of the present study were: i) to quantify the magnitude and effect of heterosis for yield and chocolate spot resistance of 10 faba bean genotypes including 90 F_1 pogenies from a diallel cross mating design; ii) to investigate the relationships among various yield components of faba bean and iii) to determine the direct and indirect effects of other yield component characters on faba bean yield.

8.2 Materials and methods

8.2.1 Faba bean germplasm

Ten faba bean parental lines having different levels of resistance for chocolate spot (CH) disease were obtained from Holetta Agricultural Research Center (HARC) and International

Center for Agricultural Research in the Dry Area (ICARD) (Table 8.1). The parental lines were crossed in a 10 x 10 full-diallel mating scheme to form 90 F_1 progenies including the reciprocals to assess the existence of maternal effect for the traits of interest .

Table 8.1 Characteristic of faba bean inbred lines used in the study

				Estimate of GCA effect for		
Parent lines	**Pedigree**	**[a] Genotypic cluster group**	**Resistant level to CH**	**CH resistance**	**Grain yield**	**Source**
ILB-4726	ICARDA	I	Resistant	Negative	Positive	ICARDA
ILB-938	ICARDA	I	Resistant	Negative	Positive	ICARDA
BPL-710	ICARDA	I	Resistant	Negative	Negative	ICARDA
Moti	ILB-4432 x kuse-2-27-33	II	Moderately resistant	Positive	Positive	HARC
Dosha	Landrace collection	II	Moderately resistant	Positive	Negative	HARC
Gebelcho	Tesfa x ILB-4726	II	Moderately resistant	Negative	Negative	HARC
Kasa	Landrace collection	II	Susceptible	Positive	Negative	HARC
NC58	National collection	III	Susceptible	Positive	Negative	HARC
CS-20-DK	National collection	III	Susceptible	Positive	Positive	HARC
Bulga-70	Landrace collection	III	Susceptible	Positive	Negative	HARC

[a] Genotypic cluster group indicated the cluster group of the parent lines based on the result of molecular analysis using SSR markers; ICARD: International Center for Agricultural Research in the Dry Area; HARC; Holetta Agricultural Research Center; GCA General combining ability; CH: Chocolate spot disease

8.2.2 Experimental sites

The experiments were conducted at three locations in Ethiopia in 2013; Holetta Agricultural Research Centre (HARC) (09º03'N, 38º30'E, and 2390 m a. s. l.). The average maximum and minimum temperature at HARC in 2013 from June to December was 22.5ºC and 8.5ºC respectively and the average rainfall received was 533.6 mm with soil pH of 4.6. Kulumsa Agricultural Research Centre (KARC) (08º00'N, 39º09'E, and 2211 m a. s. l.). The average maximum and minimum temperature at KARC in 2013 from June to December was 22.5ºC and 10.9ºC respectively and the average rainfall received was 584.1 mm with soil pH of 5.2.; Kofele research site (07º04'N, 38º48'E, and 2620 m a. s. l.). The average maximum and minimum temperature at Kofele in 2013 from June to December was 18.3ºC and 2.3ºC respectively and the average rainfall received was 1077.4 mm. with soil pH of 4.4.

8.2.3 Experimental design and management

The 90 F_1 progenies (i.e., including their reciprocals) and 10 parental lines were planted in a 10 x 10 α- lattice design in single row of 3 m at spacing and 40 cm between rows and 6 cm between plants. To minimize inter-plot interference each test genotype was bordered by a susceptible faba bean variety NC58 which had moderate performance. Fertilizer was applied at 100 kg ha $^{-1}$ of DAP at planting. The trials were kept weed-free by hand weeding at different times as per practice of the respective sites.

8.2.4 Data collection

Data for the following agronomic traits were recorded on a plot basis at all locations; days to 50% flowering (DTF) and days to physiological maturity (DTM) for the entire plot. Fifteen plants per genotype were randomly selected to record the data on number of primary branches (BB), height from ground to the first pod (HFP), number of pods per node (NPPN), number of pods per plant (NPPP), number of nodes with pods which had seed (NWP), number of nodes per plant (NNP), number of seeds per pod (NSPP) and plant height (PH). Data total biomass (BM) at harvest, hundred seed weight (HSW) from randomly selected 100 seeds from each plot and grain yield was taken from the entire plot. Grain yield adjustments were made by weighing the oven drying and adjusting to constant moisture level of 10%. The total biomass and grain yield recorded on plot basis were converted to t ha-1 for statistical analysis.

Genotype reaction to Botrytis fabae was evaluated seven days after inoculation and disease severity scoring was taken six times weekly with 7 days interval from the same five randomly pre-tagged faba bean plants per genotypes. In addition general disease assessment of response of genotypes on disease severity of chocolate spot was recorded from the whole plot once at on average at 88 days after planting. The severity of chocolate spot was recorded as percentage of leaf area infected as follows: 1%-no disease symptoms or very small specks (highly resistance); 3%-few small disease lesions (resistant); 6%-small coalesced lesions, with some defoliation (moderately resistant); 12%, large coalesced sporulating lesions, 20% defoliation (moderately susceptible); 25% large coalesced sporulating lesions, 50% defoliation and some dead plants (susceptible); and 50%-extensive, heavy sporulation, stem girdling, blackening and death of more than 80% of the plants (highly susceptible) (Bernier et al., 1993; Bernard et al., 2006).

8.2.5 Data analysis

The disease data severity score collected weekly in the field was used to calculate the area under the disease progress curve (AUDPC). Area under the disease progress curve (AUDPC) which is the most common mathematical tool used to model plant disease epidemics (Contreras-Medina et al., 2009) was calculated for each genotypes using the disease severity score according to the following formula proposed by Shaner and Finney (1977).

$$AUDPC = \sum_{i=1}^{n} 1/2[(yi + 1 + yi)(xi + 1 - xi)]$$

Where: yi = the cumulative disease severity percentage of infected plants at the ith observation (day i), xi = time (days) at the i th observation, n = total number of symptom observations.

AUDPC was calculated using Microsoft excel. The estimate of AUDPC was normalized by dividing with the total area of the graph *(i.e.* the number of days between the first and the last readings multiplied by maximum potential AUDPC), for a better visual comparison among genotypes over location (Mohapatra et al., 2008). The normalized AUDPC was referred to as the relative area under disease progress curve (RAUDPC).

ANOVA of heterosis for chocolate spot resistance and yield:

Gardner – Eberhart II

Analyses of variance (ANOVA) to detect differences among the 100 entries for each trait in the study were separately performed on the data collected across environment. An inference was made from diallel cross mating technique about heterosis and its partitions; average heterosis, variety heterosis, and specific heterosis among the ten genotypes for chocolate spot resistance and yield in SAS (SAS Institute, 2012). The following models were used to determine the sums of squares for the analysis following the notation of Gardner and Eberhart:

(i) $X_{jj'} = u + (1/2)(v_j + v_{j'}) = (B'G)_1$
(ii) $X_{jj'} = u + (1/2)(v_j + v_{j'}) + v\underline{h} = (B'G)_2$
(iii) $X_{jj'} = u + (1/2)(v_j + v_{j'}) + v\underline{h} + v(h_j + h_{j'}) = (B'G)_3$
(iv) $X_{jj'} = u + (1/2)(v_j + v_{j'}) + v\underline{h} + v(h_j + h_{j'}) + vs_{jj'} = (B'G)_4$

In each of the models, u, v_j, h, and s_{jj}, indicate the mean and variety and heterosis effects. The coefficient *v* in these models is zero when j=j' and one when j ≠ j'. Since the phenomenon of heterosis is important, the analysis maximizes the information on variety performance and the expression of heterosis of their crosses (Hallauer et al., 2010). Estimates of the variety and heterosis effects can be determined for each of the constants in the models. Data from the Gardner and Eberhart models II were analysed using the DIALLEL-SAS05 (Zhang et al., 2005) in SAS (SAS Institute, 2012).

In general, partitions of entry sum of squares were as follows:

$X_{ij} = U + ½(V_i + V_j) + vh + v(h_i + h_j) + vS_{ij}$

- U= mean effects
- Vj = variety effects
- h = average heterosis effects (Parents vs. Crosses)
- hi and hj = variety heterosis effects
- Sij = specific heterosis effects for the cross IxJ
- v = coefficient ranging from 0 - 1 when v = 0 then i=j (it's a self), when v=1 then i≠j

8Heterosis was also used to examine heterotic relationships among the lines (Falconer and Mackay, 1996). Mid-parent heterosis (MPH) determined using the mid-parent (MP) calculated as: $\mathrm{MPH} = \frac{\mathrm{F_1 - MP}}{\mathrm{MP}} \mathrm{x}\, 100$ where $\mathrm{F_1}$ is the performance of hybrids, $\mathrm{MP} = \frac{(\mathrm{P_1 + P_2})}{2}$ in which $\mathrm{P_1}$ and $\mathrm{P_2}$ are the performance values of parents, respectively. Better-parent heterosis (BPH) (Heterobeltiosis) (%) was calculated as: $BPH = \left(\frac{F_1 - BP}{BP}\right) x\, 100$ Where F_1= mean of the F_1 progenies performance, BP = the mean of the better-parent /superior parent (Sleper and Poehlman, 2006). Path co-efficient analysis among the yield and yield component traits was made using PATHSAS: the SAS computer program for path coefficient analysis of quantitative data as described by Cramer and Wehner (1999) in SAS (SAS Institute, 2012).

8.3 Results

8.3.1 Heterosis

Analysis of variance of the combined diallel cross over there environment for grain yield and chocolate spot disease is presented in Table 8.2. The mean square for the variety was highly significant ($P \leq 0.001$) for chocolate spot resistance and significant ($P \leq 0.05$) for grain yield. The heterosis mean square was highly significant ($P \leq 0.001$) for chocolate spot disease resistance and grain yield. Highly significant ($P \leq 0.001$) variety heterosis was also observed for chocolate spot resistance and grain yield. Specific heterosis was also found significant ($P \leq 0.001$) for grain yield.

Table 8.2 Combined analysis of variance of heterosis for grain yield and chocolate spot disease in the diallel over three locations in the diallel cross

Source of variation	df	GDS	RAUDPC	GY
Env	2	1487.2***	1045481.8***	124.02***
REP(Env)	3	130.0***	217250.6***	6.03**
Entry	54	160.9***	107161.1***	12.89***
Env*Entry	108	15.2*	17899.6**	1.90***
Variety SS	9	851.93***	389309.84***	3.13*
Heterosis SS	45	22.80***	50731.34***	14.84***
Average HET SS	1	2.63	48.9	10.56
Variety HET SS	9	45.89***	173197.52***	7.87***
Specific HETEROSIS	35	17.44	20688.11	16.757***
Variety x Env	18	30.09***	33923.01***	1.36
Heterosis x Env	90	12.27	14694.88	2.01***
Average HET x Env	2	13.53	77617.09***	9.55***
Variety HET x Env	18	9.89	12733.67	1.310
Specific HET x Env	70	12.84	13401.41	1.98***
Error		11.27	11352	1.0935
CV		21	21	30
R^2		88.2	85	86.8
Mean		15.4	0.29(503.6)	3.460

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; R^2: coefficient of determination; CV: coefficient of variation; RAUDPC: relative area under disease progress curve number in parenthesis is mean for AUDPC

The estimate of parent for heterosis effect and estimate of specific heterosis effect of the F_1 progenies for grain yield (t ha^{-1}) is presented in Table 8.3. Highly significant ($P \leq 0.001$) and positive estimate of heterotic effect was observed for parent ILB-4726. Similarly the parental line ILB-938 and BPL-710 had estimate of non-significant but positive effect for grain yield. On the other hand Gebecho and Dosha had significant ($P \leq 0.05$), negative estimates of heterotic effect for grain yield. Significant ($P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$) positive estimates of specific heterosis effect were recorded for 12 crosses for grain yield (Table 8.3). Highly significant estimate ($P \leq 0.001$) and positive heterotic effect were recorded for the specific hetrotic crosses ILB-4726 x Bulga-70, NC58 x BPL-710, ILB-4726 x Kasa, ILB-938 x CS-20-DK and BPL-710 x Bulga-70 for grain yield.

Table 8.3 Estimate of parent heterosis (diagonal) and specific heterosis of the cross for grain yield (t ha-1) across the three locations

	Moti	ILB-938	NC58	Dosha	ILB-4726	CS-20-DK	Kasa	BPL-710	Gebelcho	Bulga-70
Moti	**-0.676**	1.32**	0.292	-0.626	0.052	0.152	-0.307	-0.072	0.036	-0.844
ILB-938		**0.528**	1.14*	-0.006	-2.6***	1.89***	0.720	-2.56***	-1.26*	1.36**
NC58			**0.317**	-1.15*	1.48**	-2.11***	-1.74**	2.61***	1.45**	-1.99***
Dosha				**-0.906***	0.351	-0.634	0.564	0.196	1.56**	-0.260
ILB-4726					**1.378*****	1.29*	2.03***	-2.98***	-2.25***	2.63***
CS-20-DK						**0.166**	-1.67**	1.81**	1.027	-1.76**
Kasa							**-0.086**	0.787	0.787	-1.18*
BPL-710								**0.361**	-1.59**	1.81***
Gebelcho									**-0.785***	0.232
Bulga-70										**-0.297**

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

8.3.2 Mid-parent and better-parent Heterosis for grain yield and chocolate spot disease resistance

Mid-parent and better-parent heterosis and estimate of heterosis effect for grain yield

The mid-parent and better-parent heterosis for grain yield is presented in Table 8.4. The heterosis exhibited by crosses over the respective mid-parent range from -48.5% (CS-20-DK x Bulga-70) to 162.3% (NC58 x ILB-4726) for grain yield t ha^{-1}. Similarly, heterobeltiosis for grain yield t ha^{-1} ranged from -54.1% (CS-20-DK x Bulga-70) to 133.9% (NC58 x ILB-4726) over the better-parent. Among the hybrids, the cross NC58 x ILB-4726 had the highest mid-parent (162.3%) and better-parent (133.9%) heterosis. Positive heterotic effects are desirable for grain yield. Twenty-two crosses exhibited positive mid-parent heterosis and 15 crosses exhibited positive better-parent heterosis for grain yield t ha^{-1}. Crosses NC58 x ILB-4726 (162.3%) followed by ILB-4726 x Kasa (148.6%), ILB-938 x CS-20-DK (143.6%), ILB-938 x CS-20-DK (134.6%), ILB-4726 x Bulga-70 (129.7%), and CS-20-DK x BPL-710 (114.2%) had highest mid-parent heterosis. Similarly, NC58 x ILB-4726 (133.9%) followed, NC58 x BPL-710 (122.3%), ILB-4726 x Kasa (119.3%), ILB-938 x CS-20-DK (117.4%), ILB-938 x CS-20-DK (114.1%) and CS-20-DK x BPL-710 (101%). On the contrary, 23 crosses had negative mid-parent heterosis and 30 better-parent heterosis. The crosses; CS-20-DK x Bulga-70, ILB-938 x BPL-710 and NC58 x Bulga-70 had -48.5%, -46.8%, -46.9% mid-parent heterosis and CS-20-DK x Bulga-70, NC58 x Bulga-70 and BPL-710 x Gebelcho had -54.1, -53.3, and -50.7 better-parent heterosis for grain yield.

Table 8.4 Percent mid-parent heterosis (below diagonal) and better-parent heterosis (above diagonal) of the 90 F_1 progenies for grain yield (t ha-1) across the three locations in the diallel cross

	Moti	ILB-938	NC58	Dosha	ILB-4726	CS-20-DK	Kasa	BPL-710	Gebelcho	Bulga-70
Moti		10.84	-21.44	-38.73	11.91	-34.76	-37.85	-24.43	-39.47	-42.61
ILB-938	49.01		87.99	-18.16	11.91	117.38	59.00	-47.70	-35.68	42.29
NC58	1.24	99.63		-47.62	133.96	-44.32	-37.83	122.29	-2.04	-53.33
Dosha	-34.60	5.05	-35.80		-2.16	-21.90	-27.68	-17.20	-2.02	-37.16
ILB-4726	56.22	-17.96	162.29	30.86		119.32	-42.18	-45.57	82.88	114.06
CS-20-DK	-16.97	134.58	-43.36	-5.58	143.63		-27.29	101.04	-3.15	-54.09
Kasa	-20.74	71.13	-36.94	-12.36	148.98	-27.08		-9.89	-48.02	69.01
BPL-710	0.68	-46.99	133.04	5.23	-37.95	114.23	79.61		-50.73	49.81
Gebelcho	-34.62	-18.20	18.87	-0.77	-27.83	15.92	8.11	-37.97		-23.45
Bulga-70	-33.68	70.66	-46.84	-31.55	129.69	-48.48	-41.52	77.72	-17.59	

Mid-parent and better-parent heterosis and heterosis effect for chocolate spot disease resistance

The heterosis over the mid-parent and better-parent for chocolate spot disease resistance is presented in Table 8.5. The magnitude of heterosis over the respective mid-parent ranged from -29.39% (ILB-4726 x Kasa) to 116.35% (ILB-938 x ILB-4726) for chocolate spot disease resistance based on the general disease score (GDS). It ranged from -43.2% (ILB-4726 x CS-20-DK) to 176.3% (ILB-938 x ILB-4726) based on relative area under disease progress curve (RAUDPC). Heterobeltiosis for chocolate spot disease resistance ranged from -60.5% (ILB-4726 x kasa) to 111.1% (ILB-938 x ILB-4726) over better-parent. Negative heterotic effects are desirable for chocolate spot disease resistance. Twenty-nine crosses exhibited negative heterosis over mid-parent ranged from -29.4 to -4.56 and forty-one crosses exhibited negative heterobeltiosis over better-parent ranged from -60.5 to -4.5 for chocolate spot disease resistance based on the GDS. Similarly, 29 crosses exhibited negative heterosis over mid-parent ranged from -21.4 to -1.2 and 42 crosses exhibited negative heterosis over better-parent ranged from -43.2 to -1.8 based on the RAUDPC.

The best hybrids with marked performance in negative direction for chocolate spot resistance were ILB-4726 x Kasa, CS-20-DK x Gebelcho, Kasa x Bulga-70, Dosha x Gebelcho, Dosha x Kasa and ILB-4726 x Bulga-70 with -29.4%, -27.9%, -25.7%,-23.3%,-22.8% and -22.6% mid-parent heterosis respectively (Table 8.5). These crosses also recorded negative direction performance for mid-parent heterosis based on the RAUDPC. Likewise, hybrids ILB-4726 x Kasa, ILB-4726 x Bulga-70 had marked performance in negative direction ILB-938 x Kasa, NC58 x ILB-4726, and Kasa x BPL-710 with -60.5%, -

55.3%,-55.1%,-49.3%,-49.1% and -47.3% better-parent heterosis, respectively. Similar performance in negative direction and magnitude were also recorded for these hybrids based on the RAUDPC disease assessment. On contrary, the hybrids ILB-4726 x BPL-710, ILB-938 x BPL-710 and ILB-4726 x BPL-710 performed in positive direction for chocolate spot resistance with high magnitude of mid-parent and better-parent heterosis based on both GDS and RAUDPC value assessments (Table 8.5).

Table 8.5 Relative heterosis and heterobeltiosis of top twenty and bottom three faba bean hybrids for chocolate spot disease resistance based on general disease severity score (GDS) and relative area under disease progress curve values (RAUDPC)

		Chocolate spot disease (%)			
F_1 progenies	MP-GDS	MP-RAUDPC	F_1 progenies	BP-GDS	BP-RAUDPC
ILB-4726 x Kasa	-29	-4.46	ILB-4726 x Kasa	-60.52	-38.60
CS-20-DK x Gebelcho	-28	-18.86	ILB-47 26 x Bulga-70	-55.28	-37.54
Kasa x Bulga-70	-26	-16.51	ILB-938 x Kasa	-55.07	-27.30
Dosha x Gebelcho	-23	-20.25	NC58 x ILB-4726	-49.28	-39.45
Dosha x Kasa	-23	-14.71	ILB-4726 x CS-20-DK	-49.11	-43.20
ILB-47 26 x Bulga-70	-23	-5.08	Kasa x BPL-710	-47.31	-11.94
ILB-938 x Kasa	-20	-20.84	ILB-938 x NC58	-45.05	-27.92
CS-20-DK x Bulga-70	-19	-15.98	BPL-710 x Bulga-70	-44.96	-24.56
Kasa x Gebelcho	-19	-8.62	CS-20-DK x BPL-710	-44.50	-27.97
NC58 x Bulga-70	-19	-9.76	ILB-938 x CS-20-DK	-44.34	-26.52
Moti x Kasa	-17	-10.55	NC58 x BPL-710	-43.46	-29.68
NC58 x CS-20-DK	-15	-17.15	CS-20-DK x Gebelcho	-41.14	-29.44
CS-20-DK x Kasa	-14	-1.95	Kasa x Gebelcho	-40.64	-20.53
NC58 x Dosha	-14	-13.86	Dosha x Kasa	-40.20	-21.70
Dosha x CS-20-DK	-13	-15.59	Moti x Kasa	-37.13	-14.99
ILB-4726 x CS-20-DK	-12	-3.36	Dosha x ILB-4726	-37.02	-24.90
NC58 x Kasa	-12	1.04	Kasa x Bulga-70	-34.53	-19.45
NC58 x Gebelcho	-12	-4.25	Dosha x BPL-710	-32.20	-18.09
NC58 x ILB-4726	-12	2.18	ILB-938 x Gebelcho	-31.85	-22.32
Moti x CS-20-DK	-12	-17.77	ILB-938 x Bulga-70	-31.67	-7.72
ILB-4726 x BPL-710	79	120.89	ILB-4726 x BPL-710	52.23	74.64
ILB-938 x BPL-710	106	164.67	ILB-938 x BPL-710	79.49	121.41
ILB-938 xILB-4726	116	196.53	ILB-938 xILB-4726	111.08	176.28

GDS: general disease score; RAUDPC: relative area under disease progress curve value; MP: Mid-parent heterosis; BP: Better-parent heterosis

The estimate of parent *per se* and heterosis effect and estimate of specific heterosis effect of the F_1 progenies for chocolate spot disease resistance are presented in Table 8.6 and 8.7. Highly significant ($P \leq 0.001$) and negative estimate *per se* were recorded for the genotypes ILB-938, ILB-4726 and BPL-710 based on both GDS and RAUDPC assessments (Table 8.6). Nonetheless, significant ($P \leq 0.01$) and positive estimate of heterotic effect was observed for these three parents for chocolate spot resistance based on both GDS and RAUDPC assessments. Negative estimates of specific heterosis effect were observed on 19 and 21 crosses for chocolate spot resistance based on GDS and RAUDPC assessments, respectively (Table 8.7).

Table 8.6 Estimates of parent per se and heterosis for chocolate spot disease based on general disease severity score (GDS) and relative area under disease progress curve value RAUDPC value across the three locations in the diallel cross.

	Estimate of GDS		Estimate of RAUDPC	
Parameter	Per se	Heterosis GDS	Per se	Heterosis RAUDPC
Moti	2.87	0.74	79.96	76.29*
ILB-938	-11.79***	2.51*	-362.32***	152.70***
NC58	7.94***	-1.76	346.5***	-166.35***
Dosha	2.09	-1.07	79.92	-31.04
ILB-4726	-11.60***	0.43	-341.72***	47.02
CS-20-DK	7.85***	2.33*	176.50**	-63.17
Kasa	6.64***	0.88	326.77***	98.24**
BPL-710	-10.19***	2.39*	-293.32***	145.25***
Gebelcho	-0.77	-1.61	12.24	-50.56
Bulga-70	6.94***	-0.17	135.37*	-11.90

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

Table 8.7 Estimate of specific heterosis of the crosses for chocolate spot disease based on GDS (below diagonal) and based on the RAUDPC value (above diagonal) across the three locations in the diallel cross

	Moti	ILB-938	NC58	Dosha	ILB-4726	CS-20-DK	Kasa	BPL-710	Gebelcho	Bulga-70
Moti		8.89	-107.24*	61.58	45.76	-42.99	-21.86	48.35	-24.73	32.23
ILB-938	0.42		-42.43	36.34	12.50	-1.70	-61.57	18.15	-44.70	74.52
NC58	-2.46	-0.40		68.78	48.37	-14.07	47.75	-106.99*	95.95	9.87
Dosha	1.29	-1.10	1.34		18.95	-3.32	-104.56	-29.19	-60.45	11.86
ILB-4726	0.02	1.39	0.06	0.91		-66.30	-42.65	12.74	54.17	-83.54
CS-20-DK	-0.95	0.24	0.48	1.77	-1.41		96.05	62.57	-35.32	5.08
Kasa	-0.59	-1.87	2.33	-2.42	-1.54	1.99		35.81	42.38	8.66
BPL-710	0.65	0.85	-2.91	-1.18	2.15	0.69	0.83		-5.03	-36.41
Gebelcho	-0.26	-1.28	2.55	-1.61	1.42	-3.00	1.31	0.32		-22.28
Bulga-70	1.88	1.75	-0.99	1.02	-2.97	0.19	-0.03	-1.40	0.55	

*, Significant at 0.05, probability level

8.3.2 Path coefficient analysis of grain yield (t ha^{-1}), chocolate spot disease and yield components in faba bean in the diallel crosses

The direct and indirect effects of yield component characters along with their correlation coefficients with grain yield are presented in Table 8.8 and 8.9. The results of path coefficient analysis based on grain yield as a dependant variable revealed high significant ($P \leq 0.001$) and positive direct effects of total biomass (0.75), number of nodes with pods which had seed (0.56) and harvest index on grain yield (Table 8.8). However, there was high significant ($P \leq 0.001$) and negative direct effects of primary branches on grain yield. Chocolate spot disease had negative direct effects on grain yield. The results of indirect effects indicated that hundred seed weight (0.68) primary branch (0.67), number of pods per plant (0.66), number of nodes with pods which had seed (0.63), number of seeds per pod (0.55) and number of nodes per plant (0.55) had positive indirect effects on grain yield, which could be justified through the total biomass (0.75). Similarly, number of pods per node (0.53), number of pods per plant (0.53), number of seeds per pod (0.48), number of nodes per plant (0.48), primary branch (0.46) and hundred seed weight (0.46) had positive indirect effects on grain yield which could be justified via the number of nodes with pods which had seed (0.56). Consequently, it can be explained that chocolate spot disease (-0.32) and RAUDPC (-0.26) and harvest index (-0.42) had indirect and negative effects on grain yield via the total biomass (0.75). Similarly, days to flowering, chocolate spot disease (GDS and RAUDPC) and harvest index had indirect and negative effects on grain yield (Table 8.8).

The path coefficient analysis resulted in high significant ($P \leq 0.001$) positive correlation of grain yield with, number of nodes with pods which had seed (r=0.90), number of pods per plant (r=0.88), number of pods per node (r=0.83), total biomass (r=0.82), number of seeds per pod (r=0.76), number of nodes per plant (r=0.75), hundred seed weight (r=0.74), and primary branch (r=0.72). Conversely, there was significant ($P \leq 0.05$) and negative correlation between yield and chocolate spot disease assessments (Table 8.8 and 8.9). There was high significant ($P \leq 0.001$) positive correlation among most of the yield component characters (Table 8.9).

Table 8.8 Direct effect (along diagonal in bold), indirect effect (off diagonals) and total correlation (at the end in bold) of yield component characters and chocolate spot disease on grain yield (t ha-1) in the 90 F_1 progenis in the diallel crosses.

	DTF	DTM	BB	HFP	NPPN	NPPP	NSPP	NNP	NWP	PH	HSW	BM	RAUDPC	GDS	HI	GY
DTF	**0.00**	0.05	-0.03	0.03	0.00	0.00	0.00	0.00	-0.02	-0.01	0.00	0.17	-0.03	0.11	-0.31	**-0.04**
DTM	0.00	**0.06**	-0.11	0.03	0.01	-0.01	0.00	0.01	0.14	0.00	0.01	0.37	-0.02	0.12	-0.37	**0.24***
BB	0.00	0.02	**-0.36*****	0.01	0.02	-0.03	0.03	0.04	0.46	0.01	0.01	0.67	-0.01	0.04	-0.20	**0.72*****
HFP	0.00	0.05	-0.09	**0.04**	0.00	0.00	0.00	0.01	0.08	0.00	0.01	0.28	-0.02	0.11	-0.33	**0.13**
NPPN	0.00	0.01	-0.33	0.01	**0.03**	-0.03	0.03	0.05	0.53	0.01	0.01	0.66	-0.01	0.03	-0.17	**0.83*****
NPPP	0.00	0.02	-0.29	0.00	0.02	**-0.04**	0.03	0.05	0.53	0.01	0.01	0.62	0.00	0.03	-0.12	**0.88*****
NSPP	0.00	0.01	-0.29	0.00	0.02	-0.03	**0.04**	0.05	0.48	0.00	0.01	0.55	-0.01	0.02	-0.11	**0.76*****
NNP	0.00	0.01	-0.27	0.01	0.02	-0.03	0.04	**0.06**	0.48	0.00	0.01	0.55	0.00	0.03	-0.14	**0.75*****
NWP	0.00	0.02	-0.30	0.01	0.02	-0.03	0.04	0.05	**0.56*****	0.01	0.01	0.63	-0.01	0.03	-0.13	**0.90*****
PH	0.00	0.00	-0.04	0.00	0.00	-0.01	0.00	0.00	0.09	**0.07***	0.00	0.14	0.00	0.01	0.01	**0.28****
HSW	0.00	0.03	-0.33	0.02	0.02	-0.03	0.03	0.04	0.45	0.01	**0.01**	0.68	-0.02	0.07	-0.24	**0.74*****
BM	0.00	0.03	-0.32	0.01	0.02	-0.03	0.03	0.04	0.47	0.01	0.01	**0.75*****	-0.01	0.06	-0.27	**0.82*****
RAUDPC	0.00	-0.04	0.11	-0.03	0.00	0.00	-0.01	-0.01	-0.08	0.01	-0.01	-0.26	**-0.04**	-0.13	0.25	**-0.15***
GDS	0.00	-0.05	0.11	-0.03	-0.01	0.01	-0.01	-0.01	-0.11	0.00	-0.01	-0.32	0.03	**-0.15**	0.31	**-0.23***
HI	0.00	-0.05	0.15	-0.03	-0.01	0.01	-0.01	-0.02	-0.16	0.00	-0.01	-0.42	0.02	-0.09	**0.48*****	**-0.13**

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; DTF: days to 50% flowering; BB: number of primary branch per plant; DTM: days to physiological maturity; NPPN: number of pod per node; NPPP: number of pods per plant; NSPP: number of seeds per pod; NNP: number of node per plant; NWP: number of node with pod which had seed; PH: plant height; HSW: hundred seed weight; BM: total biomass; RAUDPC: relative area under disease progress curve value; GDS: general disease severity score; HI: harvest index ; GY: grain yield .

Table 8.9 Correlation coefficients for grain yield (t ha-1), yield components and chocolate spot disease for the 90 F$_1$progenies in the diallel cross

	DTF	DTM	BB	HFP	NPPN	NPPP	NSPP	NNP	NWP	PH	HSW	BM	RAUDPC	GDS	GY	HI
DTF	1	0.78***	0.07	0.71***	-0.02	-0.02	-0.09	0.01	-0.03	-0.17	0.21*	0.23*	-0.66***	-0.75***	-0.04	-0.65***
DTM		1	0.30**	0.76***	0.24*	0.25*	0.12	0.22*	0.25*	0.001	0.43***	0.50***	-0.63***	-0.81***	0.24*	-0.78***
BB			1	0.24*	0.91***	0.80***	0.82***	0.76***	0.82***	0.11	0.92***	0.89***	-0.30**	-0.32**	0.72***	-0.43***
HFP				1	0.17	0.11	0.08	0.17	0.14	0.05	0.39***	0.38***	-0.64***	-0.75***	0.13	-0.70***
NPPN					1	0.91***	0.85***	0.83***	0.94***	0.16	0.85***	0.89***	-0.15	-0.21*	0.83***	0.36***
NPPP						1	0.85***	0.83***	0.96***	0.18	0.77***	0.83***	-0.11	-0.19*	0.88***	-0.25*
NSPP							1	0.91***	0.87***	-0.02	0.78***	0.74***	-0.14	-0.15	0.76***	-0.22*
NNP								1	0.85***	0.01	0.73***	0.74***	-0.13	-0.21*	0.75***	-0.31**
NWP									1	0.16	0.81***	0.85***	-0.14	-0.20*	0.90***	-0.28**
PH										1	0.13	0.19*	0.11	-0.04	0.28**	0.019
HSW											1	0.91***	-0.46***	-0.47***	0.74***	-0.51***
BM												1	-0.35***	-0.43***	0.82***	-0.56***
RAUDPC													1	0.87***	-0.18*	0.52***
GDS														1	-0.23*	0.65***
GY															1	-0.13
HI																1

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; DTF: days to 50% flowering; DTM: days to physiological maturity; NPPN: number of pod per node; NPPP: number of pods per plant; NSPP: number of seeds per pod; NNP: number of node per plant; NWP: number of nodes with pods which had seed; PH: plant height; HSW: hundred seed weight; BM: total biomass; RAUDPC: relative area under disease progress curve value; GDS: general disease severity score; GY: grain yield ; HI: harvest index.

8.4 Discussion

8.4.1 Heterosis

The mean squares due to the variety were significant for chocolate spot resistance and grain yield indicating that the parents were diverse and with a different genetic background. Similarly, the significant mean squares due to heterosis, variety heterosis and specific heterosis for chocolate spot disease resistance and grain yield suggested the potential of the parents for heterosis. These results confirm those obtained by Zeid et al. (2004) and Alghamdi (2009).

Mid and better-parent heterosis and estimate of heterosis effect for grain yield

The heterosis percentage relative to the mid-parent and better-parent were positive for 22 and 15 crosses over the mid-parent and better-parent respectively for grain yield. The highest heterosis for grain yield of 162.3% in this study was higher than reported in other studies, for instance 129.9% by Alghamdi (2009), 119% by (Zeid et al., 2004). The high heterosis for grain yield was detected in crosses made between the introduced inbred lines (ILB-4726 ILB-938 and BPL-710) and locally released improved varieties (NC58, Kasa, Bulga-70 and CS-20-DK). This suggested these lines were genetically divergent and these parents were from different clusters. The positive correlation between genetic divergence and heterosis was reported by Das et al. (2013). This is also explained by line ILB-4726, ILB-938 and BPL-710 which had estimates of heterosis effects in the positive direction for grain yield.

In this study, the crosses NC58 x ILB-4726, ILB-4726 x Kasa, NC58 x BPL-710, ILB-938 x CS-20-DK, ILB-938 x CS-20-DK and CS-20-DK x BPL-710 had highest mid-parent and better-parent heterosis. In addition, highly significant estimates and positive heterotic effects were recorded for the specific hetrotic crosses made between the genetically divergent lines, for instance for crosses; ILB-4726 x Bulga-70, NC58 x BPL-710, ILB-4726 x Kasa, ILB-938 x CS-20-DK and BPL-710 x Bulga-70 for grain yield. The negative heterosis was found between crosses of genetically close inbred lines. For instance crosses between; CS-20-DK and Bulga-70, ILB-938 and BPL-710, NC58 and Bulga-70 had negative heterosis over the mid and better-parent for grain yield since these lines have a high degree of parental relationship genetically.

Mid and better-parent heterosis and heterosis effect for chocolate spot disease resistance

The desirable negative heterosis for chocolate spot disease resistance was obtained in crosses made between the inbred lines; ILB-4726, ILB-938 and BPL-710, Gebelco and NC58, Kasa, Bulga-70 and CS-20-DK. This could be due to line ILB-4726, ILB-938, BPL-710 and Gebelcho which had estimates of heterosis effect in the negative direction for chocolate spot disease resistance. In this study, the best hybrids which showed heterosis in negative direction over the mid and better-parent for chocolate spot resistance were between resistant lines x susceptible ones; ILB-4726 x Kasa, ILB-4726 x Bulga-70, CS-20-DK x Gebelcho, NC58 x ILB-4726, Kasa x BPL-710 and ILB-938 x Kasa. This is also explained by the difference in genetic divergence of the parental lines. On the contrary, positive heterotic effects were found between resistant lines x resistant lines; ILB-4726 x BPL-710, ILB-938 x BPL-710 and ILB-4726 x BPL-710 which performed in positive direction for chocolate spot resistance. This could be due to ILB-4726, ILB-938, BPL-710 and Gebelcho (its pedigree comprises ILB-4726) which are genetically close inbred lines. Similar results were reported by Matiello et al. (2012) for maize anthracnose and Lim and White (1978) in study of estimates of heterosis for *Colletotrichum graminicola* resistance in maize.

8.4.2 Path coefficient analysis of grain yield (t ha^{-1}), chocolate spot disease and yield components in faba bean in the diallel crosses

In the present study the maximum positive direct effect was recorded on the total biomass followed by number of nodes with pods which had seed and harvest index on grain yield t ha^{-1}. This implied that high grain yielding varieties had high total biomass, high number of nodes with pods which had seed and high harvest index. Similarly, Tadesse et al. (2011) reported the number of seeds per pods and thousand seed weight had direct effect on yield per plot in study of path analysis on some faba bean landraces from Ethiopia.

The results of indirect effects indicated that most of the yield component characters except for chocolate spot disease and days to flowering had positive indirect effects on grain yield through the total biomass and the number of nodes with pods which had seed. This was also supported by the highly significant positive correlation of most of the yield component characters number of nodes with pods which had seed, number of pod per plant, number of pods per node, total biomass, number of node per plant, thousand seed weight, and primary branches to grain yield. However, chocolate spot disease and days to flowering had negative correlation with grain yield suggesting that late flowering cultivars were generally low yielding compared to early flowering cultivars. This could be due to shortage of rainfall at the end of the growing season of the crop. Kumar et al. (2013) reported direct effect of harvest index and indirect effect number of pods per plant, number of branches per plant, number of seeds per pod, plant height and 100-seed weight for yield path analysis in faba bean.

8.5 Conclusion

In conclusion, the high mid and better-parent heterosis observed in the crosses NC58 x ILB-4726, ILB-4726 x Kasa, NC58 x BPL-710, ILB-938 x CS-20-DK, ILB-938 x CS-20-DK and CS-20-DK x BPL-710 suggests that these hybrids could be further considered in the faba bean breeding programme aiming both for segregant breeding and hybrid development in the long term. The best hybrids; ILB-4726 x Kasa, ILB-4726 x Bulga-70, CS-20-DK x Gebelcho, NC58 x ILB-4726, Kasa x BPL-710 and ILB-938 x Kasa, that exhibited negative heterosis over the mid and better-parent for chocolate spot resistance could be considered as source of segregants in faba bean breeding for disease resistance. The three crosses ILB-4726 x Kasa, ILB-4726 x Bulga-70, NC58 x ILB-4726 are recommended for faba bean breeding programme aiming for grain yield and chocolate spot disease resistance.

The path coefficients from path analysis indicated that the number of nodes with pods which had seed and total biomass had the maximum positive direct effects on the grain yield. Moreover, the greatest positive indirect effects on grain yield were attributed to hundred seed weight, primary branch, number of pod per plant, number of seed per pod and number of node per plant through their effects on the number of node with pod which had seed and total biomass. Two quantitative parameters such as number of nodes with pods which had seed and total biomass could be reliable tools for selection indices to identify the productive genotypes and to realize maximum genetic gain. Therefore, selection based on number of nodes with pods which had seed and total biomass could be used for selection of high yielding varieties in faba bean breeding programmes.

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Chapter 9

GGE Biplot and AMMI analysis of genotype x environment interaction in faba bean genotypes for grain yield and chocolate spot (*Botrytis fabae*) disease resistance.

Abstract

Faba bean (*Vicia faba*) is important legume grown world-wide as a protein source to contribute the alleviation of malnutrition for resource poor farmers. It also plays a vital role for sustainability of cropping system as input of nitrogen through biological N_2 fixation. Chocolate spot caused by *Botrytis fabae* is one of the major constraints that limit the productivity of faba bean. Although there are some exotic faba bean genotypes resistant to this disease available, the absence of high levels of stable, disease resistant sources from the landrace materials has resulted in a decrease in productivity. The present study was performed to analyse the genotype x environment (G X E) interaction for resistance to chocolate spot and grain yield of 21 faba bean genotypes at six locations in 2013. The experiment was laid out in randomized complete block design with three replicates. Disease severity and grain yield data were analysed using additive main effects and multiplicative interaction (AMMI) and GGE biplot methods for graphical display of the data. The AMMI analysis of variance (additive main effects) showed significant effects for genotypes, environment and the genotype by environment interaction. The six environments were examined for representativeness and discriminating ability. There were highly significant differences between genotype, environment and genotype x environment interaction. The effect of environment as a source of yield variability was high (61.4%). The magnitude of genotype was 20.9% and of G x E effect 13.8%. However, the effect of genotype for chocolate spot disease was high (73.4%). The magnitude for G x E (13.8%) and environment (12.7) was almost equal. Among the testing environments, Kulumsa (G3) was found the best discriminating and representative location for faba bean yield as well as chocolate spot. The AMMI and GGE biplot analysis allowed the selection of six genotypes FBColl-0012 (G2), FBColl-0034 (G11), FBColl-0055 (G14), FBColl-0025 (G5), FBColl-0049 (G21) and FBColl-0036 (G20) with highest yielding and most stable genotypes. Similarly, genotypes FBColl-0025 (G5), FBColl-0055 (G14), FBColl-0024 (G16) and FBColl-0049 (G21) were selected with low chocolate spot severity and moderate level of stability. This promising material could be highlighted and exploited in faba bean resistance breeding programmes.

Keywords: AMMI, *Botrytis fabae*; disease resistance stability; faba bean; GGE; genotype x environment interaction,

Introduction

Faba bean (*Vicia faba*) is globally the fourth most important food legume withgreat potential to contribute for the alleviation of malnutrition to resource-poor farmers. It is the principal legume in the mid and highlands of Ethiopia grown for the sustainability of cropping system and soil fertility. The average global productivity of faba bean is about 1.8 t ha^{-1}, far below the actual yield potential, because the crop is subjected to a large number of biotic and abiotic stresses (FAOSTAT, 2014). Chocolate spot caused by *Botrytis fabae* is one of the most important constraints that limit the productivity of faba bean (Stoddard et al., 2010). The relative performances of cultivars often changes from one environment to another; extensive testing is required for identifying genotypes with minimal interaction with environments. Ethiopia has diverse agro-ecological zones and faba bean varieties are bred for different zones. Thus, newly developed faba bean cultivars should exhibit great potential for yield and disease resistance with average stability over different environmental conditions for release. For quantitative traits such as yield for which the relative performances of cultivars often change from one environment to another, extensive testing is required for identifying genotypes with minimal interaction with environments, or that possess greatest yield stability.

The interaction of genotypes with environments (G x E) makes it difficult for breeders to identify the best genotypes, be it during selection or for cultivar recommendation. The presence of interactions indicates that the relative genotype performance in the tests depends essentially on the given environmental conditions. The phenotypic response of any genotype in relation to others could therefore be inconsistent, which is demonstrated by changes of the relative position of the genotypes from one environment to another. To conduct test environment evaluation, it is essential to first conduct a mega-environment analysis, that is, to investigate whether the covered growing region can be divided into mega-environments, because test environment evaluation as well as genotype evaluation becomes meaningful only when conducted within mega-environments (Yan et al., 2007).

There are various methodologies of analysis of adaptability and stability designed to evaluate a genotype group tested in a series of environments. Two frequently used models for statistical analyses of genotype x environment data have been the additive main effects and multiplicative interaction (AMMI) model (Gauch et al., 2008), that combines a univariate method for the additive method for the multiplicative effect of G x E interaction (Zobel et al., 1988) and the genotype and genotype x environment interaction (GGE) model (Yan et al., 2001). The two models have been compared for their advantage and disadvantages (Gauch, 2006; Yan et al., 2007). The GGE model has been utilized to identify breeding lines and cultivars that are resistant to *Ascochyta fabae*, rust and chocolate spot diseases in faba bean (Villegas-Ferna´ndez et al., 2009; Villegas-Fernández et al., 2011; Rubiales et al., 2012). Use of AMMI in analysing the multi-environment disease data to identify stable sources of resistance has been reported on different crops (Mulema et al., 2008; Mukherjee et al., 2013). Genotype x environment interactions is important in the development and evaluation of stable high yielder and disease resistant genotypes. Therefore, it is vital to identify chocolate spot resistant genotypes and validate the resistance stability through multi-location field evaluations for further use in breeding programmes.

There is no sufficient information on the genotype x environment interaction and stability for faba bean genotypes for yield and chocolate spot resistance in Ethiopia; therefore, the objectives of the study were: to (i) evaluate the influence of location on disease resistance and yield of faba bean genotypes, (ii) identify genotypes with high stability for chocolate spot resistance and yield, (iii) determine whether locations belong to a single mega- environment, or a diverse set, and (iv) rank locations based on discriminating ability and representativeness.

9.1 Materials and methods

9.2.1 Plant materials

The study used nineteen faba bean genotypes selected from the previous experiment conducted in 2012 (Chapter 5) based on their resistance to chocolate spot and yield including one resistant and one susceptible check (Table 9.1)

Table 9.1 Description of the faba bean genotypestested across six locations in the highlands of Ethiopia in 2013

Genotype ID	Genotype	Administrative region	Zone	Mean GY (tha^{-1}) in 2012	GDS (%) in 2012	Disease reaction in 2012
G1	FBColl-0001	Harar	Harari	3.66	9.69	MR
G2	FBColl-0012	Tigray	Tigray	4.17	9.99	MR
G3	ILB 4726	ICARDA	ICARDA	1.60	1.47	R
G4	FBColl-0030	Oromia	Arsi	3.53	9.12	MR
G5	FBColl-0025	Oromia	Arsi	4.27	12.16	MR
G6	FBColl-0011	Amhara	Wollo	4.60	11.07	MR
G7	FBColl-0002	Harar	Harar	4.22	11.40	MR
G8	FBColl-0018	Amhara	Gonder	5.24	10.93	MR
G9	FBColl-0035	Amhara	Gojjam	4.76	10.97	MR
G10	FBColl-0042	Amhara	North Shewa	4.26	11.51	MR
G11	FBColl-0034	Amhara	Gojjam	4.43	12.45	MR
G12	FBColl-0003	Harar	Harari	4.11	9.68	MR
G13	Kasa	HARC	HARC	2.66	36.78	S
G14	FBColl-0055	Oromia	South West Shewa	5.60	10.91	MR
G15	FBColl-0031	Oromia	Arsi	4.71	11.88	MR
G16	FBColl-0024	Oromia	Arsi	3.79	10.04	MR
G17	FBColl-0039	Oromia	West Shewa	4.10	13.17	MR
G18	FBColl-0008	Amhara	Wollo	3.26	12.69	MR
G19	FBColl-0057	Oromia	Wollega	4.29	11.10	MR
G20	FBColl-0049	Oromia	South West Shewa	3.19	15.38	MR
G21	FBColl-0036	Amhara	North Shewa	4.23	9.63	MR

ICARD: International Center for Agricultural Research in the Dry Area; HARC; Holetta Agricultural Research Center; R: resistant to chocolate spot, MR; moderately resistant, S: susceptible, GY: grain yield (tha^{-1}) GDS: General diseases severity score (%).

9.2.2 Description of the study area

The study was conducted in six locations. All the locations are among the principal faba bean testing sites in Ethiopia representing the faba bean belt of the country. Table 9.2 describes the study sites.

Table 9.2 Description of the six locations for testing 21 faba bean genotypes in 2013

Site	Site ID	Latitude (° N)	Longitude (° E)	Altitude (m asl)	Soil PH	Annual rain fall (mm)	Temperature	
							Minimum (o C)	Maximum (° C)
Holetta	E1	9.04	38.03	2390	4.3 (EA)	760.8	6.4	24.4
Adadi	E2	8.21	38.29	2520	7.3 (NU)	930.8	8.5	23.5
Kulumsa	E3	8.01	39.09	2730	5.2 (SA)	634.1	10.59	23.66
Bekoji	E4	7.32	39.15	2803	4.5 (VSA)	734	14.5	20.78
Assasa	E5	7.1	39.14	2366	4.9 (VSA)	871.3	6.77	22.37
Kofele	E6	7.05	38.47	2669	4.4 (EA)	1078.4	1.99	19.26

Source: National meteorology agency Ethiopia (Annual rain fall & Temperature); Soil PH denomination: VAA: Very Strong Acid, SA: Strong Acid; EA: Extreme acid; NU: Neutral. Soil PH was from soil sample of the experimental plot for respective sites in 2013

9.2.3 Experimental design and management

The experiment was conducted with 21 faba bean genotypes laid out in randomized complete block design with three replications in all locations. Plots were made up of four rows 4 m long with 0.40 m inter-row and 0.05 m intra-row spacing. Fertilizer DAP was applied at the rate of 100 kg ha^{-1} at planting for all experiments. The trials were conducted under rain-fed condition. The fields were kept free of weeds by hand weeding and other management practices were followed according to the recommendations of the specific areas. Natural disease infestation was supplemented with artificial inoculation of *Botrytis fabae* at all sites.

General disease assessment was recorded from the whole plot once at 88 days after planting. The severity of chocolate spot was recorded as percentage of leaf area infected as follows: 1%-no disease symptoms or very small specks (highly resistant); 3%-few small disease lesions (resistant); 6%-small coalesced lesions, with some defoliation (moderately resistant); 12%-large coalesced sporulating lesions, 20% defoliation (moderately susceptible); 25%-large coalesced sporulating lesions, 50% defoliation and some dead plants (susceptible) and 50%-extensive, heavy sporulation, stem girdling, blackening and death of more than 80% of the plants (highly susceptible) (Bernier et al., 1993; Bernard et al., 2006). The data for grain yield and other agronomic traits were taken following the standard practice for faba bean trial used. Grain yield was taken from the middle two rows per plot. Grain yield adjustment was made. Seeds were oven dried and adjusted to constant moisture level of 10%. The total grain yield was recorded on a plot basis and was converted to t ha^{-1} for statistical analysis.

9.2.4 Statistical data analyses

The yield data and the chocolate spot disease severity scores were subjected to analysis of variance using individual plot data for each environment separately and then combined across all six environments. Both additive main effects and multiplicative interaction (AMMI) and the genotype and genotype x environment (GGE) biplot methods were used to investigate the genotype, environment and genotype x environment interaction effects on grain yield and chocolate spot disease severity of faba bean genotype as described by Yan (2002). The AMMI model, which combines the standard analysis of variance with principal component analysis, was used to investigate the nature of genotype x environment interaction (Zobel et al., 1988). AMMI and GGE biplot analyses were done using GenStat software version 14 (Payne et al., 2012). For the GGE biplot, to visualize the performance of the genotypes in each environment and groups of environments, a polygon view was drawn by connecting genotypes that were furthest from the biplot origin such that all genotypes were enclosed within the polygon (Yan, 2002). The biplot was also used to explore the interrelationships among environments by constructing lines (environment vectors) from the biplot origin to markers for the environments. The cosine of the angle between environments corresponds to the degree of correlation between environments. The length of the vectors was also used to determine the discriminating ability of each of the test environments, with a shorter vector implying that the environment was not well represented by PC1 and PC2 (Yan et al., 2007). Spearman correlation coefficients of environments were calculated using the SAS procedure (SAS Institute, 2012).

9.2 Results

9.3.1 AMMI analysis for Grain yield

The additive main effects and multiplicative interaction (AMMI) analysis of variance of 21 faba bean genotypes tested over six environments revealed that 61.4% of the total sum of squares (SS) was attributable to the environment, 21% to the genotypes and 17.7% to genotype by environment interaction for grain yield (Table 9.3). The ANOVA indicated highly significant difference ($P \leq 0.001$) for environment, genotype and genotype x environment interaction implying a substantial variation among the genotypes as well as environments. The genotype x environment interaction sums of squares were partitioned into the first three interaction principal component axes (IPCA). The first three IPCA were highly significant ($P \leq 0.001$) for the for grain yield. The first and the second interaction principal component axis (IPCA-1 and IPCA-2) contributed to 82.6% of the total interaction with 63.5% and 19.1% of the interaction SS, respectively (Table 9.3).

A large grain yield variation explained by environments indicated that the environments were different, with large differences among environmental means causing most of the variation in grain yield. The genotype average grain yield across environment ranged from 2.1 t ha^{-1} in Holetta (E1) to 5.6 t ha^{-1} in Adadi (E2). The genotype by environment interaction was a crossover type with different yield ranking of genotype across environments (Table 9.4).

Table 9.3 AMMI analysis of grain yield and chocolate spot disease severity of 21 faba bean genotypes in six environments in 2013

	GY (t ha^{-1})				**GDS (%)**			
Source of variation	**df**	**SS**	**MS**	**Variance explained (%)**	**df**	**SS**	**MS GDS**	**Variance explained (%)**
Total	377	949.9	2.52		377	29221	77.5	
Treatments	125	792.4	6.34***		125	22935	183.5***	
Genotypes	20	166.1	8.3***	20.96	20	16839	842***	73.42
Environments	5	486.3	97.26***	61.36	5	2920	584.1***	12.73
Block	12	24.4	2.04***		12	630	52.5**	
Interactions	100	140.1	1.4***	17.68	100	3175	31.8*	13.84
IPCA1	24	77.2	3.22***	63.49	24	1120	46.6***	40.96
IPCA2	22	23.2	1.06**	19.08	22	936	42.6**	33.96
IPCA3	20	21.2	1.06**	17.43	20	700	35	25.39
Residuals	34	18.4	0.54		54	1119	20.7	
Error	240	133	0.55		240	5656	23.6	

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively; GDS: general disease severity score for Chocolate spot (%); GY: grain yield (t ha^{-1})

Table 9.4 Mean grain yield (t ha^{-1}) of 21 faba bean genotypes tested in six environments

	Mean grain yield (t ha^{-1}) and rank per each environment												Overall	
G- ID	**E1-M**	**RE1**	**E2-M**	**RE2**	**E3-M**	**RE3**	**E4-M**	**RE4**	**E5-M**	**RE5**	**E6-M**	**RE6**	**GY-M**	**Rank**
G1	1.20	8	5.28	15	3.4	8	3.13	11	3.24	2	4.18	8	3.41	16
G2	2.11	4	6.54	4	3.89	4	4.45	16	3.71	4	4.60	21	4.22	3
G3	3.06	3	1.66	21	2.89	14	1.82	15	1.44	16	3.20	11	2.35	19
G4	3.11	17	6.93	16	4.42	2	3.91	20	3.57	8	4.55	13	4.41	1
G5	2.45	19	6.51	14	3.59	11	4.42	21	3.09	13	4.06	2	4.02	6
G6	0.99	14	4.43	2	1.59	13	3.16	2	1.21	1	1.93	4	2.22	21
G7	1.10	5	4.67	5	2.99	5	3.54	5	2.88	5	4.12	20	3.22	17
G8	3.45	11	5.41	12	4.50	16	3.95	12	3.42	12	5.30	14	4.34	2
G9	1.78	20	6.35	9	2.94	1	3.81	17	2.09	11	3.70	1	3.44	15
G10	1.15	16	4.14	11	1.79	12	3.75	8	1.32	7	2.83	7	2.50	18
G11	2.33	2	6.11	17	3.62	15	4.66	4	3.04	14	4.73	15	4.08	5
G12	2.11	12	6.45	20	3.39	19	4.22	9	3.08	15	3.71	5	3.83	10
G13	1.67	21	5.01	19	3.60	20	3.61	10	3.31	20	4.69	17	3.65	13
G14	2.60	15	6.61	8	3.91	17	3.05	13	2.56	19	4.31	12	3.84	9
G15	1.93	9	8.12	1	3.31	21	4.64	19	2.48	9	4.08	9	4.10	4
G16	2.26	13	6.72	13	3.46	7	4.65	18	3.53	17	3.39	19	4.00	7
G17	2.76	1	6.07	7	3.18	9	4.15	7	1.97	21	3.84	16	3.66	12
G18	1.09	10	3.10	6	1.71	3	3.56	6	1.36	3	2.94	3	2.29	20
G19	2.68	7	5.47	10	3.24	10	3.56	1	2.13	18	3.68	18	3.46	14
G20	2.31	18	5.70	18	3.21	18	4.58	14	2.45	10	4.37	10	3.77	11
G21	2.03	6	6.74	3	3.18	6	4.54	3	1.92	6	4.81	6	3.87	8
Mean	**2.10**		**5.62**		**3.23**		**3.86**		**2.56**		**3.95**		**3.56**	

G-ID: Genotype ID in the graph; R: Rank for respective environment (Eg. RE1: Rank of genotypes at E1- Holetta), M: mean grain yield (t ha^{-1}), E1: Holetta, E2: Adadi, E3: Kulumsa, E4: Bekoji, E5: Assasa and E6: Kofele.

The first four genotypes considered as best for grain yield in all the six environments are summarized in Table 9.5. G8 was ranked firstin three environments and 4th in one environment. Similarly, G4 was second in four environments followed by G16 which was the best in three environments. G15 and G11 were identified as best genotypes in two environments (Table 9.5).

Table 9.5 The first four AMMI selections for grain yield per environment

Graph ID	**Environment**	**Mean GY (t ha^{-1})**	**1st**	**2nd**	**3rd**	**4th**
E1	Holetta	2.10	G8	G4	G3	G17
E2	Adadi	5.62	G15	G4	G21	G16
E3	Kulumsa	3.23	G8	G4	G14	G2
E4	Bekoji	3.86	G11	G16	G15	G20
E5	Assasa	2.56	G2	G4	G16	G8
E6	Kofele	3.95	G8	G21	G11	G13

GY: Grain yield (t ha^{-1})

The AMMI analysis data with IPCA1, IPCA2 and IPCA3 scores for the faba bean genotypes tested and the test environments for grain yield and chocolate spot severity are presented in Tables 9.6 and 9.7, respectively. The IPCA scores indicate how much the individual genotype or environment deviates from zero (origin). The more instability is explained by the more deviation from zero (either negative or positive direction).

Table 9.6 IPCA-1, IPCA-2 and IPCA-3 scores and graph IDs for the 21 faba bean genotypes organized based on mean grain yield (t ha-1) and GDS (%) evaluated in six environments.

Graph ID	Genotype	Mean GY (t-ha)	IPCA1	IPCA2	IPCA3	Mean GDS (%)	IPCA1	IPCA2
G1	FBColl-0001	3.405	-0.087	-0.758	0.291	21.62	-1.039	-0.865
G2	FBColl-0012	4.216	0.186	-0.367	0.255	16.69	0.211	1.096
G3	ILB 4726	2.346	-1.615	0.227	-0.443	2.13	-0.627	1.276
G4	FBColl-0030	4.413	0.051	-0.379	-0.471	7.56	-0.589	0.965
G5	FBColl-0025	4.020	0.225	0.015	-0.037	12.29	0.289	0.381
G6	FBColl-0011	2.217	0.158	0.440	0.076	28.89	1.629	-1.031
G7	FBColl-0002	3.216	-0.189	-0.403	0.569	18.09	-0.429	-0.055
G8	FBColl-0018	4.341	-0.632	-0.222	-0.195	9.64	-1.021	0.468
G9	FBColl-0035	3.444	0.403	0.058	-0.179	10.32	0.709	1.125
G10	FBColl-0042	2.497	-0.040	0.554	0.386	22.66	2.672	-0.214
G11	FBColl-0034	4.084	0.057	0.032	0.235	14.91	-0.726	-0.547
G12	FBColl-0003	3.825	0.309	-0.068	0.013	14.66	0.423	0.113
G13	Kasa	3.650	-0.310	-0.546	0.405	24.76	-0.806	-1.035
G14	FBColl-0055	3.840	0.093	-0.416	-0.731	12.95	0.079	0.473
G15	FBColl-0031	4.095	0.987	0.069	-0.257	10.59	-0.236	0.291
G16	FBColl-0024	4.002	0.409	0.015	0.116	13.76	0.828	-0.502
G17	FBColl-0039	3.663	0.110	0.450	-0.37	10.96	0.543	0.742
G18	FBColl-0008	2.291	-0.424	0.526	0.610	27.58	0.342	-2.519
G19	FBColl-0057	3.459	-0.142	0.207	-0.402	7.73	0.179	0.949
G20	FBColl-0049	3.770	0.017	0.311	0.185	15.12	-1.629	-0.729
G21	FBColl-0036	3.869	0.431	0.253	-0.055	14.09	-0.801	-0.380

Graph ID: Genotype ID in the graph: Mean GY: mean grain yield (t ha^{-1}), Mean GDS: Mean general disease severity score for chocolate spot (%)

Table 9.7 The IPCA-1, IPCA-2 and IPCA-3 scores and graph IDs for the six environments organized by environmental mean grain yield (t ha-1) and chocolate spot disease (%).

	Grain yield (t ha^{-1})				Chocolate spot severity GDS) (%)		
Graph ID	**Env. mean**	**IPCA-1**	**IPCA-2**	**IPCA-3**	**Env. mean**	**IPCA-1**	**IPCA-2**
E1	2.103	-0.969	0.756	-0.903	18.69	0.603	0.508
E2	5.620	1.838	-0.198	-0.608	16.01	-2.484	-2.550
E3	3.229	-0.509	-0.493	-0.341	14.93	0.166	-0.978
E4	3.864	0.417	1.079	0.932	9.58	-0.784	2.730
E5	2.562	-0.284	-0.807	0.644	16.54	3.373	-0.963
E6	3.953	-0.494	-0.337	0.276	14.82	-0.874	1.2522

Graph ID: Genotype ID in the graph, Env. mean: Environment mean.

AMMI biplot for grain yield

AMMI biplot was constructed for grain yield to investigate the main effects and interactions. The abscissa shows the main effects while the ordinate shows the first PCA axis (Figure 9.1). The IPCA scores of genotypes in the AMMI analysis (Table 9.6 and Figure 9.1) suggest the stability or instability of genotype over environment. The genotypes are distributed from stable genotype low yielding in quadrants II to the unstable low yielding quadrants III to the higher yielding (ideal genotype) quadrants I and unstable genotype high yielding quadrants IV (Figure 9.1). The higher IPCA scores near to zero, the more stable the genotype is over all environments tested. Further the genotypes which are characterized by means greater than the grand mean are considered as generally adapted to all environments. Accordingly, genotypes G20, G11, G4, and G14 can be considered the most stable and adapted to all environments, as their IPCA scores are close to zero with yields greater than the grand mean (Table 9.6 and Figure 9.1).

However, the genotypes with high mean performance and with large value of IPCA score are considered as having specific adaptation to the environments. Genotypes close to an environment suggests that it is better adapted to that particular environment. Hence, genotypes G8 was found better adapted to E6 (Kofele) with high grain yield but with negative interaction. G21, G16, G9 and G12 revealed specific adaptation for E4 with positive interaction. Generally G18 was the most unstable genotype identified by AMMI model with low grain yield and negative interaction. G15 had the largest positive (0.99) interaction with environment with high grain yield while, G3 had the largest negative interaction (-1.62) but low grain yield (Table 9.6).

Similarly, environments are distributed from lower yielding in quadrants II and III to the higher yielding in quadrants I and IV in the IPCA 1. From the biplots the points for environment are more scattered than the points for genotypes indicating that variability due to environments is higher than due to genotypes difference which is in agreement of ANOVA (Table 9.3). The higher yielding environments classified according to the AMMI 1 model were Adadi (E2), E4 (Bekoji) and E6 (Kofele). While, E1 (Holetta), E3 (Kulumsa) and E5 (Assasa) were lower yielding environments. In general, Adadi (E2) was the most favourable environment and E1 (Holetta) was the less favourable among the six environments included in this study. This situation is indicated in Figure 9.1 where the two environmental variations are plotted far apart from the mean.

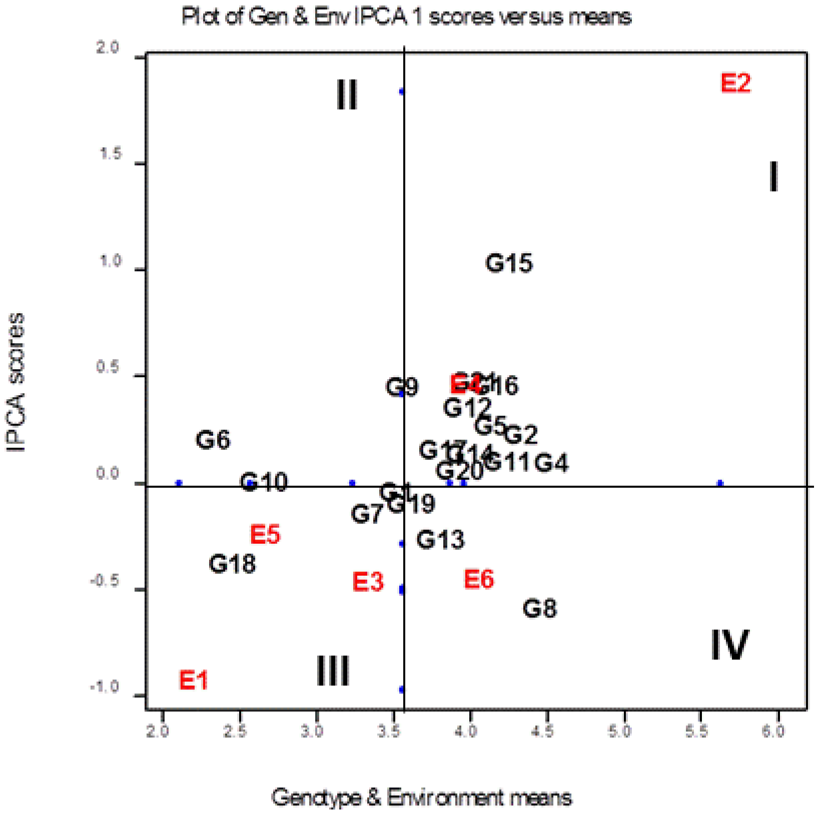

Figure 9.1 AMMI biplot of the first interaction principal component axis (IPCA1) versus mean grain yields (t ha-1) of faba bean genotypes (G1 – G21 full name Table 9.1) and environments (E1 – E6, full name in Table 9.2)

9.3.2 AMMI analysis for chocolate spot severity

AMMI analysis of variance for chocolate spot severity (GDS) indicated that there were highly significant ($P \leq 0.001$) variations for the genotype, environment and ($P \leq 0.05$) genotype by environment interaction (Table 9.3). Genotype was the main source of variation for disease expression accounting for 73.4% of total variation in the experiment, followed by genotype x environment interaction which explained 13.8% of the total variation in chocolate spot disease severity, while environment accounted for only 12.7% (Table 9.3). Hence, genotype had a greater influence on the disease severity than both environment and genotype x environment interaction. The significant G x E interaction for chocolate spot disease indicates that there was a differential response of genotypes across the environments. Highly significant ($P \leq 0.001$) variation was observed for the first two interaction principal component axes (IPCA) for chocolate spot disease severity. The first and the second interaction principal component axis (IPCA-1 and IPCA-2) contributed to 74.6% of the total interaction with 40.6% and 33.9% of the interaction SS, respectively (Table 9.3).

Environmental means of disease severity of the 21 faba bean genotypes planted in six environments are presented in Table 9.8. The chocolate spot disease severity in most of the faba bean genotypes varied greatly among the six environments. The mean severity scores across the six environments ranged from 9.6% in Bekoji (E4) to 18.7% in Holetta (E1). Thus, Holetta (E1) was most favourable for the disease expression followed by Assasa (E5) (Table 9.8 and Figure 9.2).

Table 9.8 Mean chocolate spot disease severity (%) of 21 faba bean genotypes tested in six environments in 2013

	Mean chocolate spot severity (%) and rank per each environment												Overall		
G-ID	**E1-M**	**RE1**	**E2-M**	**RE2**	**E3-M**	**RE3**	**E4-M**	**RE4**	**E5-M**	**RE5**	**E6-M**	**RE6**	**GDS-M**	**Rank**	**DR**
G1	23.66	16	28.07	18	20.79	17	15.36	19	21.15	17	20.68	18	21.62	17	S
G2	19.15	14	12.65	11	18.99	16	12.91	16	16.98	14	19.48	17	16.69	15	MR
G3	1.5	1	1.72	1	0.76	1	1.98	1	1.87	1	4.94	1	2.13	1	HR
G4	11.35	2	7.58	4	6.14	2	5.22	3	6.15	2	8.9	3	7.56	2	R
G5	16.5	10	11.6	8	11.61	9	7.61	7	14.34	12	12.11	9	12.29	8	R
G6	32.65	21	28.56	19	29.75	20	19.51	21	37.05	21	25.85	20	28.89	21	S
G7	29.73	19	20.62	16	15.52	13	11.21	15	16.01	13	15.45	14	18.09	16	MR
G8	14.62	8	12.28	9	7.80	4	6.15	4	6.98	3	10.04	4	9.64	4	R
G9	13.46	3	7.11	3	8.48	5	8.13	10	13.93	11	10.82	6	10.32	5	R
G10	30.85	20	18.24	13	21.08	18	14.45	18	33.04	20	18.27	16	22.66	18	S
G11	20.99	15	19.46	15	13.71	11	8.14	11	13.85	10	13.32	10	14.91	13	MR
G12	14.4	6	12.39	10	18.82	15	8.37	12	17.12	15	16.87	15	14.66	12	MR
G13	24.75	17	30.69	20	25.27	19	17.99	20	25.56	18	24.31	19	24.76	19	S
G14	16.42	9	11.46	7	14.34	12	7.83	9	13.50	7	14.15	12	12.95	9	MR
G15	14.03	4	11.41	6	10.03	6	6.15	5	11.06	5	10.89	7	10.59	6	R
G16	17.73	12	15.58	12	10.97	8	7.75	8	19.83	16	10.68	5	13.76	10	MR
G17	14.51	7	8.74	5	10.11	7	7.31	6	13.83	9	11.26	8	10.96	7	R
G18	27.02	18	32.5	21	33.55	21	14.18	17	32.21	19	26.03	21	27.58	20	S
G19	14.06	5	5.33	2	7.12	3	3.76	2	7.86	4	8.26	2	7.73	3	R
G20	18.32	13	21.68	17	15.71	14	8.42	13	11.26	6	15.3	13	15.12	14	MR
G21	16.76	11	18.57	14	13.04	10	8.79	14	13.78	8	13.59	11	14.09	11	MR
Mean	**18.7**		**16**		**14.9**		**9.58**		**16.5**		**14.8**		**15.095**		

G-IG: Genotype ID in the graph; R: Rank for respective environment (Eg. RE1: Rank of genotypes at E1- Holetta). E1: Holetta, E2: Adadi, E3: Kulumsa, E4: Bekoji, E5: Assasa and E6: Kofele. DR: disease reaction. HR: highly resistant, R: Resistant; MR: Moderately Resistant; S: Susceptible reaction to the disease

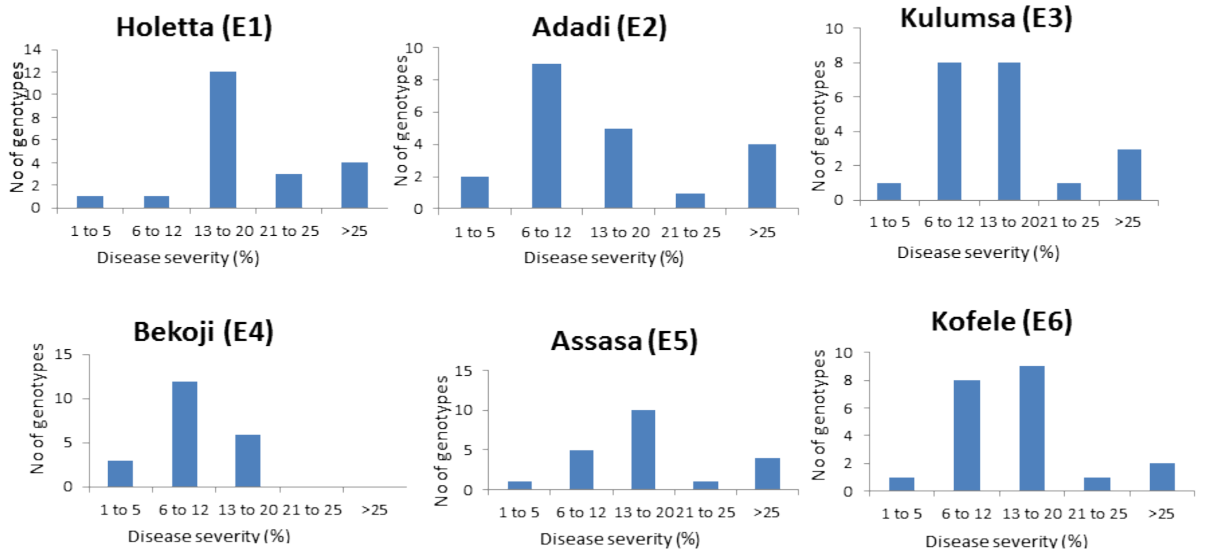

Figure 9.2 Frequency distributions for chocolate spot severity of 21 faba bean genotypes in the 6 environments in Ethiopia. The different patterns point to genotype x environment interaction.

AMMI biplot for Chocolate spot disease severity

AMMI analysis provides a graphical biplot to summarize information on the main effects and the first principal component scores of interactions (IPCA 1) of both genotypes and environments simultaneously. In AMMI (Figure 9.3), the x-coordinate indicates the main effects (means of G x E) and the y-coordinate indicates the effect of interaction, the first interaction principal component axis (IPCA 1). Values closer to the origin of the axis (IPCA 1) contributed less to the interaction than those that were further away. From the biplot grouping of genotypes are evident. The 21 genotypes were grouped into highly resistant, resistant, moderately resistant, moderately susceptible and susceptible. The resistant check genotype G3 (ILB-4726) was resistant across all environments (quadrant III) while the susceptible genotype G13 (Kasa) was susceptible across all environment (quadrant IV) (Figure 9.3 & Table 9.8). Genotypes G3, G4, G19, G15, G17, G8, G16 and G9 were resistant out of the 21 faba bean evaluated with G3, G4 and G19 as the best. In contrast G13, G18, G6, G1 and G10 were susceptible genotypes with G6 and G13 the most susceptible genotypes.

Bekoji (E4) () had a low environment score exhibiting little interaction with genotypes (quadrant III) and Adadi (E2) had a high environment score with a large negative interaction with genotypes (quadrant IV). Holetta (E1) () and Assasa (E5) had high environment scores with a high positive interaction with genotypes (quadrant I). Genotypes with IPCA 1 scores near zero had little interaction across the environment and vice versa for the environment.

Genotypes and environment combinations with IPCA 1 scores of the same sign produced positive - specific interaction effects, whereas combinations of opposite signs negative-specific interactions. The genotypes with low severity and stable across the environments were G19, G15, G17, G9 and G16. Genotype G3, G4 and G8 were unstable but resistant. However, genotypes G6, G10 and G18 were stable but susceptible to chocolate spot (Figure 9.3).

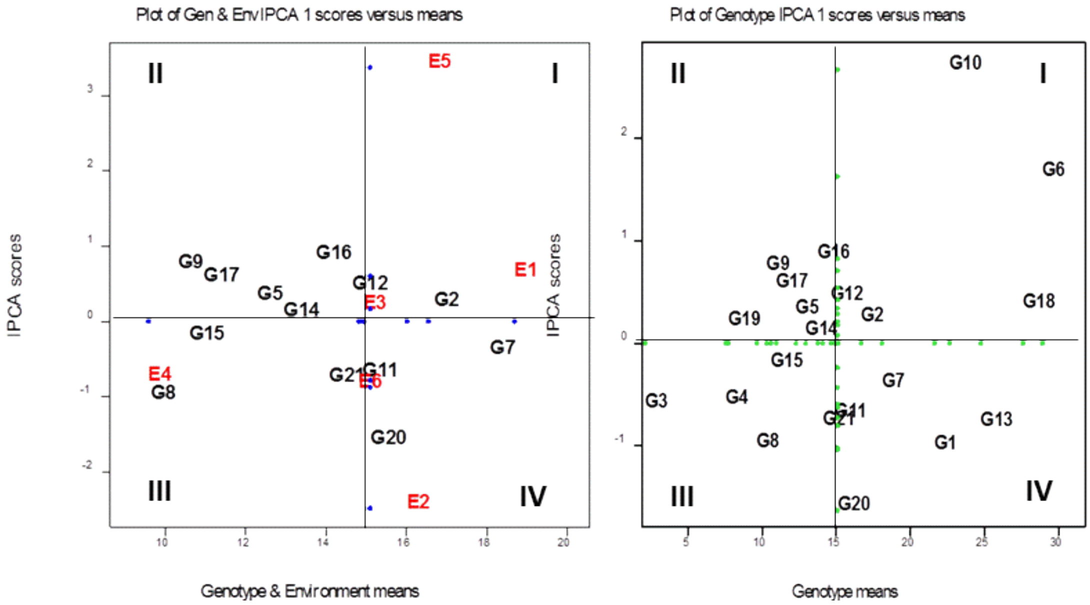

Figure 9.3 AMMI biplot of the first interaction principal component axis (IPCA1) versus mean chocolate spot disease severity (%) of faba bean genotypes (G1 - G21 full name Table 9.1) and environments (E1 – E6, full name in Table 9.2)

9.3.3 GGE biplot analysis of grain yield

The 'which –won-where' pattern and mega environments

The G x E interaction was further explored through the genotype and genotype x environment GGE biplot analysis. The GGE biplot is an invisible statistical tool for examining the performance of genotypes tested in different environments. It was constructed by plotting the first two principal components (PC 1 and 2) to explore the genotype x environment interaction. The PC1 explained 64.1% of total variation and PC2 explained 19.2%, thus together they accounted for 83.3% of the variation for the genotype and genotype x environment interaction for the grain yield of the faba bean evaluated over six locations. The polygon view of the GGE biplot indicated which cultivar performed best in which environment (Figure 9.4). The genotypes located farthest away from the biplot origin in various directions were the vertices of the polygon. In this case, six genotypes were identified as vertix genotypes. The vertex genotypes were either the highest or lowest yielding in the locations which fell within the same sectors with those genotypes indicating different genotypes won in different environments (Yan et al., 2010). Based on this, Genotypes G8 and G4 were the vertices genotype where E1, E3, E6 and E5 fell, thus were highest yielding in these environments. Genotype G15 was the highest yielding genotype at E2 and E4. Genotype G3, G18, and G6 were the vertices genotypes lowest yielding in all or some of the locations since no environment fell into their sectors. Genotypes fell within the polygon, indicating that they were less responsive than the vertex cultivars for the interaction. Four environments (E1 (Holetta), E3 (Kulumsa), E5 (Assasa), and E6 (Kofele)) fell in one sector comprising one large mega-environment, and sector two contained two environments (E4 (Bekoji) and E2-Adadi (Figure 9.4).

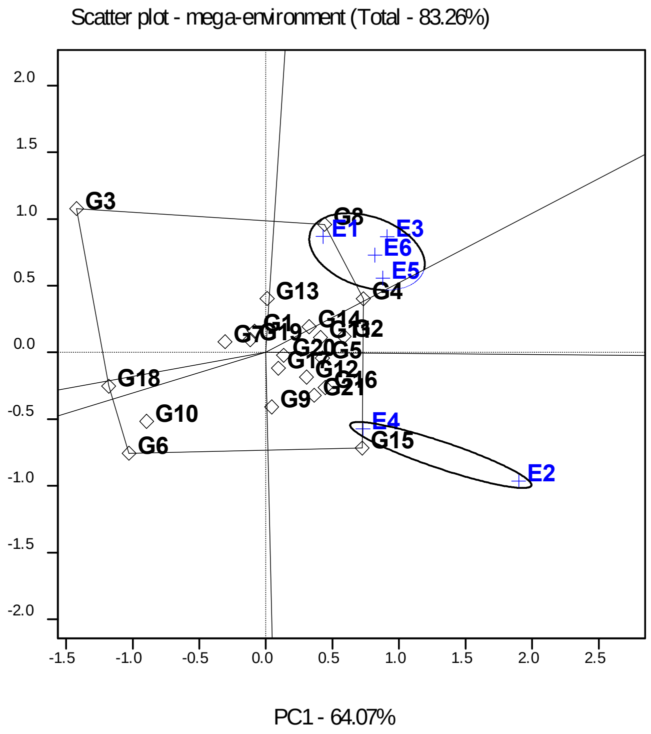
Scatter plot - mega-environment (Total - 83.26%)

Figure 9.4 'Which won where or which is best at what' based on a genotype x environment grain yield data of 21 faba bean genotypes evaluated in six environments in 2013.

Comparison based on mean performance of genotypes across environments

Grain yield of genotypes was evaluated using an average-environment coordinate (AEC) method. In the GGE biplot analysis, the AEC abscissa approximates the genotypes' contribution to G x E, which is a measure of their instability (Yan et al., 2007). The perpendicular lines to the AEC passing through the biplot origin are referred to as AEC ordinate. These ordinates are depicted as double-arrowed line in Figure 9.5. The greater the absolute length of the projection of a genotype, the less stable it is (Yan et al., 2010). A line that passes in either direction away from the biplot origin, on this axis indicates greater G X

E interaction and reduced grain yield stability (Yan and Kang, 2002). Based on these genotypes G2, G11, G14, G5, G21 and G20 were the most stable with an above average performance. Since they were located away from the AEC abscissa and had a near zero projection onto the AEC coordinate (Figure 9.5). Genotype G4, G8 and G15 were the least stable but high yielding genotypes. On the contrary, G18 and G7 were lowest yielding, but very stable genotypes. However, G3, G6 and G10 were not only low yielding but also among the least stable genotypes (Figure 9.5). The genotypes were ranked as G4>G8>G2>G11>G5>G14>G16>G21>G12>G20.

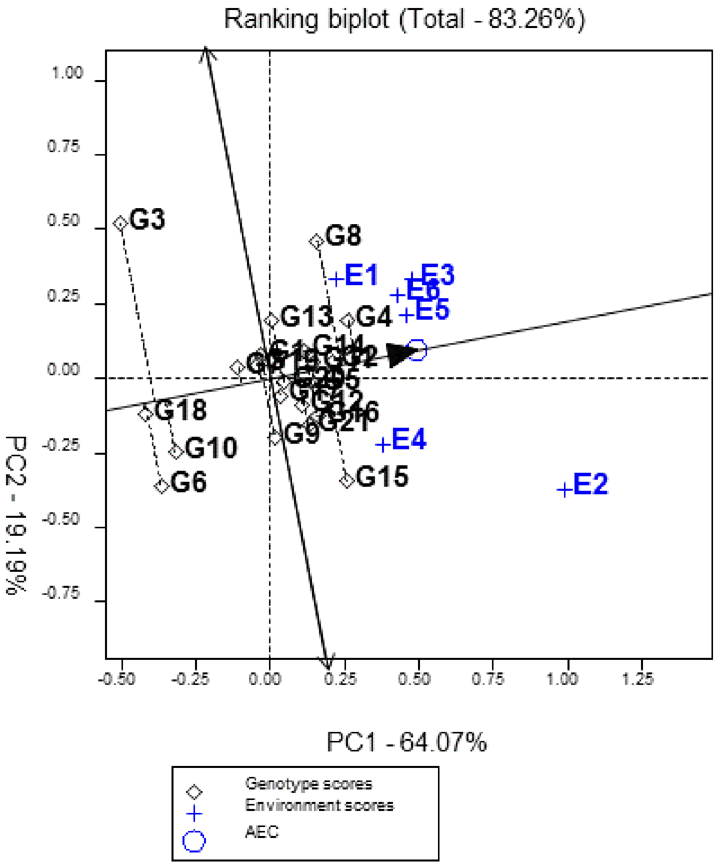

Figure 9.5 Average environment coordination (AEC) views of the GGE biplot on environment-focused scaling for the grain yield means performance and stability of genotypes

Comparison based on mean performance and stability

The GGE biplot compare the ‘ideal genotype’ with 21 faba bean genotypes shown in Figure 9.6. The small circle, which is located on the average environment coordinate (AEC) abscissa and with an arrow pointing to it, represents the ideal genotype. It has the highest yield of the entire dataset and it is absolutely stable (Yan and Kang, 2002). In this case genotype G4 was closest to the hypothetical ideal genotype and therefore, most desirable of all tested genotypes followed by G2, G11, G14, G8, G5, G16, G21, G12 and G20 in order of their stability. Whereas, genotype G3 was extremely far away from it thus it was not in the ideal genotypes category. The double-arrowed line separates genotypes with below average means from those with above average means. The arrow shown on the axis of the AEC abscissa points in the direction of higher mean grain yield performance by the genotypes and, thus ranks the genotypes with respect to mean performance and stability. Based on these the genotypes were ranked according to their projections as follows: G4> G2> G11> G14> G8>G5>G16>G21>G12>G20) (Figure 9.6). It was noticed that genotypes ranking in Figure 9.5, based on mean performance alone, and genotype ranking based on both mean performance and stability are almost identical.

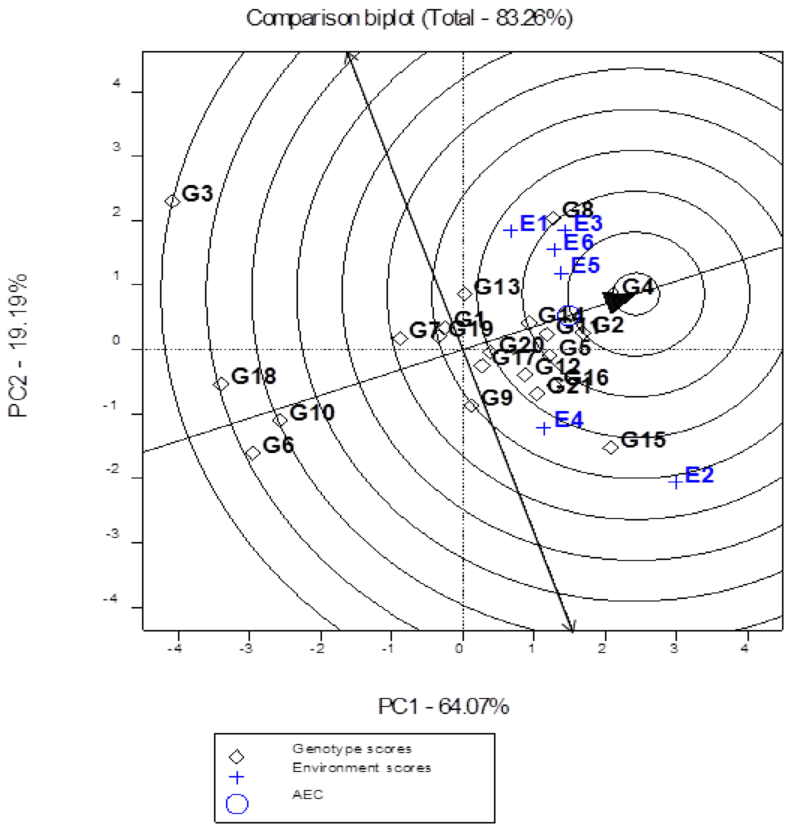

Figure 9.6 GGE biplot showing comparison of all faba bean genotypes tested for grain yield with the ‘ideal genotype’

Discriminating power and representativeness of the test environments

The GGE biplot showing association between genotypes and environments and the depicted discriminating power and representativeness to the test environment are presented in Figure 9.7. The vector view of the GGE biplot shows interrelationships among the test environments (Figure 9.7 A). The cosine of the angle between the vectors of two environments approximates the correlation coefficient between them. (Yan and Kang, 2002). The obtuse angle between E1 and E2 indicating the two environments are not correlated in discriminating the genotypes (Figure 9.7 A). This result verified the non-significant negative correlation from the spearman rank correlation coefficients (Table 9.9).

From the vector view of the biplot the length of the environments vectors measures discriminating ability. Based on this Adadi (E2) and Kulumsa (E3) were the most discriminating environments and representativeness of the other tests environments. On the contrary, Holetta (E1) and Bekoji (E4) had similar vector length and were the least discriminating environments. Kofele (E6) and Assasa (E5) had similar discriminating with similar vector length (Figure 9.7 A).

An ideal test environment should be both discriminating and representative. The small circle, defined the most discriminating and absolutely representative, on the AEC axis, with an arrow pointing to it represents the ideal environment (Figure 9.7 B). The small circle on the AEC axis with an arrow pointing to it represents the ideal environment and the arrow is used to indicate the direction of average environment axis (AEA) (Yan and Tinker, 2005). Figure 9.7 B, defined the most discriminating and absolutely representative. The absolute length of the projection from the marker of an environment onto the AEA is a measure of its representativeness, the shorter the projection, the more representative the environment. In contrast, the absolute length of the projection from the marker of an environment onto the AEA is a measure of its discriminative ability, the longer the projection, the more discriminative the environment. In the present study Adadi (E2) was the highly discriminating but least representative of the test environments. However, Assasa (E5) was found most representative with moderate discriminant to the genotypes. Kulumsa (E3) and Kofele (E6) had moderate discriminant ability but least representative of the test environments. In contrast Bekoji (E4) and Holetta (E1) were the least discriminating and least representative environments (Figure 9.7 B).

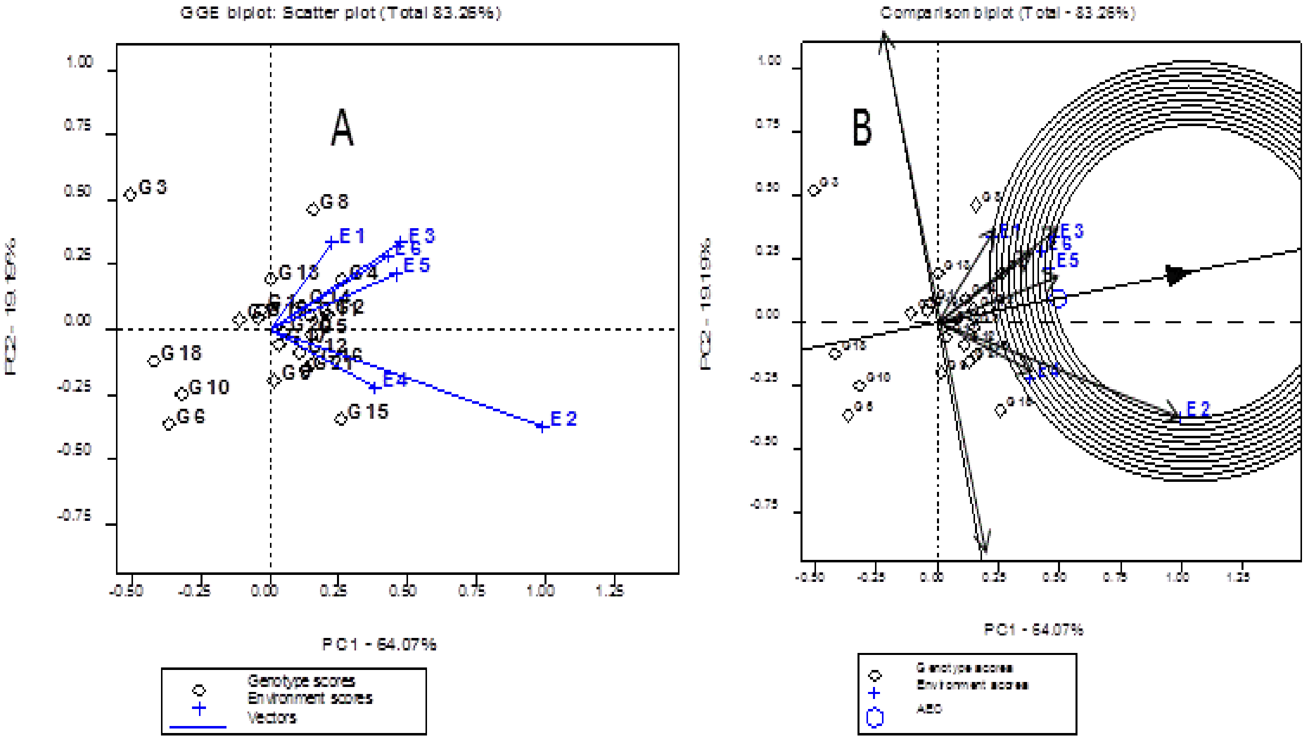

Figure 9.7 The vector view of GGE biplot shows interrelationships among the test environment (A), Comparison of environment with 'the ideal environment' (B) based on a genotype x environment yield data of 21 faba bean genotypes

Table 9. 9: Spearman correlation coefficients among the test environments for mean grain yield (t ha^{-1}) (above diagonal) and chocolate spot mean severity (%) for 21 faba bean genotypes

	E1	E2	E3	E4	E5	E6
E1	1	0.00	0.18	-0.19	0.34	-0.35
E2	-0.03	1	0.34	0.56**	0.18	0.34
E3	0.09	0.89***	1	0.55**	0.62***	0.47*
E4	0.01	0.86***	0.86***	1	0.32	0.87***
E5	-0.07	0.87***	0.86***	0.94***	1	0.33
E6	-0.09	0.79***	0.71***	0.85***	0.85***	1

9.3.4 GGE biplot analysis of chocolate spot resistance across environments

The 'which –won-where' pattern and mega environments

The results presented in Figure 9.8 showed the polygon view of the GGE biplot analysis for 21 faba bean genotypes chocolate spot disease severity. The first two principal components (PC1 87.9% and only PC2 5.5%) explained 93.4% of the total GGE variation. In the biplot view, the genotypes located at the vertex (vertex genotype) of the polygon were the best (susceptible/resistant) for the environments that fell within the sector. Therefore, genotypes G6, G18 and G10 were the vertex genotypes in the sector where the environments E1, E3, E4, E5 and E6 fall, thus had the highest chocolate spot severity percentage in these environments. Genotypes G13 and G20 were the vertex genotypes in the sector where environment E2 is found (Figure 9.8). Genotype G3 was the vertex genotype with the lowest chocolate spot diseases severity in all or some of the locations since no environment fell in their sector. Genotypes fell within the polygon, indicating that they were less responsive than the vertex cultivars (Figure 9.8).

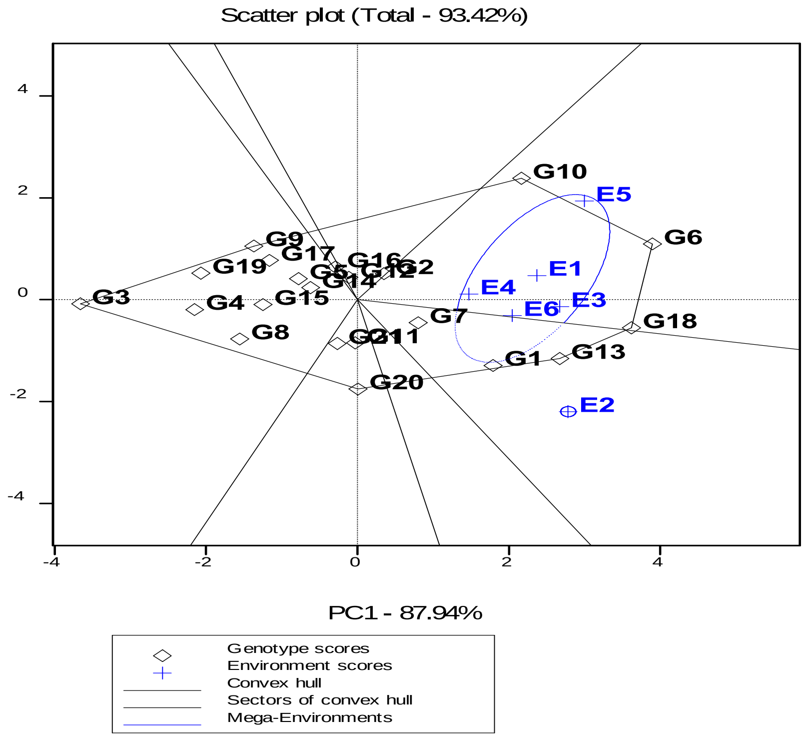

Figure 9.8 Polygon view of GGE biplot, showing which genotype is resistant in the study environment based on a genotype x environment chocolate spot disease severity data of 21 faba bean genotypes evaluated in six environments in 2013

The faba bean genotypes were assessed using an average-environment coordinate (AEC) method for chocolate spot disease severity performance (Figure 9.9). Based on these genotypes G3, G4, G15, G14, G16, G19, G8, G9 had low chocolate spot severity but least to moderate stable genotypes. G13, G6 and G10 were less stable with high percentage of disease severity. Since it was located near to the AEC abscissa and had a near zero projection onto the AEC coordinate G18 was the most stable with high disease severity (Figure 9.9). In general on this base the genotypes were ranked as G3<G4<G15<G14<G16<G19<G8=G9 for chocolate spot disease severity. The test environments fall in two sector of the polygon forming two mega-environments, E1, E3, E4, E5 and E6 as one mega-environment and E2 the second mega-environments.

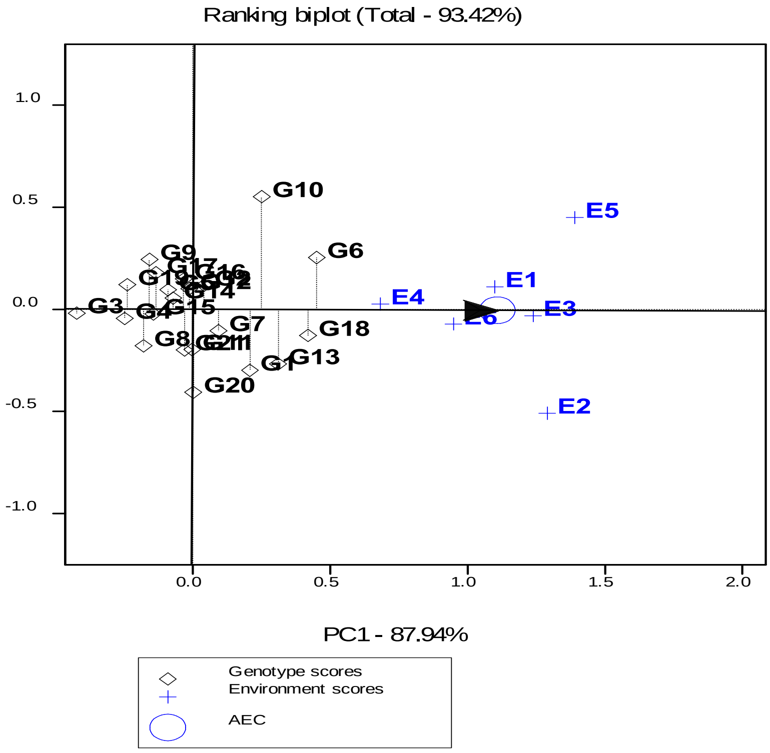

Figure 9.9 Average environment coordination (AEC) views of the GGE biplot on environment-focused scaling for the performance and stability of genotypes for chocolate spot severity.

The GGE biplot compare the ‘ideal genotype’ for chocolate spot with 21 faba bean genotypes shown in Figure 9.10. The small circle, which is located on the average environment coordinate (AEC) abscissa and with an arrow pointing to it, represents the ideal genotype (susceptible genotype). It had the highest chocolate spot disease severity percentage of the entire dataset. In this case, genotype G18 was closest to the hypothetical ideal genotype and therefore, most susceptible of all tested genotypes followed by G6, G13 and G10. Whereas, genotype G3 was extremely far away from the ideal genotype and thus, it is desirable genotype for low disease severity. Generally, the genotypes were ranked for chocolate spot disease severity score according to their projections for the ideal genotype as follows: G3< G4=G19=G8<G9=G15=G17< G5= G 14=G16 =G21<G12=G11=G20 (Figure 9.10).

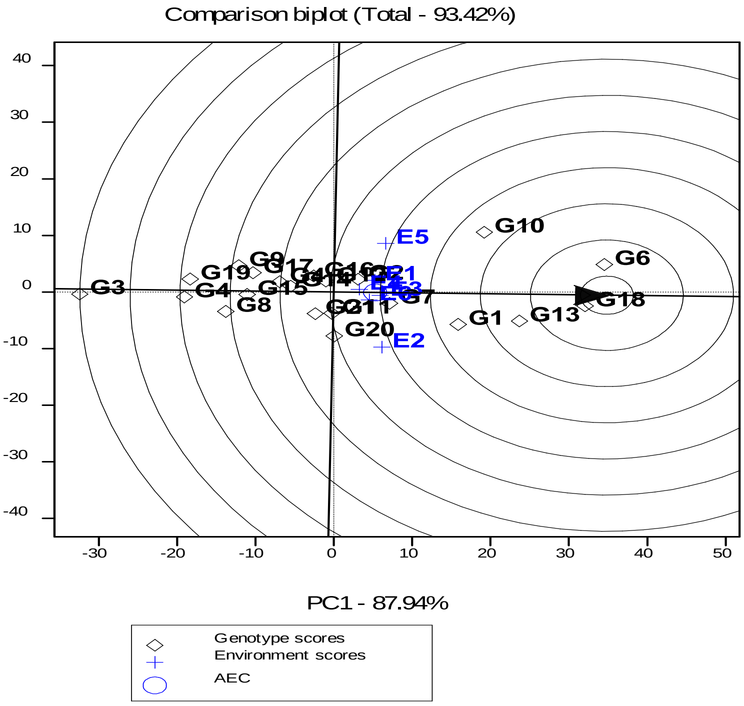

Figure 9.10 GGE biplot showing comparison of all faba bean genotypes tested for chocolate spot disease severity with the 'ideal genotype'

Discriminating power of the test environments

The interrelationships and discriminative ability among environments is presented in Figure 9.11. Thus of the six environments E2 and E5 had the longest vectors and high positive PC1 scores, suggesting that they were more discriminating of the genotypes than the other environments (Figure 9.11 A). Environment E4 had short vector and PC2 close to zero, suggesting less discriminating ability for the genotypes. Further the angle between any two environment vectors is a measure of their correlation coefficient between them (Yan, 2002). In this study, the angle between all the six environments were acute angle (< 90^o) indicating positive correlations among them (Figure 9.11 A). This was also supported by the positive correlation among test environments in the spearman correlation coefficients except the non-significant but negative correlation between E1 with E2, E5 and E6 (Table 9.9).

Based on these requirements, E3 (Kulumsa) was the most discriminant to the genotypes and representative to the test environment for chocolate spot disease followed by Holetta (E1) (Figure 9.11). Adadi (E2) and Assasa (E5) were highly discriminating but least representative to the test environments. Bekoji (E4 was found the least discriminate environment for the genotypes for chocolate spot disease.

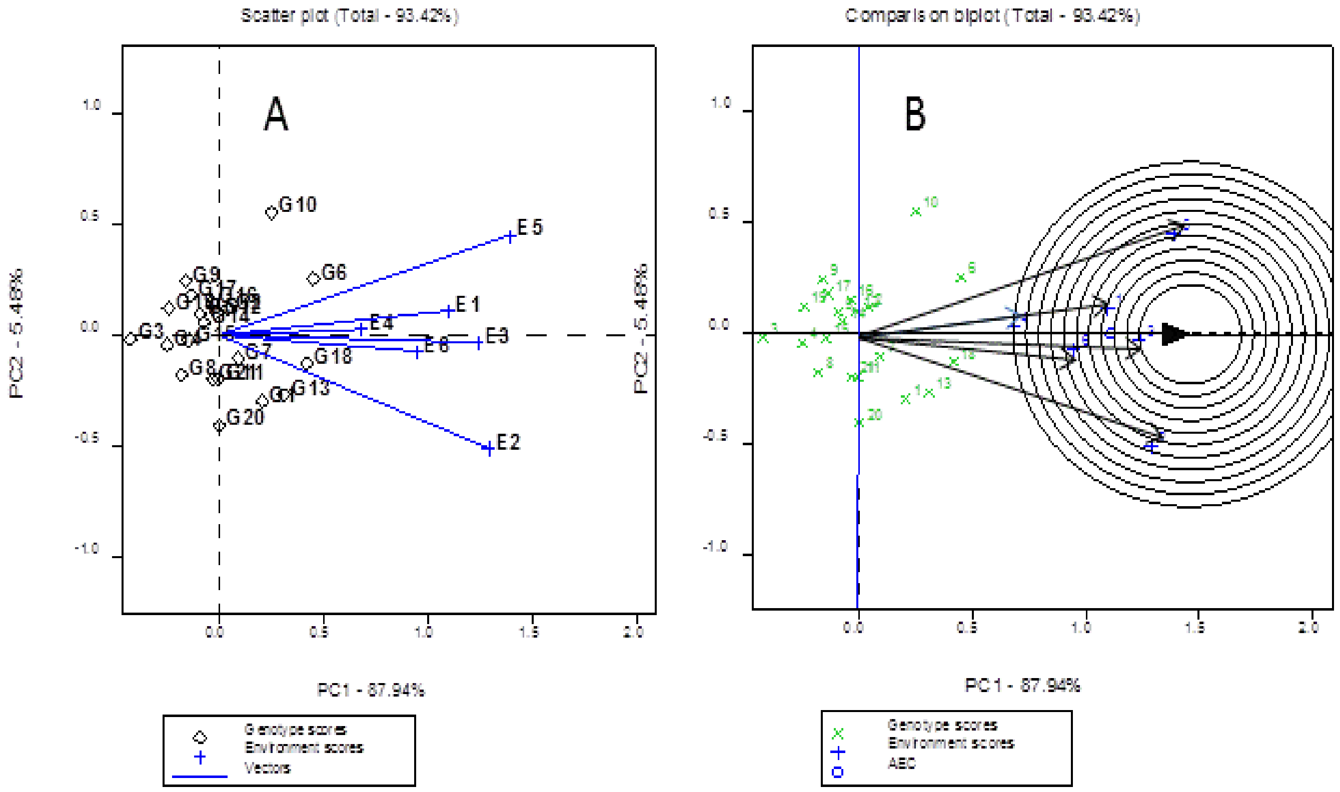

Figure 9.11 GGE biplot based on chocolate spot severity score (%) for six environments: Vector view showing the discriminating ability and representativeness of the environments (A), Comparison of environment with 'the ideal environment' (B)

9.3 Discussion

9.4.1 AMMI analysis

The additive main effects and multiplicative interaction (AMMI) analysis revealed highly significant variation for the effect of genotypes (G) environments (E), and genotype x environment interaction (GEI). The results of this study indicate that grain yield performances for the tested faba bean genotypes were highly (61.4%) influenced by environment effect followed by GEI. These results are consistent with the findings of the largest (88.5%) proportion of total variation attributed to environment in AMMI analysis of some faba bean accessions in Ethiopia (Fikere et al., 2008). The presence of significant G x E for grain yield suggested the need of testing faba bean genotypes across different environments before recommendation. AMMI biplot revealed that genotypes G20, G11, G4, and G14 were the most stable and adapted to all environments. However, G8 had specific adaptation at E6 (Kofele). Similarly, G21, G16, G9 and G12 were adapted to E4. In the present study, Adadi (E2) was the highest yielding environment and Holetta (E1) the lowest yielding. This can be explained by the neutral nature of the soil at Adadi but extremely acidic at Holetta. Acidic soil was reported as one of the the biotc factors that reduce nodulation and yield of faba bean (Zerihun and Abera, 2014)

AMMI analysis revealed genotype was the main source of variation for chocolate spot disease expression, followed by GEI, suggesting differential responses of the genotypes. Genotype and GEI equal contribution for disease expression were reported on faba bean genotypes evaluated in different environments (Villegas-Fernández et al., 2011). In the present study, Holetta (E1) was the most favourable for disease expression followed by Assasa (E5). In contrast E4 (Bekoji) was the least environment for the disease expression. Genotypes G3, G4, G19, G15, G17, G8, G16 and G9 were identified as resistant to moderately resistant with G3, G4 and G19 as the best. In contrast, G13, G18, G6, G1 and G10 were susceptible with G6 and G13 the most susceptible genotypes. Genotypes G19, G15, G17, G9 and G16 were resistant and stable across the environments. Genotype G3, G4 and G8 were unstable but resistant. G6, G10 and G18 were stable but susceptible.

9.4.2 GGE biplot analysis

Grain yield

The GGE biplot provided a visual depiction of the relationship among the genotypes and test environments. The polygon view of the GGE biplot indicated the presence of crossover G x E interaction as described by Yan and Kang (2002). Hence, genotypes G8 and G4 were found highest yielding at E1, E3, E6 and E5 and genotype G15 was the highest yielding genotype at E2 and E4. Based on the average-environment coordinate (AEC) in the GGE biplot, genotypes G2, G11, G14, G5, G21 and G20 were the most stable with an above average performance. In general, based on both mean grain yield performance and stability, genotypes were ranked as G4 > G2> G11> G14> G8>G5>G16>G21>G12>G20.

The GGE biplot revealed variability among the test environment and the crossover G x E interaction also signifies the test environments could be divided into mega-environments. In this study the environments fell within two sector of the polygon view suggesting two mega-environments. Mega-environment 1 comprised E1 (Holetta), E3 (Kulumsa), E5 (Assasa), and E6 (Kofele) and mega-environment 2 comprised two environments E4 (Bekoji) and E2 (Khodadadi et al.). The GGE biplot revealed Adadi (E2) as the most discriminating environment for faba bean genotypes for grain yield followed by Kulumsa (E3), Kofele (E6) and Assasa (E5). In contrast, Bekoji (E4) and Holetta (E1) were the least discriminating environments. However, Adadi (E2) was the least representative test environment. On the other hand Assasa (E5) was the most representative environment followed by Kofele (E6), Kulumsa (E3), Bekoji (E4) and Holetta (E1) for grain yield. An ideal test environment should effectively discriminate genotypes and represent the environments (Yan and Kang, 2002). Thus, in this study among all the six environments, Kulumsa (E3) represented the ideal testing environment with high discriminating ability for the genotypes and moderate in representativeness of the test environment for faba bean grain yield.

GGE biplot analysis for chocolate spot disease severity

Stability of resistance to chocolate spot is crucial for the success of breeding programme. In the present study, the polygon view of the GGE biplot revealed that genotypes G3, G4, G15, G14, G16, G19, G8, and G9 had low chocolate spot severity, but were the least stable genotypes. On the other hand, G13, G6 and G10 had high disease severity but least stable. G18 was the most stable genotype with high diseases severity. The test environments fell in two sectors of the polygon forming two mega-environments, E1, E3, E4, E5 and E6 as one mega-environment and E2 the second mega-environments. Genotypes were ranked as: G3< G4=G19=G8<G9=G15=G17< G5= G 14=G16 =G21<G12=G11=G20 for stability to chocolate spot disease resistance. Variation in stability of faba bean genotypes for chocolate spot disease resistance was observed in different locations (Villegas-Ferna´ndez et al., 2009).

The GGE biplot comparison of the ‘ideal genotype’ for chocolate spot with 21 faba bean revealed that genotype G3 was a desirable genotype for its low disease severity. Whereas, G18 was closest to the hypothetical ideal genotype and therefore, most susceptible of all tested genotypes followed by G6, G13 and G10. According to their projections from the ideal genotype, the genotypes can be grouped as; G3 (4.9%), G4 (8.9%), G19 (8.3%), G8 (10%), G9 (10.8%), G15 (10.9%), G17 (11.3%) for very low diseases severity but unstable, while genotypes G5 (12.1%), G14 (14.2%), G16 (10.7%), G21 (13.6%) and G20 (15.3%) were moderately stable and with low disease severity below the average mean. However, G20 (15.3), G2 (19.5%), G7 (15.5%), G1 (20.7%), G10 (18.3%), were stable but with higher disease severity. The closest genotypes to the hypothetical ideal genotype were G13 (24.3%), G6 (25.9%) and G18 (26%) very stable but very susceptible to chocolate spot disease. The per cent in parenthesis is the mean of disease severity of the respective genotypes across the six locations. Therefore, from these genotypes G5, G14, G16, G21 and G20 were selected for their lower disease severity for chocolate spot with moderate stability.

The GGE biplot of vector view revealed that Adadi (E2) and Assasa (E5) had more discriminating ability of the genotypes for chocolate spot disease than the other environments. In contrast, environment E4 had less discriminating ability for the genotypes.

However, the GGE biplot comparison of environment with ‘the ideal environment’ revealed environment E3 (Kulumsa) as the most discriminating and representative environment for chocolate spot disease followed by Holetta (E1). This indicated these environments are the most efficient for evaluating the potential of genotypes for chocolate spot resistance. Bekoji (E4) had less discriminating ability for the genotypes for chocolate spot disease. An ideal test environment should effectively discriminate genotypes and represent the environments (Yan and Kang, 2002). Therefore, among the six environments Kulumsa (E3) represented the ideal testing environment for chocolate spot disease and would be appropriate for selecting best faba bean genotypes resistant to chocolate spot.

9.4 Conclusion

The result of this study indicated that the yield performances of newly selected faba bean genotypes were highly influenced by environment and genotype x environment interaction. The magnitude and genotypic differences had high contribution to the total variation for chocolate spot disease severity. Both the GGE biplot and AMMI analysis provided almost similar results in terms of stability and performance of the genotypes. Among the genotypes FBColl-0012 (G2), FBColl-0034 (G11), FBColl-0055 (G14), FBColl-0025 (G5), FBColl-0049 (G21) and FBColl-0036 (G20) all from landrace collection were the highest yielding and most stable genotypes across the six environments. In this study the AMMI and GGE biplot analysis of the test genotypes for chocolate spot disease resistance revealed that genotypes FBColl-0025 (G5), FBColl-0055 (G14), FBColl-0024 (G16) and FBColl-0049 (G21) had moderate stability and resistance to chocolate spot disease. In addition, G20 FBColl-0030 (G4), FBColl-0057 (G19), FBColl-0018 (G8), FBColl-0035 (G9), FBColl-0031 (G15) and FBColl-0039 (G17) were also low in chocolate spot severity but found unstable. These could be utilised as a promising material for faba bean breeding programme future development of elite chocolate spot resistant cultivars. Considering the additive gene action mode of inheritance for chocolate spot resistance (Chapter 5), the less stable yet resistant genotypes could be exploited as a source using different breeding strategy such as back crossing to broaden genetic base of resistance and hence increase its stability. The GGE biplot clustered the six environments into two mega environments. Environment I included Holetta (E1), Kulumsa (E3), Assasa (E5), and Kofele (E6) while environment II represented by Bekoji (E4) and Adadi (E2). Among the testing environments Kulumsa (G3) was found the best discriminating and representative test location for faba bean evaluation for yield as well as chocolate spot resistance.

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Chapter 10

An overview of research findings

10.1 Introduction

Faba bean is one of the leading food security legume crops in Ethiopia widely grown in the mid and high altitude agro ecology. Potential faba bean yields are considerably affected by biotic and abiotic constraints in the country. Among the biotic constraints, chocolate spot caused by *Botrytis fabae* is the major problem and imposes a significant yield loss. The research focus for this study was to explore for chocolate spot resistance and high yield faba bean breeding strategies. The first step was a survey in the major faba bean growing area to identify farmers' preferred traits, their perception for chocolate spot disease and constraints that threaten faba bean production in Ethiopia. It was crucial to assess the genetic diversity and identify the resistance sources from the local landrace which are adapted to the environment as the basis for a breeding programme to develop chocolate spot resistant varieties. In order to formulate effective breeding strategies the genetics of inheritance, combining ability and heterosis of faba bean genotypes for grain yield and chocolate spot disease were determined. Finally stability analysis was carried out to identify stable genotypes and suitable testing sites in Ethiopia. This chapter summarised the research objectives and highlight the core findings and their implications for faba bean high yield potential and chocolate spot disease resistance breeding and opinions for future research.

10.2 Summary of major findings

10.2.1 Participatory assessment of production threats, farmers' desired traits and selection criteria of faba bean (*Vicia faba* L.) varieties: opportunities for faba bean breeding in Ethiopia.

The highlights of the study were:

- Faba bean was identified as major legume crop used as major source of protein, break crop for improvement of soil fertility and cash crop for resource poor farmers.
- Unavailability of improved faba bean seed and chocolate spot disease were among the major constraints reducing production and productivity of faba bean in the mid and highlands of Ethiopia.
- Majority of the farmers were aware of chocolate spot with various names for it at different districts

- Farmers predominantly grew local faba bean landrace varieties with low yield and very few used improved varieties.
- Farmers preferred the local landraces for their adaptability to the environment, tolerance to frost, early maturity, good food taste and straw yield.
- Breeding opportunities exist for improving the yield and disease resistance of local faba bean varieties which already possess farmer preferred traits and involving the farmers in the selection process at different stages of the breeding.

10.2.2 Molecular genetic diversity study of faba bean (Vicia faba L.) landrace collections from the Ethiopian highlands using SSR markers.

- The PIC and genetic diversity value averaging 0.62 and 0.63 respectively indicated that genetic diversity among faba bean germplasm was high.
- The genotypes were divided into three major distinct clusters.
- The clusters I and II comprised accessions collected from farmers and improved varieties and Cluster III comprising of genotypes from ICARDA.
- It was revealed that 68.54% of molecular variation in the faba bean landraces was due to variation within population and 31.46% accounted for the variation among populations which can be exploited by breeding programmes.
- The 30 SSR markers will be recommended for use in faba bean breeding programmes, because they were highly polymorphic.

10.2.3 Phenotypic diversity among Faba bean (*Vicia faba* L.) landraces from the Ethiopian Highlands and ICARDA

- The genotypes were categorized into three phenotypic clusters and different sub-groups.
- Six principal components were identified explaining more than 80% of the total variation.
- The Shannon Weaver diversity index, ranged from 3.82 to 7.15 revealed high diversity among and within the genotypes.
- The observed high variation among the faba bean genotypes would be exploited in breeding new varieties with desired traits for the target regions.

10.2.4 Genetic variability of faba bean genotypes to chocolate spot (*Botrytis fabae)* resistance, yield and some agronomic traits.

- There were highly significant differences among the landraces for reaction to the disease and agronomic traits.
- Significant positive correlation was recorded between reaction of genotypes in the field and greenhouse disease data.
- Chocolate spot disease severity rating was positively and highly correlated with AUDPC value
- The highest level of resistance was found in the ICARDA lines, ILB-4726, ILB-938 and BPL-710.
- At least 18 landrace collections displayed significantly lower disease reaction than the susceptible check.
- The following landraces FBColl-0030, FBColl-0036, FBColl-0003, FBColl-0001, FBColl-0012, FBColl-0024, FBColl-0055, FBColl-18, FBColl-0035, FBColl-0011, FBColl-0057, FBColl-0002, FBColl-0042, FBColl-31, FBColl-0034, FBColl-0008, FBColl-0039 and FBColl-0032 were identified as promising sources of resistance against *Botrytis fabae*.
- Overall resistance was highly heritable, suggesting that phenotypic selection can be exploited to improve chocolate spot resistance in faba bean varieties.

10.2.5 Gene action determining grain yield and Chocolate Spot (*Botrytis fabae*) Resistance in the Faba Bean Diallel cross.

- Chocolate spot disease assessment at 88 days after planting was found with better precision for discriminating the genotypes. In addition, there was high and positive correlation between AUDPC values for disease severity and the final disease severity assessment.
- Significant variation was observed for chocolate spot disease resistance and yield among the genotypes of Faba bean (*Vicia faba* L.).
- Both GCA and SCA effects were highly significant for chocolate spot resistance.
- The GCA was predominant signifying that additive gene effects were more important than non-additive effects; hence, selection would be effective to enhance disease resistance.
- SCA effects were predominant for grain yield suggesting that the non-additive gene action was in control and that the hybridization strategy would be effective to improve yield.
- Reciprocal effects were generally negligible (<10%) for both yield and disease resistance. This indicated that maternal or cytoplasmic effects played a minor role in the governance of resistance to chocolate spot and yield.

- The genotypes x Environment and SCA x Environment effects were highly significant for yield indicating the need to perform multi-environment trials before varieties are recommended to farmers.
- The line ILB-4726, which combined good disease resistance with high grain yield potential, would be recommended for programmes that emphasise chocolate spot resistance in faba bean.

10.2.6 The diallel cross analysis of yield components in faba bean

- The GCA and SCA effects differed significantly for all of the characters except plant height. This indicates that both additive and non-additive genetic effects played a significant role in the inheritance of the secondary traits.
- The reciprocal effect was negligible for all traits and suggested that maternal effects were not important for yield components.
- The GCA was larger than SCA for only two traits, while the SCA effects were predominant for nine traits, suggesting that genes with non-additive effects were preponderant for the yield components and that hybridization would be effective to improve the varieties.
- Narrow sense heritability ranging between 70% and 95%, indicating that selection strategies would be effective.

10.2.7 Heterosis and Path Analysis for Grain Yield and chocolate spot disease resistance in Faba Bean

- Promising experimental hybrids were identified that displayed high grain yield (t ha^{-1}) and up to 162.3% better parent (BP) and up to 133.9% mid parent (MP) heterosis
- The crosses NC58 x ILB-4726, ILB-4726 x Kasa, NC58 x BPL-710, ILB-938 x CS-20-DK, ILB-938 x CS-20-DK and CS-20-DK x BPL-710 would be recomended for faba bean breeding for grain yield.
- Minimum mid parent heterosis was -29.4% and better parent heterosis was -60.5% was obtained for chocolate spot resistance
- Crosses ILB-4726 x kasa, ILB-4726 x Bulga-70, CS-20-DK x Gebelcho, NC58 x ILB-4726, Kasa x BPL-710 and ILB-938 x Kasa are recommended for chocolate spot resistance breeding.
- The three crosses ILB-4726 x Kasa, ILB-4726 x Bulga-70, NC58 x ILB-4726 are recommended for faba bean breeding aiming for grain yield and chocolate spot disease resistant.

- Hundred seed weight, primary branch, number of pods per plant, number of seeds per pod and number of nodes per plant had indirect effect for grain yield.
- The number of nodes with pods which had seed (NWP), and total biomass (BM) had positive direct effects on the grain yield. They can be used for selection of high yielding varieties in faba bean breeding programmes.

10.2.8 GGE Biplot and AMMI analysis of genotype x environment interaction in faba bean genotypes for grain yield and chocolate spot (*Botrytis fabae*) disease resistance.

- The study confirmed significant genotype x environment interaction for grain yield and chocolate spot resistance. Cross over interaction was detected and the six environments grouped into two mega-environments
- The AMMI biplot depicted the genotypes on the basis of their adaptation patterns. The GGE biplot also clustered the environments and identifies the winning genotypes in each environments
- Among the testing environments Kulumsa (G3) was found the best discriminating and representative location for faba bean yield as well as chocolate spot.
- Six genotypes FBColl-0012 (G2), FBColl-0034 (G11), FBColl-0055 (G14), FBColl-0025 (G5), FBColl-0049 (G21) and FBColl-0036 (G20) were found to be high yielders and the most stable genotypes.
- Genotypes FBColl-0025 (G5), FBColl-0055 (G14), FBColl-0024 (G16) and FBColl-0049 (G21) displayed low chocolate spot severity and moderate level of stability.
- This promising material could be exploited in faba bean resistance breeding programmes.

10.3 Implications of the findings for breeding faba bean for higher yield and chocolate spot resistance.

The majority of the farmers continued to grow local varieties because they possessed several desired traits that were lacking in improved varieties released previously. Future research should be conducted through participatory breeding approach in order to incorporate farmers’ requirements. The improved varieties especially the introduced varieties possessed some desired traits that could be used as parent for hybridization to improve trait shortfalls in the local varieties. Overall, among the desirable traits farmers anticipated includes high grain yields, early maturity, resistant to chocolate spot, tolerant to frost, good food taste and high straw yield.

The majority of farmers were aware of chocolate spot disease with different name but most of them were not aware of the cause. The control measures by farmers were very minimal due to lack of knowledge and farmers cannot afford to buy fungicides to control the disease. Cultural practises are not effective for controlling chocolate spot. Therefore, breeding for chocolate spot resistance would be the best option for managing chocolate spot in Ethiopia. The study suggested the necessity of extension to improve knowledge on disease identification and management strategies.

There is considerable genetic diversity which could be useful for faba bean breeding in the mid and high lands of Ethiopia. The SSR genetic markers were useful and provided three distinct groups. These enable breeders to exploit the genetic resource for breeding for traits of interest.

Considerable resistant levels were identified from the study of evaluation of faba bean regionally adapted landraces and introduced germplasms for chocolate spot disease resistance. This can then be used to improve the susceptible but farmers' preferred and adapted germplasm. This study has identified source of resistance for chocolate spot. Nevertheless additional sources from different genetic backgrounds are required to broaden the genetic base.

Positive correlation coefficients between AUDPC values for chocolate spot disease severity and general disease severity score suggested that discriminating of the genotypes using the two disease assessments was by large similar. Therefore, a single disease severity assessment at 88 days after planting would be adequate for screening large numbers of germplasms.

The genetic study indicated presence of sufficient genetic variability among the parental lines. The predominance of additive gene action for chocolate spot resistance indicates that genetic improvement can be achieved by accumulating favourable alleles from parents. This could be achieved using conventional breeding methods such as recurrent selection and backcross breeding. Moreover, additive genes coupled with high heritability in the inheritance of chocolate spot resistance suggests that selection should be effective in early segregation generations. The importance of non-additive gene action controlling grain yield and most of yield components suggested that breeding gain can be realized through hybridization strategy in the programme.

The ultimate goal in any breeding programme is to develop high yielding, stable genotypes. Using the AMMI and GGE biplot models, this study identified FBColl-0012 (G2), FBColl-0034

(G11), FBColl-0055 (G14), FBColl-0025 (G5), FBColl-0049 (G21) and FBColl-0036 (G20) genotypes as high performing genotypes that were stable in all six test environments. The genotypes FBColl-0025 (G5), FBColl-0055 (G14), FBColl-0024 (G16) and FBColl-0049 (G21) had low chocolate spot severity and moderate level of stability which can be used as genetic source for disease resistance and high yield breeding programmes. The experimental hybrids that exhibited high level of heterosis for grain yield and chocolate spot resistance can be recommended for further testing and releases.