

**Breeding for Common Bacterial Blight Resistance in Common Bean
(*Phaseolus vulgaris* L.) in Ethiopia**

By

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ABSTRACT

The common bean (*Phaseolus vulgaris* L., $2n=2x=22$) is a commodity crop in Ethiopia cultivated by about 3.6 million smallholder farmers. In the country, common bean is annually cultivated across an estimated area of 306,187 ha with a net production of 520,979 tons. Despite the increased area of production and economic value of common bean, the mean productivity of the crop is relatively modest (1.7 tons ha^{-1}) in Ethiopia. This is still lower compared to the potential yields of the crop reaching up to 4.5 tons ha^{-1} . The low productivity of the crop is attributable to a multitude of abiotic, biotic and socio-economic constraints. Limited access to high yielding varieties, drought stress, fungal and bacterial diseases, insect pests, poor soil health are among the major constraints affecting common bean production and productivity in the country. Over 50 common bean varieties were released to enhance yield gains under optimal growing conditions in Ethiopia. However, most of the released varieties have succumbed to the common bacterial blight (CBB) disease caused by *Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans*. The common bacterial blight causes significant yield loss varying from 20 to 100% necessitating development and deployment of CBB resistant and farmer-preferred common bean cultivars for sustainable production and economic gains in the country. Hence, there is a need for development and deployment of new varieties with durable resistance and farmer-preferred traits.

The objectives of the study were to: (i) identify constraints affecting common bean production and productivity and to identify farmers' perception on the constraints, and their trait preferences for inclusion in common bean breeding programs, specifically for disease resistance breeding; (ii) identify new sources of CBB resistance from a diverse panel of genotypes; (iii) select common bean parents and families with good combining ability effects and heritability for CBB resistance and agronomic traits for variety development; and (iv) introgress and track CBB resistance genes or quantitative trait loci (QTL) in selected susceptible commercial common bean genotypes through marker-assisted selection for cultivar development.

During the first study, a participatory rural appraisal (PRA) was conducted in two major common bean growing regions, Oromia and Southern Nations, Nationalities and Peoples' Region (SNNPR) in Ethiopia. Data were collected using semi-structured questionnaires and focused group discussions with 255 farmers. Key inferences were made based on quantitative and qualitative data

analyses. Drought stress (reported by 46.3% of the respondent farmers), diseases (24.4%), insect pests (12.6%) and lack of seeds of improved varieties (12.2%) were identified as the most severe constraints to common bean production across the study areas. Among the identified biotic constraints of common bean, CBB was ranked as the most devastating disease reported by 63.5% of the respondents. Only 9.8% of the respondents reported using introduced common bean varieties with disease resistance and better agronomic traits. A significant proportion of the respondent farmers (28.6%) did not use any disease control methods. Yield loss due to diseases was reported to reaching up to 70% in the study areas. Hence, CBB resistance and other production constraints, agronomic attributes and farmer-preferred traits are the main drivers of common bean improvement in Ethiopia.

In the second study, 110 genetically diverse accessions were evaluated for CBB resistance and better agronomic traits at three hotspot sites (Melkassa, Arsi Negelle and Mieso) for two seasons (2017 and 2018) in Ethiopia. Data on mean disease severity on leaf (SL) and mean disease severity on pod (SP), the area under disease progress curve (AUDPC), number of pods per plant (PPP), number of seeds per pod (SPP) and grain yield (GY) were collected. Data were subjected to standard analysis of variance and principal component analysis. The genotype \times site interaction (G \times E) had significant effect on all assessed traits. This indicated the presence of marked variation among tested genotypes in CBB resistance across the testing sites. Genotypes including SEC21, SEC23, SMC21, VAX6, SEC12, SEC25, SMC22, VAX5, SEC20, SEC22, SEC24, SEC26, SMC16, SMC24, VAX6, SEC25, SEC21, SEC23 and SMC21 exhibited lower values of SL, SP and AUDPC which are useful genetic resources for future CBB resistance breeding programs. Genotype Nasir provided a mean grain yield of 3.45 ton/ha followed by VAX1 (2.86 ton/ha) and Hawassa Dume (2.83 ton/ha). **The reaction of the Hawassa Dume and VAX6 was resistance but Nasir was susceptible.** CBB-resistant and high yielding genotypes had the higher PPP and SPP making them ideal candidates for common bean breeding in Ethiopia or similar agro-ecologies emphasizing CBB resistance and enhanced agronomic traits.

During the third investigation, eight selected CBB resistant common bean genotypes were crossed with four susceptible farmer-preferred common bean genotypes using a line \times tester mating design. The F₂ generation were evaluated at Melkassa and Arsi Negelle Agricultural Research stations in

Ethiopia using an alpha lattice design with two replications. Disease parameters such as SL, SP and AUDPC and agronomic traits such as the number of pod per plant (PPP), number of seed per pod (SPP) and grain yield (GY) were recorded. Genetic analysis was done through heritability and combining ability estimates. Results showed that the inheritance of all CBB resistance parameters is largely attributable to additive gene effects. There were high heritability values for grain yield ($H^2 = 0.70$). The heritability values of yield components varied from 0.66 to 0.7 revealing the contributions of additive genes in conditioning trait inheritance. Parents such as SEC12, SEC21, SEC20, SEC24 and SEC25 had negative and significant general combining ability (GCA) effects for CBB severity for leaf and pod infection. The F₂ generation such as Nasir/SEC24, Red Wolaita/SMC21, Mexican142/SMC21, Mexican142/SEC25, Awash1/SEC22, Red Wolaita/SEC12, Nasir/SEC22, Nasir/SEC20 and Awash1/SEC12 were best specific combiners and selected for CBB resistance breeding. These generation displayed better agronomic attributes with significant and negative specific combining ability (SCA) effect for SL and AUDPC. The selected parents and population are useful genetic resources for future breeding of CBB resistant and agronomically superior transgressive segregants for common bean variety development in Ethiopia.

In the last study, 16 breeding populations were developed. The new populations were field phenotyped at two CBB hotspot sites (Melkassa and Arsi Negelle) in Ethiopia and genotyped using three selected and diagnostic single nucleotides polymorphism (SNP) markers (CBB_SAP6_801, CBB_06_TC_9138316 and CBB_SU91_g91004686) at Intertek in Sweden. The F₂ progenies and parents involved in each cross were evaluated at both sites and data were collected on CBB severity on leaf (SL) and severity on pod (SP). Significant ($P < 0.001$) variations were recorded among test genotypes for CBB severity. Analyses of the segregation F₂ populations for SL and SP at indicated a genetic ratio of 1:3:1 involving resistant: moderately resistant: susceptible individuals, respectively and suggesting that CBB resistance was conditioned by multiple genes. Significant genotype variation was observed based on SNP analyses with 23% of the total variation was attributable to among the assessed populations. The SNP markers explained 22% (marker CBB_SAP6_801) and 87% (CBB_06_TC_9138316) of the total variations present in the test populations making them a marker of choice for future genetic analysis of CBB resistance. The study has selected CBB resistant individuals with QTL associated with the two SNP markers useful for marker-assisted selected and development of breeding populations in common bean.

Overall, the present study identified agronomically superior and CBB resistant common bean parents and new families that will be subjected for multiple environment evaluations and stability analysis for cultivar development and release in Ethiopia.

Declaration

I, Kidane Tumsa, declare that:

- 1 The research reported in this thesis, except where otherwise indicated, is my original research.
- 2 This thesis has not been submitted for any degree or examination at any other University.
- 3 This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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Signed:



Kidane Tumsa

As the candidate's supervisor, we agree to the submission of this dissertation



Prof. H. Shimelis(Supervisor)

Prof. Mark Laing(Co-supervisor)

Dr. Mukankusi Clare (Co-supervisor)

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Dedication

The thesis is dedicated to my mother Mamite Argacho who devoted her time for my success.

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Abbreviations

AUDPC	Area under disease progress curve
CBB	Common bacterial blight
CGIAR	Consultative Group for International Agriculture Research
CIAT	International Center for Tropical Agriculture
CV	Coefficient of variation
DF	Degrees of freedom
NPPP	Number of pods per plant
NSPP	Number of seeds per pod
GY	Grain yield
GCA	General combining ability
H ²	Broad sense heritability
h ²	Narrow sense heritability
LSD	Least significant difference
MAS	Marker-assisted selection
PCA	Principal component analysis
QTL	Quantitative trait loci
SCA	Specific combining ability
SL	Severity on leaf
SP	Severity on pod
UKZN	University of KwaZulu-Natal

INTRODUCTION TO THESIS

Background

The common bean (*Phaseolus vulgaris* L., $2n=2x=20$) is a major legume crop cultivated worldwide as source of food and cash income (Gepts et al., 2008; Mukankusi et al., 2019). It is an alternative and a relatively cheap source of protein, complex carbohydrates, fiber, minerals, vitamins and folate for more than 500 million people in the tropics (Broughton et al., 2003; Miklas et al., 2006; Mukankusi et al., 2019). It serves as the major staple food to more than 100 million people in Africa, with per capita consumption of 40 to 60 kg per person per year. African countries such as Rwanda, Kenya and Uganda are the leading consumers of common bean in the world (Blair et al., 2013; Mukankusi et al., 2019).

Based on the report of the Food and Agriculture Organization of the United Nations, global production of common bean was 26.8 million tons in 2016 (FAO, 2016). Over 80% of the production is in the tropics. The leading common bean producers in the world are India (with total annual production of 2.64 million tons), Brazil (3.02 million tons) and Myanmar (2.65 million tons) (FAOSTAT, 2017). In Sub-Saharan Africa (SSA) Tanzania, Uganda, Kenya and Ethiopia are the largest producers (FAOSTAT, 2017). However, the recurrence of common bacterial blight has become a major yield and quality limiting factor of common bean production globally including in Ethiopia (CSA, 2018). The common bacterial blight (CBB) disease of common bean incited by the *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and its variant *X. axonopodis* pv. *phaseoli* var. *fuscans* (*Xaf*) is one of the major production constraints in most production regions worldwide (Tar'an et al., 2001; Perry and Peter, 2016). The disease is widely distributed and under severe epidemics it causes a significant yield loss depending on cultivar susceptibility, environmental condition, crop growth stages, among others (Viteri et al., 2014; Perry and Peter, 2016).

Common bean production constraints and breeding objectives in Ethiopia

The common bean is an important legume crop in Ethiopia, providing food and income security to millions of smallholder farmers. In terms of quantity of common bean production, Ethiopia is ranked 10th in the world and 4th in Sub Saharan Africa (SSA) after Tanzania, Uganda and Kenya (FAOSTAT, 2017). About 496,600 tonnes of common bean valued at 283.4 million US Dollars was marketed globally in 2016. During the same period Ethiopia exported 184,300 tons (37% of

the global export) with a monetary value of 128.6 million USD (FAOSTA, 2016). In Ethiopia common bean is cultivated on an estimated area of 306,187 ha with a net production of 520,979 tones. In the country the total number of households producing common bean in 2017/18 cropping season was 3.6 million. About 81.4 % of common bean is produced in Oromia and Southern Nations, Nationalities and Peoples' Region (SNNPR) regions of the country (CSA, 2018).

Despite the increased area of production and economic value of common bean, the mean productivity of the crop is modest compared to other African countries (1.7 tons ha⁻¹) (CSA, 2018) in Ethiopia. [There is a yield gap between the mean national productivity and the potential yield of the crop](#) (Muthoni et al., 2017). The low productivity of the crop is caused by an array of abiotic, biotic and socio-economic constraints in the country. Use of low yielding varieties, drought stress, fungal and bacterial diseases, insect pests, poor soil health are among the major constraints to common bean production in the country (Katungi et al., 2011; Asfaw et al., 2012; Amsalu et al., 2018). More than 50 common bean varieties have been released between 1960s to 2010s to enhance yield gains under optimal growing conditions (Amsalu et al., 2018). However, most of the released varieties succumbed to CBB needing development of new varieties with durable resistance and farmer-preferred traits.

Breeding for CBB resistance in common bean

Genetic variation is key in plant breeding programs. Recently, modern phenotyping and genotyping platforms were developed to determine genetic variation, screen and select parents for breeding for different purposes including disease resistance. The use of resistant common bean varieties with durable resistance is believed to be the most effective, economical and environmentally friendly approach to control CBB. Although there are limited sources of CBB resistance in *P. vulgaris*, interspecific hybridization with related species has shown promise in some breeding programs (Singh and Miklas, 2016; Singh and Munoz, 1999; Singh, et al., 2014). Successful transfer of resistance genes from genetically related species (e.g. from secondary and tertiary gene pool) to common bean has been reported (Singh and Miklas 2016; Viteri, et al. 2014). Scarlet runner bean (*P. coccineus* L.) and tepary bean (*P. acutifolus*) are reportedly the major source of CBB resistance (Yu et al., 1998; Welsh and Grafton, 2001). However, most derived lines with CBB resistance were poorly adapted when deployed to diverse agro-ecologies in Africa and other tropical production regions (Kelly and Bornowski, 2018). Hence, developing common bean

cultivars that are high yielding, locally adapted, farmer-preferred with durable CBB resistance is an overriding consideration for successful disease management and yield gains (Osdaghi et al., 2009). Common bean lines with CBB resistance were developed worldwide including XAN-159, XAN-160 and XAN-161 (McElroy 1985). Common bean lines such as HR45 and HR67 that exhibited higher levels of CBB resistance were also developed from crosses between *P. vulgaris* × *P. acutifolius* (Park et al., 2007). Also, the novel VAX-lines (VAX1 to VAX6) were developed through gene stacking from variable sources (Singh and Munoz, 1999; Singh, et al., 2014). Screening of a large number of test genotypes and breeding populations under greenhouse or field conditions enables selection of individuals with desirable traits for breeding. However, phenotypic selection requires technical skill, material and financial resources (Witcombe and Virk, 2001). Use of molecular markers could significantly complement the efficiency of phenotypic selection in conventional plant breeding programs (Gupta et al., 2010).

The inheritance of CBB resistance, based on leaf and pod severity, is reportedly conditioned by few to several genes (Tryphone et al., 2012). The expression of CBB resistance is dependent on the genetic background of the source of resistance, environmental conditions, disease pressure, crop growth stage and parts of the plant infected (Kelly et al., 2003; Singh and Schwartz, 2010; Durham et al., 2013). These factors and the presence of multiple genes and at least 24 quantitative trait loci (QTL) across all the 11 linkage groups have reportedly made breeding of CBB resistance complicated (Singh and Schwartz, 2010). Hence, use of phenotypic traits and diagnostic molecular markers can aid in the introgression CBB resistance genes and tracking of QTLs transferred into susceptible common bean genotypes.

Molecular markers are widely used in disease resistance breeding programs for genetic analysis and to fast track and pyramid candidate genes, among others (Miklas et al., 2006; Mukankusi et al., 2019). The most commonly used marker systems in CBB resistance breeding include sequence characterized amplified region (SCAR) and simple sequence repeats (SSR) markers (Yu et al., 2000). SSR marker such as BC420 is reportedly associated with linkage group B6 (Yu et al., 2000), while marker SU91 was linked to QTL on linkage group B8 and SAP6 on linkage group B10, all conferring CBB resistance in common bean (Miklas et al., 2000; Yu et al., 2004). Common bean lines possessing high level of CBB resistance were developed through phenotypic and marker-assisted selections. These included USDK-CBB-15 (Miklas et al., 2006b), USWK-CBB-17

(Miklas et al., 2006c), USCR-CBB-20 (Miklas et al., 2011) and ABC-Weiing (Mutlu et al., 2008) Recently the SCAR and SSR markers were converted to single nucleotide polymorphism (SNP) and allowed genotyping in gel-free systems through various service providers (Song et al., 2015; Mukankusi et al., 2019). SNPs are valuable markers for marker-assisted selection because of their abundance, stability and simplicity for genotyping (Shi et al., 2011).

Rationale of this study

Common bean is regarded as the ‘white gold’ for being an export commodity in Ethiopia. For instance, the export value of white beans increased markedly from 17.9 million to 100 million USD during the period between 1989/90 and 2012/13. Development and deployment of resistant varieties is the most economic and sustainable option to mitigate the effect of the disease. However, breeding for resistance to CBB is not well developed and has received limited attention in legume breeding programs in Ethiopia.

In the course of cultivar development, inclusion of farmer preference in breeding objectives is likely to improve adoption of a newly developed cultivar. Client-preferred varieties have substantial market share and penetration. Hence, identification and incorporation of farmers preferred traits and their needs through participatory rural appraisal (PRA) studies is among the important approaches to be adopted during breeding for disease resistance and enhanced yields. As the adaptation of most CBB resistance sources are limited to specific agro-ecology, multi-environment evaluations of candidate common bean parental lines would allow selection of parents with CBB resistance and complementary agronomic traits. This enables population development for successful CBB resistance breeding and deployment of best adapted lines under variable disease pressure and pathotypes in the hotspot and target production areas. Therefore, identification of new sources of CBB resistance with desirable agronomic traits from a diverse panel of genotypes from different sources is mandatory for effective breeding. Genetic information of the potential parental lines and their progenies is derived from combining ability tests based on economic traits. This will facilitate identification of productive and CBB resistant common bean cultivars. The combining ability effects of the selected parents and their progenies should be assessed to develop new breeding populations adapted to local production conditions. Phenotypic screening of a large number of populations in greenhouse or field conditions facilitates selection of progenies with multiple traits including CBB resistance. Use of complementary molecular

markers could significantly improve the efficiency of phenotypic screening in conventional breeding methods for gene transfer and tracking during marker-assisted selection (MAS).

Aim

The aim of this study was to contribute to the development of CBB resistant, high yielding and farmer-preferred common bean varieties in Ethiopia.

Specific objectives

The specific objectives of this study were:

1. To identify constraints affecting common bean production and productivity and to identify farmers' perception on the constraints, and their trait preferences for inclusion in common bean breeding programs, specifically for disease resistance breeding.
2. To identify new sources of CBB resistance from a diverse panel of genotypes, which can be used to develop CBB resistant common bean varieties.
3. To select common bean parents and families with good combining ability effects and heritability for CBB resistance and agronomic traits for variety development.
4. To introgress and track CBB resistance genes/QTL in selected susceptible commercial common bean genotypes through marker-assisted selection for marker-assisted cultivar development.

Hypotheses

The major hypotheses tested in this study were:

- i. Data obtained through surveys involving common bean farmers from the two growing regions would allow documenting the current common bean production, constraints and scoping mechanisms to guide breeding for CBB resistance.
- ii. There exists phenotypic variability among common bean genotypes for CBB resistance and economic agronomic traits when evaluated under multiple environmental conditions.
- iii. Test genotypes and progenies have good combining ability effects for CBB resistance and agronomic traits for selection.
- iv. [There is significant phenotypic and genotypic correlations that exists between CBB resistance and agronomic traits based on phenotypic and SNP analyses for marker-assisted selection.](#)

Outline of thesis

This thesis consists of five different chapters in accordance with the number of objectives (see Table 0.1). Chapter 1 is written as a separate review paper, while chapters 2 to 5 are written as discrete research papers, each following the format of a stand-alone research paper, including the published chapter, followed by a general overview and implications of findings from the study. The literature review and four experimental chapters of the study made the thesis chapters that were condensed into discrete but inter-dependent papers according to the University of KwaZulu-Natal's major thesis format. There are some overlaps and unavoidable repetitions of references and some introductory information between chapters. Chapter 3 was published in the Journal of Phytopathology (<https://doi.org/10.1111/jph.12951>). Chapter 2 was submitted for publication to Phytopathology: Manuscript ID: JPHY-20-238.

Table 0.1 Thesis structure

Chapter	Title
-	Introduction to the Thesis
1	Review literature
2	Farmers' perceptions on production, production constraints, trait preference and disease management options in two major common bean growing regions of Ethiopia: implications for common bacterial blight disease resistance breeding
3	Identification of sources of resistance to common bacterial blight in common bean in Ethiopia
4	Combining ability and gene action controlling common bacterial blight resistance and agronomic traits in common bean
5	Introgression of common bacterial blight resistance and aided by marker assisted selection in common bean (<i>Phaseolus vulgaris</i> L.) in Ethiopia
-	General overview and implications of the study

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CHAPTER ONE: A REVIEW OF THE LITERATURE

Abstract

The common bean (*Phaseolus vulgaris* L., $2n=2x=22$) is an important grain legume and a valuable source of protein and cash particularly for low income households in developing countries. In Ethiopia, the crop is cultivated by millions of small-scale farmers for food security, and local and international markets. The common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. axonopodis* pv. *phaseoli* var. *fuscans* (*Xaf*) is one of the major diseases of common bean in tropical and subtropical regions including Ethiopia. CBB causes significant yield loss reaching up to 20–100% necessitating development and deployment of cultivars with durable resistance possessing economic traits. This chapter reviews the research findings on CBB resistance breeding including on the causal agent of CBB, its dissemination and symptoms. Further, the chapter summarizes various disease management strategies recommended for CBB each with its pros and cons. This is followed by a discussion on host plant resistance breeding approach as an effective, economical and environmentally friendly method that can be deployed to resource poor farmers. Important highlights are provided on progress made on identification and introgression of CBB resistance genes/quantitative trait loci (QTL) from genetically related species of the common bean such as the scarlet runner and tepary beans followed by genetic analyses on the inheritance and genomic regions conditioning CBB resistance. Efforts that have been made to develop lines and cultivars that possessed CBB resistance with their breeding methodologies are identified and discussed. Different molecular marker systems and their use in marker-assisted selection (MAS) to improve the efficiency of conventional breeding are presented. Finally, the use of participatory rural appraisal (PRA) as multidisciplinary research tool in identifying the needs and preferences of farmers, and the advantages of integrating these in plant breeding programs are highlighted. Information presented in this chapter may enhance common breeding efforts emphasizing CBB resistance and farmer-preferred traits.

Key words: Common bean, common bacterial blight, Ethiopia, marker-assisted selection, *Phaseolus vulgaris*, participatory rural appraisal, quantitative trait loci, resistance breeding

1.1 Introduction

The common bean (*Phaseolus vulgaris* L., $2n=2x=22$) is one of the relatively cheap sources of protein and human nutrition for many rural and urban populations especially in developing countries (Broughton et al., 2003; Miklas et al., 2006a). It is an important source of dietary fiber, vitamins (0.9 -1.2 mg/100gm), riboflavin (0.14 - 0.27 mg/100gm), niacin (1.16 - 2.68 mg/100gm), folic acid (0.17 mg/100gm) and vitamin B6 (Mazza, 1998). Common bean is also well-known for its low fat content and for being free from cholesterol, which are desirable to reduce the risks of cancer, diabetes and heart diseases in humans (Matella et al., 2006). Domestication of common bean started in the regions of South America, Central America and Mexico before its expansion globally (Singh et al., 1991) including to Africa, Europe, Asia and Oceania (Singh et al., 1991; Singh and Miklas, 2015).

In sub-Saharan Africa over 200 million people depend on common bean as a primary staple food (Broughton et al., 2003; FAO, 2017). In 2016, global production of common bean was 26.8 million tonnes (FAOSTAT, 2016) and over 80% of the global bean production is contributed by tropical countries. With a total annual production of 520,979 tonnes, Ethiopia ranks 10th in the world and fourth in Africa (FAOSTAT, 2017). Approximately 3.4 million smallholder farmers produce common bean in Ethiopia for household consumption and cash income. Currently, the national average yield of the common bean is 1.7 ton ha⁻¹ (Central Statistics Authority, 2018) compared to the potential yield reaching up to 4.5 ton ha⁻¹ (Muthoni et al., 2017). The low yields in the country are attributed to different production constraints such as the common bacterial blight disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and its variant *X. axonopodis* pv. *phaseoli* var *fuscan* (*Xaf*). CBB is one of the major diseases of common bean in tropical and subtropical production regions of the world (Tar'an et al., 2001; Duncan et al., 2011). The disease is ranked as the fourth in Africa causing an estimated yield loss of 220,000 ton year⁻¹ (Wortmann et al., 1998). Of the reported losses, 66% occurs in East Africa and nearly 32% in Southern Africa (Wortmann et al., 1998). Among many diseases affecting common bean in Ethiopia, CBB is one of the most destructive and widespread (Fininsa, 2001; Tadesse et al., 2006) especially during the periods of warm and humid weather conditions. Different management options have been recommended to minimize losses due to CBB. However, most of the control strategies are unsustainable and difficult to implement especially under small-scale growers condition (Zaumeyer and Meiners, 1975; Gilbertson et al., 1992). Incorporating host plant resistance has been proposed as an effective

and economic strategy to mitigate yield loss caused by CBB (Rodrigues et al., 1999; Miklas et al., 2003; Shi et al., 2011a). Although there are limited sources of CBB resistance in common bean, interspecific hybridization has been utilized to transfer resistance genes from genetically related species (secondary and tertiary genepool) to common bean. Scarlet runner bean (*Phaseolus coccineus* L.) and tepary bean (*P. acutifolus*(A. Gray) Wootton & Standl.) are the major source of CBB resistance (Yu et al., 1998; Welsh and Grafton, 2001) but most of the sources of resistance are poorly adapted to the diverse agro-ecologies (Silva et al., 1989). Hence, developing cultivars with durable CBB resistance and yield stability across the target production environments is essential for the successful management of the CBB (Osdaghi et al., 2009).

Field evaluation in the hotspot areas and screening in the greenhouse with disease inoculation are the common screening methods to select genotypes with CBB resistance (Singh and Munoz, 1999). Based on field experiment using resistant and susceptible cultivars with and without inoculation variable CBB incidences were observed by many studies. For instance phenotypic and genotypic screening of some breeding lines developed from the donor VAX- and RMX lines using the CBB isolates *Xf260* and *Xf410* in a greenhouse condition revealed selection of resistant progenies within a population. However, the population mean had intermediate level of resistance indicating the quantitative nature of CBB inheritance to *Xap* (Kachulu et al., 2011). Evaluation of CBB resistance among inter-gene pool double cross populations under greenhouse and field conditions indicated that different resistant breeding lines were found through phenotypic and marker-assisted selection (MAS) (Duncan et al., 2012).

In plant breeding programs, screening of a large number of populations in greenhouse or field conditions requires the expertise, material and financial resources to select progenies with multiple traits including CBB resistance (Witcombe and Virk, 2001). Use of complementary molecular markers could significantly improve the efficiency of phenotypic screening in conventional breeding methods (Gupta et al., 2010). Different breeding methods such as recurrent selection and backcross breeding, are facilitated by MAS specifically at early generation selection for disease resistance breeding in the common bean (Miklas et al., 2003). In the common bean breeding, molecular markers have played unique role in genetic analysis, gene tagging and pyramiding of disease resistance genes including CBB resistance (Pedraza Garcia et al., 1997; Kelly and Miklas, 1999; Miklas et al., 2006a). Incorporation of farmers preferred traits and their needs identified

through participatory rural appraisal (PRA) studies is also among the important approaches to be adopted during breeding for disease resistance and enhanced yields.

1.2 Common bacterial blight

1.2.1 The pathogen and its occurrence

Common bacterial blight disease is caused by *Xanthomonas campestris* pv. *phaseoli* Smith (Dye) (synonym: *X. axonopodis* pv. *phaseoli* [Smith]) and *X. campestris* pv. *phaseoli* var. *fuscans* (Burkholder) Starr & Burkholder (synonym: *X. fuscans* subsp. *fuscans* sp. nov.). CBB is a major bacterial disease of common bean worldwide (Tar'an et al., 2001; Duncan et al., 2011). The pathogen has been reported in different states of the USA such as Michigan (Weller and Saettler, 1980), Nebraska and Colorado (CIAT, 1981), from countries of Latin America such as Colombia, Chile (Schuster and Coyne, 1975), Brazil and Mexico (Crispin and Campos, 1976). The disease was also confirmed to be present in Europe, Asia and Australia (Mengesha and Yetayew, 2018). In Africa, CBB has been reported as a major disease in Kenya (Njungunah et al., 1980), Malawi (Edje, 1981), Uganda, Kenya, Burundi (Opio, 1993) and Tanzania (Karel and Autrique, 1989). It has also been reported in Angola, Mauritius, Lesotho and Mozambique. In South Africa the disease has been reported in provinces of KwaZulu-Natal and Limpopo and other parts of the country (Melis, 1987). In Zimbabwe, the disease has been reported in the smallholder and large-scale commercial farming sectors (Giga, 1989). CBB had been reported in Ethiopia (Imru, 1985; Fininsa, 2001).

1.2.2 Epidemiology of CBB

The CBB is a warm temperature disease of common bean. It causes greater damage to the crop at temperatures between 28 and 32 °C and lowest temperature for occurrence of the CBB is 16 °C (Harveson and Schwartz, 2007; Viteri et al., 2014). High temperatures, rainfall, and humidity favor rapid disease progress and higher yield loss (Hailu et al., 2017). The pathogen is spread by rain, soil, plant debris, irrigation water, animals, insect pests such as the leaf-miners and whiteflies (Kaiser and Vakili, 1978). Debris from diseased plants has been considered as possible survival media to overwinter the CBB (Leben, 1981; Purseglove, 1988). It was reported that *Xap* can survive in dry leaves under laboratory conditions for more than six years (Gilbertson, 1988). Isolation of *Xap* have been successfully made from bean debris kept in the greenhouse for 12 months in Zimbabwe (Karavina et al., 2008), while pathogen survival was reported from more than

18 months old dried leaves kept in the laboratory in Sudan (Opio, 1993). The pathogen has also been reported to overwinter in weed debris under field conditions (Cafati and Saettler, 1980). Survival of *Xap* on or within infected bean seed is one of the most effective means of the bacteria's survival (Cafati and Saettler, 1980; Weller and Saettler, 1980; Leben, 1981). Contaminated seed is the primary source of inoculum (Gilbertson et al., 1990; Grum et al., 1998), and it is the most effective means for both local and widespread dissemination of the pathogen.

1.2.3 Infection, symptoms and host range of the CBB

Common bacterial blight causing pathogens enter host plant tissues through natural openings such as stomata and hydathodes or through wounds caused by damages (Beattie and Lindow, 1995). The resultant infection causes gradual disintegration of the middle lamella. The pathogens can also enter the stem through the stomata of the hypocotyls and epicotyls and invade vascular tissues. The bacterium then exits from stomata and then spreads to secondary sites of infection. The presence of sufficient bacterial colonies in the xylem tissue may cause wilting by obstructing the vessels of the cell walls (Yoshii, 1979). The CBB pathogens can also enter pod sutures from the vascular system of the pedicel and pass into the funiculus through the raphe, leading into the seed coat to infect the cotyledon when the seed germinates.

Leaf symptoms of CBB infection initially appear as water-soaked spots on the underside of leaves and leaflets. The spots then enlarge irregularly, and adjacent lesions frequently merge. As the lesions enlarge and coalesce, the plants appear to have been burnt. Lesions can be found at the margin and in intervention areas of the node. Infected regions appear flaccid, and are encircled by a narrow zone of lemon-yellow tissue which later turns brown and necrotic. Higher disease severity may cause defoliation or stem girdling. Dead leaves may remain attached to the plant up to maturity. Symptoms consist of lesions that are generally circular, slightly sunken and dark red-brown. Lesions on pods vary in shape and size depending on age of the pod. Under high humidity conditions, pod lesions are frequently covered with bacterial ooze (Melis, 1987). Symptoms on white or light-colored seeds are evident as butter-yellow or brown spots distributed throughout the seed coat or restricted to the hilum area (Mabagala and Saettler, 1992). If infection occurs during pod and seed development, seed may change size and color (rot, shrivel or wrinkled) and such seed shows poor germination and seedling development.

The common bean is the major host of the CBB causing *Xap* pathogen. Other hosts of *Xap* include scarlet runner bean (*P. coccineus*), tepary bean (*P. acutifolius*), soybean (*Glycine max* L.), lablab bean (*Dolichos lablab* (L.)), lupine (*Lupinus polyphyllus* Lindl.), Georgia velvet bean (*Mucuna pruriens* L. (syn. *Stizolobium deeringianum* Bort), fuzzy bean (*Strophostyles helvola* (L.) Elliott), moth bean (*V. aconitifolia* (Jacq.) Marechal), adzuki bean (*V. angularis* (Willd.) Ohwi & H. Ohashi), mung bean (*V. radiata* (L.) Wilczek), and cowpea (*V. unguiculata* (L.) Walp.) (Harveson and Schwartz, 2007).

1.2.4 Management of the CBB

Various disease management strategies are recommended to control CBB in common bean. Table 1.1 summarized the potential uses and limitations of different management options. These strategies include cultural practices such as use of pathogen-free seed, agronomic practices and application of crop protection chemicals such as Kocide-101 (77 % copper hydroxide w/w). However, some of the recommended options are mostly ineffective and the use of chemicals is limited due to increased cost of production, human safety and environmental impact (Zanatta et al., 2007). The use of genetic resistance is reported to be the most effective, economic and environmentally friendly approach (Rodrigues, 1999; Miklas et al., 2003; Shi et al., 2011b).

Table 1.1 Reported common bacterial blight (CBB) management options, potential benefits and limitations

Management options	Potential benefits	Perceived limitation	Reference
Antibiotic seed treatment	Reduces initial inoculum from seed surface	Potential buildup of antibiotic resistance to soil microbiology, high cost of production and only remove external seed infestation	McMullen and Lamey (2000); Ararsa et al. (2018)
Plant extracts	Reduces initial inoculum from seed surface	Limited access across all common bean growing regions and only reduce bacteria from seed surface	Ararsa et al. (2018)
Foliar spray of bactericides	Reduces initial inoculum and dispersal	High cost of production and mostly effective when only applied before infection	Schwartz and Galvez (1980); Ararsa et al. (2018)
Crop residue management	Reduces initial inoculum	Removal of organic minerals with residue	Saettler (1989); Fikre (2004)
Intercropping/mixed cropping	Reduces the epidemics of CBB	Competition among crops results in yield reduction	Fininsa (1996); Fikre (2004); Ararsa et al. (2018)
Crop rotation	Reduces the epidemics of CBB	Due to epiphytic nature of the pathogen, infection can occur in the field	Schwartz and Galvez (1980); Vandemark et al. (2008); Duncan et al. (2011)
Varietal mixture	Inhibits the dispersal of the bacteria due to differential reaction of the varieties	Mixture of different market classes drastically reduces market value	Fikre (2004)
Host resistance	Limits initial infection and reduce disease progression	Lack of high level of resistance in common bean, highly affected by environment and pathogenic variation and linkage drag	Rodrigues et al. (1999); Miklas et al. (2006c); Shi et al. (2011a); Duncan et al. (2012)
Integrated disease management (IDM)	Reduces initial inoculum and disease progress rate	High cost and lack of awareness of mode of application	Balachew et al. (2015); Ararsa et al. (2018)

1.3 Breeding for CBB resistance

1.3.1 Sources of CBB resistance

A summary of CBB resistant lines/cultivars thus far developed, sources of resistance, their gene pool and mode of inheritance are provided in Table 1.2. The highest level of CBB resistance has been reported in tepary bean (*P. acutifolius*) (Zapata et al., 1985; Arnaud-Santanal et al., 1993; Singh and Munoz, 1999), followed by scarlet runner (*P. coccineus*) (Singh and Munoz, 1999; Singh et al., 2001; Miklas et al., 2006a). The introgression of the resistance from the two species into *P. vulgaris* has been achieved through interspecific hybridization (Durham et al., 2013). But some introgression required additional techniques such as embryo rescue and congruent backcrosses to generate successful progenies (Singh and Munoz, 1999). A major achievement in breeding for CBB resistance was started by development of XAN-159, XAN-160 and XAN-161 lines with higher levels of resistance introgressed from the recurrent backcross populations (McElroy, 1985) followed by development of OAC 88-1 (Scott and Michaels, 1992). Common bean lines such as HR45 and HR67 that exhibited high levels of CBB resistance were also obtained from a cross between *P. vulgaris* × *P. acutifolius* (Park et al., 2007). The VAX-lines (VAX = *vulgaris acutifolius Xanthomonas*) (VAX 1 to VAX6) were developed through gene pyramiding from different source (Singh and Munoz, 1999; Singh et al., 2001).

Table 1.2 Common bacterial blight resistant lines/cultivars developed with sources of resistance, genepool and mode of inheritance

Lines/cultivars	Source	Gene-pool	Mode of inheritance	Reference
VAX1 and VAX2	<i>P. acutifolius</i> L.	Mesoamerica	Quantitative and dominant	Singh and Muñoz (1999); Singh et al. (2001)
OAC-88-1	<i>P. acutifolius</i> L.	Mesoamerica (small, white)	Quantitative with three linked dominant genes	Scott and Michaels (1992); Bai et al. (1997); Tar'an et al. (2001)
OAC-Rex	<i>P. acutifolius</i> L.	Mesoamerican	Quantitative	Michaels et al. (2006)
XAN 159	<i>P. acutifolius</i> L.	Andean (black-mottled)	Quantitative with additive and partial dominance	Vandemark et al. (2008); Viteri et al. (2014)
XAN-160 and XAN-161	<i>P. acutifolius</i> L.	Mesoamerican (medium, speckled)	Quantitative with additive effect (one major and a few minor genes)	McElroy (1985); Arnaud-Santana et al. (1994); Jung et al. (1997); Yu et al. (2004); Mutlu et al. (2005); Liu et al. (2008)
HR 67	<i>P. acutifolius</i> L.	Mesoamerican (navy)	Quantitative with two major genes	Miklas et al. (2006c)
Wilk 2 and Wilk 4	<i>P. acutifolius</i> L.	Mesoamerican	Quantitative with one dominant gene	Singh (1999)
G 17341 and NY79-3776-1	<i>P. acutifolius</i> L.	Mesoamerican	Quantitative with one dominant gene	Singh (1999)
XR235-1-1	<i>P. coccineus</i> L.	Mesoamerican	Two recessive genes	Yu et al. (1998); Freytag et al. (1982)
TARS VCI-4B	<i>P. coccineus</i> L.	Mesoamerican	One recessive gene	Miklas et al. (1994)
ICB-3	<i>P. coccineus</i> L.	Mesoamerican	Quantitative inheritance	Miklas et al. (1999)
ICB-6, ICB-8, and ICB-10	<i>P. coccineus</i> L.	Mesoamerican	Quantitative inheritance	Miklas et al. (1999)
C1,C2, C3 and C4	<i>P. coccineus</i> L.	Mesoamerican	Quantitative inheritance	Park and Dhanvantari (1987)
Colima 9	<i>P. vulgaris</i> L.	Mesoamerican	Quantitative with dominant gene	Duncan et al. (2011)
BAC 5, BAC 6, BAC 14, BAC 16 and BAC 31	<i>P. vulgaris</i> L.		Quantitative with additive effect (one major and a few minor genes)	Jung et al. (1996)
GN Nebraska #1 Sel 27 and GN Montana no. 5	<i>P. vulgaris</i> L.	Mesoamerican	Polygenic with at least one major-gene	Saettler (1989); Miklas et al. (2003)
BAT93	<i>P. vulgaris</i> L.	Mesoamerican	Quantitative with four QTL	Nodari et al. (1993)
Jules, Harris and Star	<i>P. vulgaris</i> L.	Andean	Polygenic	Coyne and Schuster (1974); Coyne et al. (1994)
Montcalm and Chase	<i>P. vulgaris</i> L.	Andean	Quantitative with one major-gene	Viteri et al. (2014)
USDK-CBB-15, USPT-CBB-5, USWK-CBB-17 and USCR-CBB-20	<i>P. vulgaris</i> L.	Andean (red kidney)	Quantitative with major and minor genes	Miklas et al. (2006b); Miklas et al. (2011); Miklas et al. (2006b); Miklas et al. (2006c)
Pyramided (multiple sources)	Pyramided (multiple sources)	Mesoamerican (navy)	Quantitative with partial dominance and additive (one major and a few minor genes)	Arnaud-Santana et al. (1994); Park and Dhanvantari (1994)
VAX3, VAX4, VAX5 and VAX6	Pyramided (multiple sources)	Mesoamerica	Quantitative with dominant mode of inheritance	Singh and Muñoz (1999); Singh et al. (2001)

1.3.2 Combining ability and heritability of CBB resistance

The inheritance of CBB resistance is reported to be qualitative or quantitative (Tar'an et al., 2001; Singh and Schwartz, 2010; Tryphone et al., 2013) is controlled by one to several genes contributing in the form of resistance to leaf and pod infections. Expression of resistance genes depends on genetic background of the resistance source, environmental condition, disease pressure, crop growth stage and plant part of the infected area (Kelly et al., 2003; Santos et al., 2003; Singh and Schwartz, 2010; Durham et al., 2013). In most genetic analyses, it has been established that inheritance to CBB resistance is quantitative and the mode of gene action is mainly additive, often with dominance and epistasis effects (Tar'an et al., 2001; Singh and Schwartz, 2010; Tryphone et al., 2013). For instance, up to five genes were found to be responsible in controlling CBB resistance in common bean (Fourie et al., 2011). In contrast, two major genes were also reported to control CBB in parental genotypes, used as sources of resistance in Malawi (Chataika et al., 2011). In addition to the major gene effects, CBB resistance has been associated with minor genes (Silva et al., 1989; Chataika et al., 2011). Inheritance of CBB resistance in the cultivar Montana No. 5 was polygenic with at least one major-gene involved (Miklas et al., 2003).

Different levels of heritability estimates have been reported among breeding lines. Arnaud-Santana et al. (1994) reported low heritability values that ranged between 0.08 and 0.15 for leaf and pod severity to CBB, while Tryphone et al. (2012) reported moderate heritability for foliar resistance (0.32). High heritability estimates (0.49-0.76) for leaf and pod reactions have also been reported (Ariyaratne et al., 1995). Heritability as high as 0.8 was reported in lines derived from the cross between HAB-52 and BAC-6A (Ferreira et al., 2004). There were varied reports on the significant effect of additive gene action for leaf resistance with heritability estimates varying from 0.18 to 0.87 (Silva et al., 1989), 0.30-0.60 (Ariyaratne et al., 1999), 0.52-0.60 (Arnaud-Santana et al., 1994) and from 0.09 to 0.93 (Singh et al., 1991). Breeding towards incorporating additive genes is useful for successful population improvement towards more resistant phenotypes (Tryphone et al., 2012).

Information on the combining ability effects of parents through their progeny tests for economic traits is essential in identifying productive and CBB resistant common bean cultivars (Parviz et al., 2016). The combining ability effects are quantified in terms of the estimates of general combining ability (GCA) and specific combining ability (SCA). The GCA is the average performance of a

line in hybrid combinations and is due to additive genes action. The SCA refers to crosses that do relatively better or worse than would be expected based on the average performance of the lines involved and is due to non-additive gene action. In CBB resistance breeding, negative GCA and SCA effects of lines are desirable (Bokmeyer et al., 2009; Mukankusi et al., 2011). There are contradictory reports regarding the preponderant gene action conditioning CBB resistance. For example, Rodrigues et al. (1999) reported that GCA effects were predominant over SCA for CBB resistance based on leaf severity assessment. Conversely, Trindade et al. (2015) reported that SCA effects were more important than the GCA in conditioning CBB resistance. Heritability for CBB resistance ranged between low (0-30) and medium (30-60) (Tar'an et al., 2001; Singh and Schwartz, 2010; Tryphone et al., 2013).

1.3.3 Mechanisms of CBB resistance in common bean

Host plant resistance to CBB refers to the ability of the plants to hinder the growth, development and spread of the pathogen due to the existence of several defense mechanisms. The defense mechanisms include the presence of physical barriers that inhibit the pathogen to penetrate the plant, such as thick cuticle layer, size and location of stomata (Agrios, 2005). Host plants can also use physiological mechanisms such as the release of chemical compounds (phenols, tannins and avenalin) into its environment that inhibits the pathogen (Agrios, 2005). Resistant genotypes of common bean use such physiological mechanisms to reduce or inhibit the movement of bacteria in plant tissues and thereby reduce the accumulation of bacteria attacking the plant or internal tissues in seeds (Aggour et al., 1989; Goodwin et al., 1995). Susceptible genotypes accumulate larger bacterial populations in the leaves that translocate rapidly through vascular tissues than resistant and partially resistant genotypes (Goodwin et al., 1995). Some of the resistant or partially resistant common bean breeding genotypes do not show internal seed infection (Aggour et al., 1989).

1.3.4 Phenotyping for CBB resistance

Reliable and accurate phenotyping techniques are an essential component of CBB resistance breeding. Table 1.3 presents the breeding methods and screening protocols used to develop bean genotypes with resistance to CBB. Genes conditioning monogenic inheritance and regulating the expression of CBB may be evaluated in the laboratory, while complex polygenic resistance must be examined under field conditions. Field phenotypic evaluation using different types of

inoculation methods, is one of the screening methods used to select high levels of CBB resistance (Singh and Munoz, 1999). Evaluation of CBB resistance of inter-gene pool double cross population under greenhouse and field conditions indicated that 12 resistant breeding lines were found through phenotypic selection and 6 lines were obtained through MAS (Duncan et al., 2012). A report from field experiment using two resistant and four susceptible cultivars following non-inoculated and leaf inoculated plants indicated that CBB incidence was very low in the non-inoculated than the inoculated experiment (Gillard et al., 2009).

Table 1.3 Common bacterial blight (CBB) resistant lines/cultivars developed with diverse screening protocols and breeding methods.

Lines/cultivars developed	Breeding method	Screening protocol	Reference
ICB-3, ICB-6, ICB-8 and ICB-10	Recurrent selection	Phenotypic (greenhouse and field) screening	Saettler (1989); Miklas et al. (1999)
HR67	Single cross	Phenotypic (greenhouse and field)	Park et al. (2007)
HR45	Backcrossing	Phenotypic (greenhouse and field)	Park and Dhanvantari (1994); OBoyle and Kelly (2004)
USDK-CBB-17	Marker assisted backcrossing	Phenotypic (greenhouse and field) and MAS	Miklas et al. (2006c)
USDK-CBB-15	Modified backcrossing	Phenotypic (greenhouse with leaf inoculation) and MAS	Pedraza Garcia et al. (1997); Miklas et al. (2000); Miklas et al. (2003)
USCR-CBB-20	Backcrossing	Phenotypic screening	Miklas et al. (2011)
XAN-159, XAN-160, XAN 161	Recurrent backcrossing	Phenotypic (greenhouse and field)	Vandemark et al. (2008)
VAX-1, VAX-2, VAX 3, VAX 4, VAX 5 VAX 6	Interspecific crosses followed by backcrossing	Phenotypic (greenhouse and field)	Schuster and Coyne (1971); Singh, (1999); Urrea et al. (1999)
12 resistant breeding lines obtained from Wilk-2 X DRK 2//DRK 1 X VAX-3	Double cross	Phenotypic screening and MAS	Duncan et al. (2012)
C1,C2, C3 and C4	Inter-specific crosses followed by backcrossing	Phenotypic screening (field)	Park and Dhanvantari (1987)
TARS VCI-4B	Interspecific cross followed by recurrent selection	Phenotypic screening (greenhouse)	Miklas et al. (1996)
Chase	Pedigree breeding	Phenotypic screening (field and greenhouse)	Miklas et al. (2003)

1.4 Marker-assisted selection (MAS) in CBB resistance breeding

Marker assisted selection is reportedly complements and improves the efficiency of conventional crop breeding programs. Different methods such as backcrossing breeding, are better facilitated by MAS specifically at early generation selection (Miklas et al., 2003). In plant breeding programs, screening of a large number of populations under greenhouse or field conditions is demanding (Witcombe and Virk, 2001). Hence, use of high throughput molecular markers could significantly complement and improve the efficiency of phenotypic screening in conventional breeding methods (Gupta et al., 2010). In common bean breeding, molecular markers have played great role in developing lines with high level of resistance through gene pyramiding including CBB resistance genes (Pedraza Garcia et al., 1997; Kelly and Miklas, 1999; Miklas et al., 2006a; see also Mondo et al 2019). These markers include the simple sequence repeat (SSR) or microsatellites (Tar'an et al., 2001), sequence-characterized amplified region (SCAR) and SNPs.

Common bacterial blight resistance genetic studies identified QTLs associated with different markers such as SCAR marker BC420 on linkage group (LG) B6 (Yu et al., 2000), SU91 on LG B6 and SAP6 on LG B10 (Pedraza et al., 1997), SSR marker PVctt001 on LG B5 (Miklas et al., 2000; Yu et al., 2000; Tar'lan et al., 2001) and single nucleotide polymorphism (SNP) markers (Galeano et al. 2009; Blair et al. 2013). Especially, the three markers, SAP6, SU91 and BC420 are highly recommended and utilized for genetic analysis and for CBB resistance breeding programs (Pedraza Garcia et al., 1997; Yu et al., 1998; Jung et al., 1999; Miklas et al., 2000; Yu et al., 2000). The SAP6 marker is associated with resistance gene originated from the common bean cultivar Montana No. 5 introgressed into other Middle American and Andean genotypes (Miklas et al., 2003). Although the level of SAP6-associated resistance is relatively low (Miklas et al., 2003), it is consistently found in common bean genotypes with improved levels of CBB resistance. SU91 has been the most useful marker, which is associated with CBB resistance from *P. acutifolius* L.(Pedraza Garcia et al., 1997). Resistance associated with SU91 and was introgressed into common bean through XAN-159 breeding line, and has been associated with high levels of resistance (McElroy, 1985).

The BC420 marker is also associated with resistance from *P. acutifolius*, found in XAN-159 (Linkage group 6) (Yu et al., 2000). The BC420 marker was mapped on linkage group 7 in a population derived from the cross of HR67 and OAC95-4 (Yu et al., 2004). A total of 22 QTLs

have been identified for CBB resistance useful in MAS for CBB resistance (Duncan et al., 2011). Markers with high level of reproducibility, low cost and high-throughput are useful to aid phenotypic selection programs (Gupta et al., 2010). Such markers include, the SNPs that have been widely exploited in genetic analysis of most crop species given that many markers can be used to genotype large set of segregating populations in short period of time with minimum cost (Varshney et al., 2006). Markers detected through marker-trait association studies using one single mapping population may not prove useful for all breeding programs designed to improve the trait of interest. This is attributed to the following reasons : parents of a proposed cross often have different genetic backgrounds, and they may not exhibit polymorphism for the selected marker (Miklas et al., 2006a). In CBB resistance breeding, markers associated with CBB resistance QTL was successfully validated especially in relation to the background of the parental lines (Yu et al., 2000).

1.5 Farmer needs and preferences in a common bean variety

Studies have shown that adoption of new varieties is limited mostly due to non-deliberate exclusion of specific needs and preferences of farmers by breeding programs (Keneni et al., 2004). More than 60 common bean varieties with different traits have been released and registered for production in Ethiopia (Amsalu et al., 2016). However, only a few varieties are being utilized by farmers in the country. Common bean breeders must therefore seek to satisfy farmers' trait preferences. Different studies have been conducted to collect information on the constraints of production and their impact involving bean growers (Katungi et al., 2010; Fininsa, 2001).

The participatory rural appraisal method has been used extensively and successfully in gaining preliminary information from farmers to guide variety development and to enhance variety adoption rate (Gebretsadik et al., 2014). The PRA enables researchers to gather primary information from participants and apply statistical and inference analyses to identify useful trends for decision making and to designing new varieties. The PRA was used to identify farmers preferred wheat cultivars with rust resistance (Hei et al., 2015) and sorghum varieties with striga resistance in Ethiopia (Gebretsadik et al., 2014). Therefore, PRA is an important multidisciplinary tool, which can be used to assess the production constraints and identify farmer-preferred attributes of new varieties, and to circumvent the production challenges while satisfying farmer and market preferences.

1.6 Conclusions and future prospects

Breeding for CBB resistance remains the most economic, effective and readily applicable option that can be deployed to resource poor farmers. But, most of the cultivars being grown by farmers have low resistance to CBB and incorporating resistance to these cultivars can improve the productivity of the crop in Ethiopia. New and novel sources of CBB resistance should be explored and used in new variety development and release. The new sources need to be evaluated in diverse environments in the targeted production agro-ecologies and the prevailing strains of the pathogen to identify CBB resistant and high yielding genotypes. The efficiency of CBB resistance breeding could be enhanced by combining phenotypic and marker-assisted breeding techniques. Incorporation of farmers' needs and preferences in a new variety development remains the main driver to release farmer-preferred varieties with CBB resistance and economic traits for sustainable common bean production and productivity in Ethiopia.

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CHAPTER TWO

FARMERS' PERCEPTIONS ON PRODUCTION, PRODUCTION CONSTRAINTS, TRAIT PREFERENCES AND DISEASE MANAGEMENT OPTIONS IN TWO MAJOR COMMON BEAN GROWING REGIONS OF ETHIOPIA: IMPLICATIONS FOR COMMON BACTERIAL BLIGHT DISEASE RESISTANCE BREEDING

Abstract

The common bean (*Phaseolus vulgaris* L.) is an important legume crop in Ethiopia. Common bacterial blight (CBB) disease is among the major constraints to common bean production in the country. The objectives of the study were to identify constraints affecting common bean production and productivity, and to identify farmers' perception on the constraints, and their trait preferences for inclusion in common bean breeding programs, specifically for disease resistance breeding. A participatory rural appraisal (PRA) study was conducted in Oromia and Southern Nations, Nationalities and Peoples' Regions (SNNPR) of Ethiopia. Data was collected using semi-structured questionnaires and focused group discussions from a total of 255 respondents during 2017. Inferences were made based on quantitative and qualitative statistics. The study identified drought stress (reported by 46.3% of the respondent farmers), diseases (24.4%), insect pests (12.6%) and lack of seeds of improved varieties (12.2%) as the most severe constraints to common bean production across the study zones. Among the biotic constraints of common bean, CBB was ranked as the most devastating disease by 63.5% (out of 40.0% of the total respondents) across study zones. Only 9.8% of the respondents mentioned as the existing varieties possessed disease resistance traits. A significant proportion (28.6%) of the farmers did not use any disease control methods and the yield loss due to the disease, as estimated by the respondents, can reach up to 70%. Improving resistance to CBB will reduce yield losses, improve productivity and enhance adoption of newly released varieties across the two major bean growing regions.

Key words: *Phaseolus vulgaris* (L.), participatory rural appraisal (PRA), farmers' preference

2.1 Introduction

The common bean (*Phaseolus vulgaris* L.) is globally valued as a cheap source of protein especially for low income households in developing countries (Broughton et al., 2003; Miklas et al., 2006a). In Ethiopia, the common bean is a source of food and sustains the livelihoods of millions of people as an income generating crop. In terms of common bean production, Ethiopia is ranked tenth in the world and fourth in Africa after Tanzania, Uganda and Kenya (FAOSTAT, 2017). In 2016, the common bean exports from Africa were about 496,600 tonnes valued at 283.4 million US Dollars. Ethiopia accounted for about 37% of these exports, by exporting 184,300 tonnes valued at 128.6 million USD (FAOSTAT, 2016). The cultivation of common bean extends over an area of 306,187 ha in Ethiopia with a net production of 520,979 tons. The national average yields of the white and red types of common bean are 1.7 ton ha⁻¹ (CSA, 2018). The total number of households producing the common beans in 2017/18 cropping season were 3.6 million, with 81.4 % of the total being from Ethiopia's two main bean growing areas of Oromia and Southern Nations, Nationalities and Peoples Region (SNNPR) (CSA, 2018).

The low average yields of common bean obtained in Ethiopia is attributed to different abiotic, biotic and socio-economic constraints. Among the major disease constraints is common bacterial blight, caused by *Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *fuscans*, which causes substantial yield losses in common bean (Fininsa, 2001). The disease is prevalent in many parts of Ethiopia, causing estimated yield losses of more than 22% especially in the central and eastern regions of the country (Fininsa, 2003). Consequently, inclusion of farmers' knowledge on common bean disease as production and mitigating strategies is important in designing breeding for disease resistance.

The participatory rural appraisal (PRA) method has been used extensively and successfully in gaining preliminary information from farmers that can be used in devising intervention strategies (Gebretsadik et al., 2016). The PRA enables researchers to gather primary information from participants and apply [inferential statistical analysis](#) to identify useful trends for decision making and designing intervention strategies. Girma, et al. (2018) identified that anthracnose resistance and tolerance to bird attack were the most preferred traits by sorghum producing farmers in western Ethiopia through PRA. Disease resistance was also identified as a preferred trait in wheat

and sorghum. [Hei et al. \(2015\)](#) used PRA and identified that farmers preferred wheat cultivars with rust resistance while [Gebretsadik et al. \(2016\)](#) found that Striga resistance formed part of the criterion used by farmers to select sorghum varieties for production in Ethiopia. Thus, PRA provides an important tool to assess production constraints and identify important traits to circumvent the production challenges while satisfying farmer and market preferences. The farmers have experience in cultivation and marketing of the crop and thus have practical knowledge of constraints affecting crop productivity and consumption. For breeders, information obtained from farmer participation can be used to formulate breeding objectives taking into cognizance most of the farmers' expectations. Inclusion of farmer preference in breeding objectives is likely to improve adoption of a newly developed cultivar at the primary production level and may also have substantial market penetration if it possesses consumer preferred qualities. Hence, to promote the uptake of improved common bean varieties, the production constraints faced by farmers and their varietal preferences must be documented for inclusion during varietal development. Therefore, the objectives of this study were (1) to identify constraints affecting common bean production and productivity among farmers in the major common bean producing regions, and (2) to identify farmers' perception, and trait preferences for inclusion in common bacterial blight resistance breeding program in Ethiopia.

2.2 Materials and Methods

2.2.1 Description of the study areas

A participatory rural appraisal (PRA) was conducted in two major common bean production regions of Ethiopia, i.e., Oromia and SNNPR. From each region, two administrative zones were further selected based on the number of households growing beans and total land area under bean production (CSA, 2018). [East Shoa is characterized by hot to warm semi-arid weather conditions and situated at an elevation of 1000-2000 meter above sea level \(masl\). The mean annual temperatures and mean annual rainfall vary from 16-28.5°C and 650-750 mm, respectively. West Arsi zone is situated in an elevation of 1500-2200 masl with mean annual temperatures varying from 16-27.5°C. The Wolaita zone is located in an altitude ranges of 1400-1700 masl with mean annual rainfall ranging from 400-1300 mm. The mean annual temperature of this zone varies from 21-27.5 °C. The Sidama zone is situated at 1100- 2000 masl and the mean annual temperature ranges between 18.5 - 22.5 °C. The mean annual rainfall of this zone varies from](#)

1000 - 1500 mm (USAID, 2010). The four zones are among common bean growing regions in the country. The total number of households, total production area and productivity of common bean in the selected regions are shown in Table 2.1.

Table 2.1 Number of households, cultivated area, total production and productivity of common bean in the major common bean production regions of Ethiopia (CSA, 2018)

Region	Type	No. of households	Area (ha)	Production (tonnes)	Productivity (tonnes ha ⁻¹)
Tigray	White	5,238.0	2,104.0	-	-
	Red	9,839.0	1,227.7	1,595.7	1.3
Amhara	White	265,497.0	38,040.9	60,884.8	1.6
	Red	294,458.0	29,608.6	52,091.1	1.8
Oromia	White	613,765.0	41,834.4	71,788.0	1.7
	Red	1,075,638.0	84,060.2	159,786.5	1.9
Benishagul	White	19,168.0	2,046.2	3,843.6	1.9
Gumz	Red	61,267.0	3,154.7	5,488.9	1.7
SNNPR	White	81,800.0	5,142.3	8,618.7	1.7
	Red	1,159,608.0	97,694.2	152,962.7	1.6
Harari	White	1,029.0	3.3	-	-
	Red	1,623.0	7.9	13.2	1.7
Dire Dawa	White	4,274.0	206.5	309.8	1.5
	Red	7,154.0	319.4	571.3	1.8
Sub total	White	990,771.0	89,377.4	145,444.9	
Sub total	Red	2,609,587.0	216,072.7	372,509.3	
Grand total		3,600,358.0	305,450.2	517,954.2	

Note: ha=hectares

2.2.2 Sampling techniques

A multi-stage cluster sampling method was used based on information on common bean production statistics at zone level provided by the Ministry of Agriculture of Ethiopia. Two representative districts from each zone were selected and from these districts, one to three peasant associations (PAs) were considered for the survey based on the number of households

participating in common bean production (Table 2.2). Based on the information of production obtained from zones, 20 to 43 farmers who cultivated common bean in 2015/16 cropping season were selected from each district. A total of 255 smallholder farmers participated in individual interviews and 112 key informants were included in focus group discussions across the four zones. The key informants included agricultural development agents, PA leaders and community elders. A zone was referred to as the larger unit of administration in the region, whereas PA was the smallest unit of administration in the rural community (Table 2.2). The research team comprised of multidisciplinary members, including a plant breeder, an agronomist, an extensionist and an agricultural economist from Melkassa Research Center (MARC). Agricultural officers from zonal and district level offices of the Ministry of agriculture and PA level agricultural development agents assisted with enumeration.

Table 2.2 Number of participant farmers in individual interview and focus group discussion in selected zones and districts

Region	Zones	Districts	No. of interviewees			No. of focus group discussants		
			Male	Female	Total	Male	Female	Total
Oromiya	East Shoa	Adami Tulu	30	2	32	35	4	39
		Jido						
		Kombolcha						
		Boset	37	6	43	22	1	23
SNNPR	Sidama	Dugda	17	3	20	10	1	11
		Hawasa	27	4	31	26	1	27
		Zurea						
Oromiya	West Arsi	Arsi Negele	25	6	31	28	4	32
		Shalla	37	2	39	28	3	33
SNNPR	Woliata	Boloso Sore	28	7	35	23	1	24
		Sodo Zuria	22	2	24	22	1	23
	Total		223	32	255	183	16	212

2.3.3 Data collection

Semi-structured questionnaire interviews, focus group discussions, transect walks and matrix ranking (Loader and Amartya, 1999) were used to gather the primary data. Semi-structured questionnaires were designed to gather information including production practices, constraints and their importance as perceived by the farmers, trait preferences, popular varieties grown, perceived losses due to common bacterial blight infection and indigenous methods to control common bacterial blight. Triangulation through the use of multiple sources was used to avoid bias and inaccurate information from respondents, and to ensure clarity of questions and cross verification of the responses. Transect walks were conducted in each farmer's field for qualitative observations of farmers' cropping systems, the extent of common bean blight prevalence and, control strategies. The farmers' group discussions (FGDs) involved situational analyses and preference ranking. The FGDs were categorised by demographic characteristics such as age, gender and role in the household and were conducted to gain insight into farmers' current circumstances, indigenous knowledge on pest management, trait preferences and opportunities for enhancing agricultural productivity. Secondary data were collected from zonal and district agricultural offices of the respective zones and districts surveyed in the study. Leaflets with photos of leaf samples showing common bacterial blight and other major diseases of common bean were used as guides during individual interviews and focus group discussions to assess farmers' ability to differentiate the diseases.

2.2.4 Data analysis

Descriptive statistics and frequencies were deduced and pair-wise comparisons among districts variables were achieved through cross-tabulation using the Statistical Package for Social Science (SPSS) version 24 (SPSS, 2017). During analysis, non-parametric and parametric tests were carried within and between zones to test significant differences and to draw comparisons. Statistical inferences were based on the [Pearson](#) Chi-square test statistic. Qualitative data from FGDs was used to expound some quantitative data obtained from the questionnaire interviews.

2.3 Results

2.3.1 Household and demographic characteristics

The majority of respondents in all the four zones were males, who constituted 87.5% compared to females (12.6%) (Table 2.3). About 7.7% of the interviewed farmers were less than 29 years old, whereas 70.1% were between 30 and 50 years old. The remaining 22.3% of the respondents

were more than 50 years old. Significant differences were observed among zones ($P < 0.05$) in family size (Table 2.3). A higher proportion (69.7%) of the respondents had family sizes of between 3 and 8 people per household, while 28.5% respondents had family sizes of more than 8 people per household. Only 1.8% of the interviewed farmers had families with less than 3 people per household. Significant differences ($P < 0.05$) were observed with respect to the level of education among farmers in the four zones (Table 2.3). The proportion of participant farmers that had attended peasant education was 26.6%, while 42.7% attended primary school. The highest proportion of respondents with primary school education was from East Shoa (55.8%) and this may be attributed to the high availability of primary schools in East Shoa. Only 5.3% of the farmers were illiterate, while about 2.0% attended college education.

Table 2.3 Proportion of respondents based on sex, family size, age group, level of education in the 4 study zones

Variables	Zones				Average
	East Shoa	Sidama	West Arsi	Wolaita	
Sex					
Male	88.4	87.1	88.6	84.7	87.2
Female	11.6	12.9	11.4	15.3	12.8
Chi-Square test	df = 3	$X^2 = 0.559$	P-value = 0.91		
Age group					
≤ 29	8.4	6.5	5.7	10.2	7.7
30 - 49	63.2	74.2	70.0	72.9	70.1
≥ 50	28.4	19.4	24.3	16.9	22.3
Chi-Square test	df = 6	$X^2 = 3.956$	P-value = 0.683		
Family size					
< 3	4.2	0.0	1.4	1.7	1.8
3 - 8	65.3	83.9	50.0	79.7	69.7
> 8	30.5	16.1	48.6	18.6	28.5
Chi-Square test	df = 6	$X^2 = 20.404$	P-value = 0.002		
Education					
Illiterate	7.4	3.2	5.7	5.1	5.3
Peasant education	29.5	22.6	25.7	28.8	26.6
Primary school	55.8	41.9	35.7	37.3	42.7
Secondary school	5.3	32.3	28.6	27.1	23.3
College	2.1	0.0	4.3	1.7	2.0
Chi-Square test	df = 12	$X^2 = 24.695$	P-value = 0.016		

Note: df = Degrees of freedom; X^2 = Chi-Square; P = Probability.

2.3.1 Sources of income for respondent farmers

A large proportion of the sampled farmers (62.0%) obtained their income from crop and livestock production (mixed agriculture), while only 10.6% depended on crop production only (Table 2.4). About 7.8% participated in small business, like owning small shops and selling of food and household items during market days in addition to crop production and livestock husbandry. In addition, 6.7% of the sampled households engaged in some jobs as hired labor to supplement their major sources of income.

Table 2.4 Proportion (%) of respondents who obtain incomes from different sources in 2016/17 cropping season

Source of income	Zone				Total
	East Shoa	Sidama	West Arsi	Wolaita	
Crop production solely	3.8	1.2	2.7	2.8	10.5
Livestock production solely	0	0	0.4	0	0.4
Crop and livestock production	23.1	8.5	18.7	11.4	61.7
Crop production and small business	2.8	0.4	0.4	2	5.6
Crop production and labor hiring	2	0	2.4	2	6.4
Livestock production and labor hire	0	0	0	0.4	0.4
Crop production, livestock production and	2	2	1.2	2.7	7.9
Crop production, livestock production and	3.1	0	1.6	2	6.7
Crop production, livestock production,	0.4	0	0	0	0.4
Total	37.2	12.2	27.5	23.1	100
Chi-Square test	df = 24		$X^2 = 26.11$	P-value = 0.348	

Note: df = Degrees of freedom; X^2 = Chi-Square; P = Probability

2.3.2 Sources of labor for farm operation

Family labor (labor household members) constituted the highest proportion (35.3%) of labor sources across the zones followed by a combination of family labor complemented by hired labor (23.5%) (Table 2.5). Neighbors and extended relatives also complemented family labor to variable extent across the different zones. In East Shoa, integration of labor from different sources (family based, relatives, neighbor and hired) accounted for a larger proportion of labor compared to other zones where family based labor was more dominant. Supporting family based labor by hiring additional labor during peak seasons was also common across all zones.

Table 2.5 Sources of labor (%) for farm operation in the study zones

Source of labor	Zones				
	East Shoa	Sidama	West Arsi	Wolaita	Total
Family based	9.0	5.9	10.6	9.8	35.3
Relatives	-	-	-	0.4	0.4
Neighbor	-	-	-	0.8	0.8
Hired	-	1.2	2.8	1.2	5.1
Family based and relatives	0.4	-	-	-	0.4
Family based and neighbor	7.8	1.2	2.8	5.9	17.7
Family based and hired	8.2	3.5	7.8	3.9	23.5
Relatives and hired	-	-	-	0.4	0.4
Family based, relatives and neighbor	0.8	-	-	-	0.8
Family based, relatives, neighbor	11.0	0.4	3.5	0.8	15.7
Total	37.3	12.2	27.5	23.1	100.0
Chi-Square test	df =27	X ² = 64.65		P-Value = 0.000	

Note: df = Degrees of freedom; X² = Chi-Square; P = Probability.

2.3.3 Land holdings and major crops grown for different purposes

The size of land holdings of the farmers who participated in the survey ranged from 0.08 ha to 10 ha across the four zones (Table 2.6). The largest average land size was recorded in East Shoa (mean = 2.6 ±1.5 ha) followed by West Arsi (mean = 2.2 ±1.6 ha), while smallest land-holdings were observed in Wolaita (mean = 0.9±07 ha).

Table 2.6 Mean, standard deviation and range of land sizes in hectares owned by the households in different zones

Statistics	Zones			
	East Shoa	Sidama	West Arsi	Wolaita
Mean	2.6	1.0	2.2	0.9
SD	1.5	0.5	1.6	0.7
Range	0.5 - 10.0	0.08 - 2.0	0.5 - 10.0	0.3 - 3.0

Note: SD = standard deviation

Maize was the major crop in terms of cultivation area across all zones followed by the common bean (Figure 2.1). The average plot size allocated for maize production was highest in East Shoa

(with an average of 1.09 ha coverage), followed by West Arsi (0.72 ha), while Wolaita recorded the least hectareage under maize production (0.17 ha). The average area allocated to common bean production ranged from 0.31 ha in Wolaita to 0.68 ha in West Arsi. Tef was also considered as a major crop in East Shoa and West Arsi with an allocated area of 0.37 and 0.24 ha, respectively. Wheat, barley, sorghum and millet were also among the food crops grown in the study areas, although these crops covered a relatively small proportion of the total arable land (less than 0.15ha each).

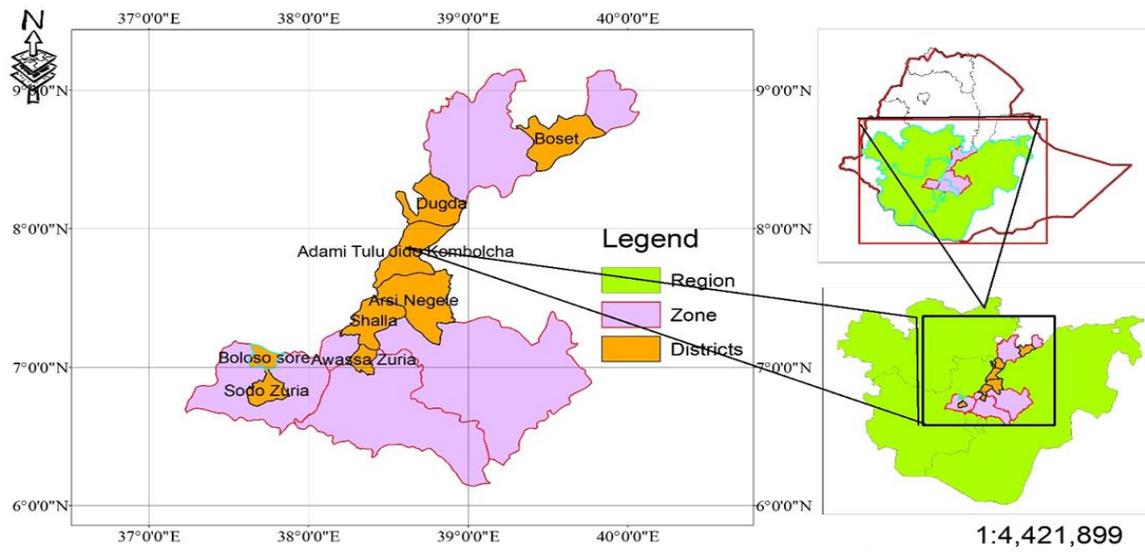


Figure 2.1. Map of Ethiopia showing the study zones

The major purposes of production were income generation (commercial), consumption, animal feed and a combination of these purposes (Table 2.7). In East Shoa, 17.3% of the households (produced maize for consumption and commercial purposes only, while 12.6% produced maize for cattle feed in addition to consumption and commercial purposes. Similarly, higher proportions of the households in the other zones produced maize for multiple purposes including the animal feed. A larger proportion (19.2%) of the respondents in East Shoa produced *tef* for commercial, food and feed purposes compared to other zones, where a high proportion of the participant farmers did not grow *tef* at all in the 2015/16 cropping season. Most households across zones produce common bean for consumption and commercial purposes. A relatively larger proportion of interviewed farmers in East Shoa (13%), and Wolaita (9.1%), produced common bean for

commercial and consumption purposes compared to Sidama (2%) and West Arsi (3.2%). In East Shoa, where small white pea beans for export dominated about 11.0% produced common bean for mainly for income generation. Across the zones, 10.6% in East Shoa and 9.4% in West Arsi produced wheat for both commercial and consumption purposes. In East Shoa, 5.1% of the farmers participated in sorghum production for different purposes. Millet was also produced for multiple purposes by 8.6% in West Arsi and 3.6% in Wolaita. However, the majority of the farmers did not produce some of the listed crops during the 2015/16 cropping season. [The respondent farmers did not produce some of the major crops during the study season due to crop growth season differences as well as choice in the crop type and rotation systems.](#)

Table 2.7 Frequency of household participating crop production for different purposes across zones in 2015/16 cropping season.

Crop	Zone	Production purposes (%)					
		Did not plant	Income	Food	Food and income	Income, food and feed	Total
Maize	East Shoa	1.6	0.4	5.5	17.3	12.6	37.3
	Sidama	0.4	-	2.8	4.7	4.3	12.2
	West Arsi	0.8	-	5.5	16.9	4.3	27.5
	Wolaita	4.3	0.4	7.5	6.7	4.3	23.1
Tef	East Shoa	13.3	-	1.2	3.9	19.2	37.3
	Sidama	11.4	-	-	0.4	0.4	12.2
	West Arsi	14.1	0.8	1.6	5.5	5.1	27.5
	Wolaita	17.7	-	-	2.4	3.1	23.1
Common bean	East Shoa	-	11.0	2.0	18.4	2.0	37.3
	Sidama	-	0.8	1.2	8.6	1.6	12.2
	West Arsi	-	1.6	1.6	22.8	1.6	27.5
	Wolaita	-	2.0	7.1	14.1	-	23.1
Wheat	East Shoa	24.3	0.8	1.6	10.6	-	37.3
	Sidama	12.2	-	-	-	-	12.2
	West Arsi	16.9	0.8	0.4	9.4	-	27.5
	Wolaita	22.4	-	0.4	0.4	-	23.1
Barley	East Shoa	35.3	-	0.4	1.6	-	37.3
	Sidama	12.2	-	-	-	-	12.2
	West Arsi	25.9	-	0.4	1.2	-	27.5
	Wolaita	22.8	-	0.4	-	-	23.1
Sorghum	East Shoa	25.5	-	5.1	5.1	1.6	37.3
	Sidama	12.2	-	-	-	-	12.2
	West Arsi	25.5	-	0.4	0.8	0.8	27.5
	Wolaita	23.1	-	-	-	-	23.1
Millet	East Shoa	36.5	-	0.8	-	-	37.3
	Sidama	11.8	-	-	0.4	-	12.2
	West Arsi	18.8	-	5.1	3.5	-	27.5
	Wolaita	23.1	-	-	-	-	23.1
Enset	East Shoa	37.3	-	-	-	-	37.3
	Sidama	7.5	-	2.0	2.8	-	12.2
	West Arsi	27.5	-	-	-	-	27.5
	Wolaita	19.6	-	2.0	1.6	-	23.1

2.3.5 Bean production area

Among the major crops, common bean ranked the first in Wolaita and second in the other zones in terms of area under production (Table 2.8). Different varieties were grown across the zones based on the agro ecological suitability. Among the study zones, only 0.4% of the respondents in

West Arsi, grew common bean on more than 8 ha and the remaining zones allocated between 0.1 and 4.5 ha. Higher percentages of farmers from Wolaita (18.8%) and East Shoa (18.4%) produced common bean on less than 0.5 ha. About 17.3% and 13.3% in East Shoa and West Arsi, respectively, produced common bean on plot sizes ranging between 0.5 and 2 ha.

Table 2.8 Area cropped with bean in four zones during the 2015/2016 main cropping season in Rift Valley and Southern regions of Ethiopia.

Area (ha)	Zone				Total
	East Shoa	Sidama	West Arsi	Wolaita	
< 0.5	18.4	9.4	12.9	18.8	59.6
0.5 to 2	17.3	2.8	13.3	4.3	37.7
2 to 3	0.8	-	0.8	-	1.6
3 to 4.5	0.8	-	-	-	0.8
> 8	-	-	0.4	-	0.4
Total	37.3	12.2	27.5	23.1	100.0

Chi-Square test df =12 $X^2 = 29.76$ P-Value = 0.003

Note: df = Degrees of freedom; X^2 = Chi-Square; P = Probability

2.3.6 Common bean varieties grown across zones

In East Shoa, a total of 58.2 ha of land was used for bean production. Farmers grew eight different known varieties (Table 2.9). Unknown or unidentified varieties were produced by 13.3% of respondents on an area of 19.44 ha. Generally, bean production in East Shoa was dominated by the small white common bean type mostly grown for export market. Awash 1, was the most common cultivar in East Shoa grown on an area of 16.13 ha, followed by Mexican 142 which covered 6.7 ha. Most of the unknown varieties were suspected to be a mixture of Awash 1 and Mexican 142. In East Shoa, local varieties (mostly small white) covered an area of 2.38 ha while the remaining 38.39 ha were covered by some other improved varieties of common bean.

In Sidama and Wolaita, the small red type of common bean was the most popular variety as well as in some districts of West Arsi zone. The improved variety, Hawasa Dume was dominant in Sidama, and grown by 5.9% of the total participants on 4.4 ha of land. About 78.6% of the participant farmers in West Arsi produced the widely adapted variety, Nasir on 35.8 ha of land. In the same zone, the variety Nasir and Dinknesh covered about 9.6 ha. In Wolaita zone, 12.2% of the total participant farmers produced Nasir on 8.3 ha of land, while Hawassa Dume was grown by 2.7 % of participant farmers on 2.76 ha of land. Red Wolaita was ranked third among the most common varieties grown in Wolaita.

Table 2.9 Proportion of farmers and area covered by different common bean varieties in 2015/2016 cropping season across surveyed zones

Zone	Variety	Frequency	Percent	Total area (ha)
East Shoa	Unknown	34	35.8	19.4
	Mexican 142	7	7.4	6.8
	Nasir	7	7.4	5.0
	Awash 1	25	26.3	16.1
	Local	7	7.4	2.4
	Deme	11	11.6	4.8
	Roba	1	1.1	0.3
	Nasir and Mexican 142	3	3.2	3.5
	Sub-total	95	100.0	58.2
Sidama	Unknown	7	22.6	0.1
	Mexican 142	1	3.2	1.0
	Hawasa Dume	15	48.4	4.4
	Awash 1	2	6.5	0.8
	Local	1	3.2	0.0
	Deme	4	12.9	1.5
	Hawasa Dume and Deme	1	3.2	0.5
	Sub total	31	100.0	8.3
West Arsi	Unknown	3	4.3	0.8
	Mexican 142	1	1.4	0.3
	Nasir	55	78.6	35.8
	Local	7	10.0	1.6
	Dinknesh and Nasir	3	4.3	9.6
	Nasir and Roba	1	1.4	0.3
	Sub total	70	100.0	48.3
Wolaita	Unknown	2	3.4	0.6
	Nasir	31	52.5	8.3
	Hawasa Dume	7	11.9	2.8
	Local	2	3.4	1.5
	Deme	1	1.7	0.1
	Nasir and Red Wolaita	4	6.8	1.3
	Dimtu	5	8.5	1.6
	Dinknesh	1	1.7	0.3
	SER 119	1	1.7	0.6
	Red Wolaita	4	6.8	1.0
	Nasir and Deme	1	1.7	0.3
Sub-total	59	100.0	18.4	
Total		255	100	133.2

2.3.7 Major seed sources for common bean varieties

In East Shoa, 41.2% of the respondent farmers obtained seed from the local grain market and 37.2% of them used farmers' saved seed (Table 2.10). About 12.7 and 7.4% of the respondent farmers in East Shoa obtained seed from agricultural extension and research centers, respectively. Respondent farmers who accessed seed from research centers were 1.7 and 7.1% in Wolita and West Arsi zones, respectively. Agricultural extension and local markets were the major seed sources for farmers in Sidama. As seed sources, agricultural extension, local market and farmers saved seed were used by 35.7, 30 and 27.1% of the farmers in West Arsi, respectively. A high proportion (64.5%) of respondent farmers planted seed obtained from agricultural extension in Wolaita, while 18.7 and 11.9% of the farmers used retained seed or seed sourced from local markets, respectively.

Table 2.10 Proportion of respondent farmers who received seed of different varieties in 2015/16 main production season

Zone	Variety	Farm saved seeds	NGO	Agricultural extension	Local market	Seed company	Research center	Total
East Shoa	Unknown	11.7	1.1	6.3	14.7	0	2.1	35.8
	Mexican 142	1.1	-	1.1	3.2	-	2.1	7.4
	Nasir	1.1	-	2.1	2.1	1.1	1.1	7.4
	Awash 1	14.7	-	3.2	8.4	-	-	26.3
	Local	4.3	-	-	3.2	-	-	7.4
	Deme	4.3	-	-	7.4	-	-	11.6
	Roba 1	-	-	-	1.1	-	-	1.1
	Nasir and Mexican	-	-	-	1.1	-	2.1	3.2
	Total	37.2	1.1	12.7	41.2	1.1	7.4	100
Sidama	Unknown	-	-	9.7	9.7	-	3.2	22.6
	Mexican 142	-	-	3.2	-	-	-	3.2
	Hawasa Dume	-	3.2	38.7	3.2	3.2	-	48.4
	Awash 1	-	-	-	6.5	-	-	6.5
	Local	3.2	-	-	0	-	-	3.2
	Deme	-	-	6.5	6.5	-	-	12.9
	Hawasa Dume and	-	3.2	-	-	-	-	3.2
	Total	3.2	6.4	58.1	25.9	3.2	3.2	100
West Arsi	Unknown	1.4	-	-	2.9	-	-	4.3
	Mexican 142	-	-	-	1.4	-	-	1.4
	Nasir	18.5	-	34.3	18.6	-	7.1	78.6
	Local	5.8	-	-	4.3	-	-	10
	Dinknesh and Nasir	1.4	-	1.4	1.4	-	-	4.3
	Nasir and Roba 1	-	-	-	1.4	-	-	1.4
	Total	27.1	-	35.7	30	-	7.1	100
Wolaita	Unknown	1.7	-	1.7	-	-	-	3.4
	Nasir	8.5	1.7	35.6	5.1	-	-	52.5
	Hawasa Dume	1.7	-	6.8	3.4	-	-	11.9
	Local	-	-	3.4	-	-	-	3.4
	Deme	-	-	1.7	-	-	-	1.7
	Nasir and Red	-	-	5.1	1.7	-	-	6.8
	Dimtu	5.1	-	1.7	1.7	-	-	8.5
	Dinknesh	-	-	1.7	-	-	-	1.7
	SER 119	-	-	1.7	-	-	-	1.7
	Red Wolaita	1.7	1.7	3.4	-	-	-	6.8
	Nasir and Deme	-	-	1.7	-	-	-	1.7
Total	18.7	3.4	64.5	11.9	-	1.7	100	

2.3.8 Traits of existing common bean varieties grown by the farmers

Based on the evaluation of existing varieties, most of the respondents stated grain yield and market preferences (seed shape and color) as the major merits that the varieties possessed (Table 2.11). Different varieties were grown in different study zones suggesting the dissimilarities of the preferences of the respondent farmers. For instance, about 22.1% of the interviewed farmers preferred Awash1 for its better grain yield in East Shoa, but in Sidama, Hawasa Dume was preferred by 45.2% of the participant farmers for the same traits. About 61.4 and 47.5% of the respondent farmers from West Arsi and Wolaita, respectively, preferred Nasir for its high grain yield. In East Shoa (9.6%), Sidama (9.7%), West Arsi (11.5%) and Wolaita (3.4%) grew the existing varieties for their market traits. Only a small proportion of the participant farmers (3.3% from East Shoa, 1.4% from West Arsi and 5.1% from Wolaita) mentioned as the existing varieties possessed disease resistance, otherwise most of them mentioned that the existing varieties lack resistance to major diseases including CBB. Other traits such as adaptability to their agro ecologies and early maturity were also mentioned by a few of the respondent farmers across the surveyed zones. [Previous breeding efforts of common bean were encouraging on CBB resistance breeding. However, market-led breeding approaches could provide major impetus for these efforts.](#)

Table 2.11 Common bean varieties grown across the study zones and ranking of their farmer-preferred attributes

Zone	Variety	Better grain	Disease tolerance	Market value	Adaptability	Good test	Early maturing	Total
East	Unknown	3.0	1.1	2.1	-	2.1	2.1	35.8
Shoa	Mexican142	8.5	1.1	1.1	-	-	-	10.7
	Nasir	6.3	-	1.1	-	-	-	7.4
	Awash1	22.1	-	2.1	2.1	-	-	26.3
	Local	5.3	-	-	-	1.1	1.1	7.4
	Deme	6.3	1.1	3.2	-	-	1.1	11.6
	Roba1	1.1	-	-	-	-	-	1.1
	Total	52.6	3.3	9.6	2.1	3.2	4.3	100
	Sidama	Unknown	16.1	-	6.5	-	-	-
Mexican142		3.2	-	-	-	-	-	3.2
Hawasa Dume		45.2	-	-	3.2	-	-	48.4
Awash1		6.5	-	-	-	-	-	6.5
Local		-	-	-	-	3.2	-	3.2
Deme		9.7	-	3.2	-	3.2	-	16.1
Total		80.7	-	9.7	3.2	6.5	-	100
West		Unknown	-	-	2.9	-	1.4	-
	Mexican142	1.4	-	-	-	-	-	1.4
Arsi	Nasir	61.4	1.4	5.7	8.6	1.4	-	78.6
	Local	4.3	-	2.9	1.4	1.4	-	10
	Dinknesh	-	-	-	2.9	1.4	-	4.3
	Roba1	1.4	-	-	-	-	-	1.4
	Total	68.5	1.4	11.5	12.9	5.6	-	100
	Wolaita	Unknown	3.4	-	-	-	-	-
Nasir		47.5	1.7	3.4	-	-	-	52.6
Hawassa Dume		8.5	-	-	-	1.7	1.7	11.9
Local		3.4	-	-	-	-	-	3.4
Deme		1.8	1.7	-	-	-	-	3.5
Red Wolaita		6.8	1.7	-	-	1.7	-	10.2
Dimtu		8.5	-	-	-	-	-	8.5
Dinknesh		3.2	-	-	-	-	-	3.2
SER119		1.7	-	-	-	1.7	-	3.4
Total		84.8	5.1	3.4	-	5.1	1.7	100

2.3.9 Abiotic, biotic and socio-economic constraints affecting common bean production

Drought stress was ranked, as the most severe constraint to common bean production, by 46.3% of the respondent farmers across study zones (Table 2.12). Common bean diseases were identified as the second most important constraints to common bean production by 24.4% of the respondents across all zones. Insect pests and lack of improved varieties were ranked third and fourth most severe constraints to common bean production by 12.6 and 12.2% of the respondents, respectively. In addition, lack of access to credit, inadequate market information, shortage of land and occasional flooding were also considered to have negative impact on common bean production.

Table 2.12 Abiotic and biotic stresses ranked as severe constraints by participant farmers across study zones (%)

Constraints	East Shoa	Sidama	West Arsi	Wolaita	Total	Rank
Drought	15.3	5.1	11	14.9	46.3	1
Diseases	6.3	5.1	7.1	5.9	24.4	2
Insect pests	4.7	1.2	5.5	1.2	12.6	3
Limited source of seed	8.6		2.4	1.2	12.2	4
Lack of market information	2		1.2		3.2	5
Shortage of land		0.4			0.4	7
Flood	0.4	0.4	0.4		1.2	6
Total	37.3	12.2	27.6	23.2	100	
X ²	47.577					
df	18					
P- value	0.000					

Note: df = Degrees of freedom; X² = Chi-Square; P = Probability.

2.3.10 Major diseases and insect pests of common bean

With respect to the biotic constraints of common bean, common bacterial blight (CBB) was identified as a major problem by about 63.5% of the respondents (out of 40.0% respondents who identified biotic stresses as constraints) across study zones (Table 2.13). Rust and anthracnose were mentioned by 8.6 and 7.5% as the second and third major diseases, respectively. Among the insect pests, leaf miners (by 2.0% of the respondents), aphids (by 2.8%) and bean stem maggot (by 5.5%) were considered as the constraints to common bean production across the study zones. The African bollworm was identified as a major pest only in East Shoa.

Table 2.13 Percentage of participants identifying disease and insect pests as major constraints of common bean production across study zones.

Constraints	Zones				Total
	East Shoa	Sidama	West Arsi	Wolaita	
No information	0.0	0.0	0.0	0.8	0.8
Common bacterial blight	31.0	5.9	17.7	9.0	63.5
Rust	0.0	2.0	3.9	2.8	8.6
Halo Blight	0.0	1.6	0.4	2.8	4.7
Angular leaf spot	0.4	0.4	0.4	0.8	2.0
Anthraco nose	1.6	1.2	0.0	4.7	7.5
Root rot	0.0	0.0	0.0	0.8	0.8
Bacterial brown spot	1.2	0.0	0.0	0.0	1.2
Leaf miner	1.6	0.0	0.4	0.0	2.0
Bean stem maggot	0.0	0.8	4.3	0.4	5.5
Aphid	0.8	0.4	0.4	1.2	2.8
African boll worm	0.8	0.0	0.0	0.0	0.8
Total	37.3	12.2	27.5	23.1	100.0
Chi-square test	df = 33	X ² = 112.173	P-value = 0.000		

Note: df = Degrees of freedom; X² = Chi-square; P = Probability

2.3.11 Estimation of yield loss in common bean due to common bacterial blight

According to the information from surveyed participants, common bean yields were severely compromised by CBB infections. However, the losses estimated by the respondents, varied significantly ($P < 0.05$) across the study zones (Table 2.14). About 42.8% of the respondents across the four zones estimated that yield losses due to CBB were between 31 and 50 %, while 33.3% of the farmers estimated the losses to range between 10 and 30%. Among the respondents, 16.1% estimated losses due to the common bacterial blight to range between 51 to 70% while 5.1% of the respondents estimated the losses to be less than 10%.

Table 2.14 Estimation of yield loss due to CBB by participant farmers across surveyed zones

Yield loss	Zone				Total
	East Shoa	Sidama	West Arsi	Wolaita	
less than 10 %	3.1	0.0	0.8	1.2	5.1
10 – 30 %	17.7	3.9	3.1	8.6	33.3
31 – 50 %	12.6	5.9	13.7	10.6	42.8
51 – 70 %	3.5	0.8	9.4	2.4	16.1
Above 70 %	0.4	1.6	0.4	0.4	2.8
Total	37.3	12.2	27.5	23.1	100.0
Chi-square test	df = 12	X ² = 56.750	P-value = 0.000		

Note: df = Degrees of freedom; X² = Chi-Square; P = Probability.

2.3.12 Control options used by the farmers

The results showed that farmers used a number of control strategies to mitigate losses against CBB across the study zones (Table 2.15). Generally, about 30.2% of the respondents used chemicals to control the disease, whereas 28.6% did not use any form of control options in the study zones. About 13.7% of the farmers who use chemicals as control options were from West Arsi followed by Wolaita (7.5%). Crop rotation was also one of the control options stated by 25.1% of the respondents out of which 11.8% were from East Shoa and 5.9% were from West Arsi. Other control options such as land preparation and timely weed control were also identified by 5.1 and 3.9% of the respondents across all study zones.

Table 2.15 Proportion of participant farmers (%) who used control options to reduce losses due to CBB across surveyed zones

Control options	Zone				Total
	East Shoa	Sidama	West Arsi	Wolaita	
No option used	15.3	2.8	1.6	9.0	28.6
Crop rotation	11.8	3.9	5.9	3.5	25.1
Application of chemicals	5.1	3.9	13.7	7.5	30.2
Good land preparation	0.4	0.8	3.9	0.0	5.1
Rouging diseased plants	1.2	0.4	0.8	0.8	3.1
Timely planting	0.0	0.4	0.0	0.0	0.4
Avoid movement in the field after rain	0.4	0.0	0.8	0.0	1.2
Weed control	2.4	0.0	0.4	1.2	3.9
Application of manure	0.0	0.0	0.0	0.4	0.4
Use of resistant varieties	0.8	0.0	0.0	0.4	1.2
Use traditional control method	0.0	0.0	0.4	0.4	0.8
Total	37.2	12.2	27.45	23.14	100.00
Chi-square test	df = 30	X ² = 82.244	P-value = 0.000		

Note: df = Degrees of freedom; X² = Chi-Square; P = Probability. CBB=common bean bacterial blight

2.4 Discussion

2.4.1 Implications of demographic characteristics on common bean production

The purpose of this study was to gain insights into the constraints affecting common bean production and identify farmer-preferred traits that could be included in common bean breeding programs in Ethiopia. The proportion of the male participants in the study were higher compared to that of females across study zones in agreement with the report by Katungi et al., (2010) that revealed that bean production and marketing in Ethiopia is dominated mostly by men and a few women who were household heads. The larger proportion (over 75 %) of the participant farmers were aged between 15 and 50 years and this is directly related with the active labor for production.

In this study, a large proportion (98%) of the respondents had family sizes of between 3 and 19 persons per household, which is also paramount for labour provision since family based labor accounted for about 35.3% of farm labour. This corroborates findings by Katungi et al., (2010) and Gebretsadik et al., (2014) who found that family size has a direct contribution to availability of labor for farm operations in Ethiopia. Over 75% of the respondents had low level of education or were illiterate, in concurrence with Katungi, et al., (2010), who also identified low level of education among constraints of common bean production in Ethiopia. Level of education influences farming systems and technology adoption, with higher level of education among farmers being positively correlated with better farming practices and adoption of improved technologies (Gebretsadik et al., 2014). The large proportion of farmers (61.7%) who depended on mixed farming may be attributed to the fact that Ethiopia has a large rural population which depends on primary agriculture for income. The study zones were previously reported under common bean production zones (USAID, 2010), which was confirmed by the majority of farmers who cultivated the crop across the zones.

2.4.2 Biotic and abiotic constraints reducing common bean production and productivity

Over 46% of the respondents identified drought as the most severe constraint to common bean across the four study zones of the two regions confirming previous reports that drought stress is the single most important constraints of common bean. Katungi et al., (2010) found out that drought was the most important constraint in common bean production, with a probability of occurrence estimated at 38% and causing yield losses of up to 22% in Ethiopia. Other authors also reported that drought or low moisture stress is among the major constraint limiting common bean production especially, in the southern region of Ethiopia (Asfaw et al., 2006; Yayis et al., 2015). The study also identified major common bean diseases to be among the major problems, followed by insect pests and limited access to improved varieties. This also agreed with the report by Katungi et al. (2010) that diseases, insect pests and poor access to new improved varieties are important impediments to common bean productivity in Ethiopia.

Common bacterial blight is one of the major diseases of common bean causing significant yield losses in the world (Saettler and Hall, 1991; Fourie and Herselman, 2002) and in Ethiopia (Fininsa, 2001; Abiy, 2006). According to this study, about 42.8% of the respondents across the four zones estimated the yield loss due to CBB to be between 31 and 50%, which agreed with

Opio (1996) and Wortmann (1998), who estimated that yield losses due to CBB were around 40%. In addition, 33.3% of the respondents estimated the losses to be between 10 and 30%, which was consistent with Fininsa (2001), who estimated yield losses of about 22% in Hararghe Highlands, Ethiopia. The ability of the farmers to identify that CBB could cause yield losses of this magnitude shows that they have practical experience that should be taken into cognizance when devising strategies for controlling the disease.

While the majority of the interviewed farmers acknowledged that the current varieties were high yielding and had a high market value, only a small proportion of the farmers (9.8%) mentioned disease resistance among the trait in the existing varieties. This indicates that disease resistance has been given less priority by common bean breeding programs in Ethiopia. Farmer's perception and understanding of crop disease might be different to that of trained people. This may reflect poor information dissemination by extension service providers or the lack of basic education of among farmers that was impeding their access to information. High yield and quality traits (seed color and shape) to meet market preferences have been the main objectives of the common bean variety improvement programs in Ethiopia. Consequently, most of the commonly cultivated varieties are highly susceptible to common bacterial blight.

2.4.3 Common bean blight control options available to farmers

Only 30.2% of the farmers applied chemicals while 25.1% used crop rotation as a strategy to control common bacterial blight, resulting in yield losses of up to 50%. While chemical control may be used as a component of an integrated approach to disease management in seed and commercial production, most small holder farmers cannot afford to procure the necessary pesticides (Dursun et al., 2002). Chemicals such as streptomycin, which effectively controlled most external bacteria, could reduce primary inoculum of common bacterial blight (McMullen and Lamey, 2000). Though, it was economical, copper-based bactericides could reduce the speed of spread of bacterial infections (Schwartz and Galvez, 1980; Gilbertson et al., 1992; Fininsa, 2003). Agronomic practices such as deep ploughing, which prevents the common bacterial blight from over-wintering within debris (Gilbertson et al., 1990) and crop rotation with non-host alternative crops (Schwartz and Galvez, 1980), could be used in the control of common bean blight. However, crop rotation as a control strategy is often not feasible because some of the

zones, such as Sidama and Wolaita, have land shortages. Hence, developing disease resistant varieties appears to be the most sustainable and viable strategy to control common bacterial blight in Ethiopia.

2.5 Conclusions

Drought stress, diseases, insect pests and limited access to improved varieties were identified by farmers as the most important constraints limiting common bean production. Among the major diseases of common bean, common bacterial blight was identified to be the most severe disease in the four zones studied. Most of the available varieties lacked resistance to common bacterial blight according to the farmers' observations. Common bacterial blight was estimated to cause up to 70% yield losses with substantial impact on food production and income generation based on the farmers observations. The farmers indicated that yield, market value and disease resistance were their preferred traits in improved bean varieties. Developing cultivars with common bacterial blight resistance, high grain yield potential and high market value was identified as the most viable option to improve common bean productivity in Ethiopia. The low level of education could hamper adoption of new technologies, such as improved seeds, although research and extension agencies will have to play a significant role in information and seed dissemination to enhance the adoption rates of new varieties.

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CHAPTER THREE

IDENTIFICATION OF SOURCES OF RESISTANCE TO COMMON BACTERIAL BLIGHT IN COMMON BEAN IN ETHIOPIA

Abstract

Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans*, is one of the major biotic constraints limiting common bean (*Phaseolus vulgaris* L.) production and productivity in Ethiopia. The objective of this study was to identify new sources of CBB resistance from a diverse panel of genotypes to develop CBB resistant common bean varieties. One hundred and ten diverse accessions were evaluated for CBB resistance at three hotspot sites (Melkassa, Arsi Negelle and Mieso) for two seasons (2017 and 2018) in Ethiopia. Data on mean disease severity on leaf (SL) and mean disease severity on pod (SP), the area under disease progress curve (AUDPC), number of pods per plant (PPP), number of seeds per pod (SPP) and grain yield (GY) were collected. Data were subjected to standard analysis of variance and principal component analysis. The genotype × site interaction (G × E) had significant ($P < 0.05$) effect on all assessed traits. This indicated the presence of marked variation among tested genotypes in CBB resistance across the testing sites. Genotypes including SEC21, SEC23, VAX6, SEC12, SMC22, VAX5, SEC20, SEC22, SEC24, SEC26, SMC16 SMC24 and SEC25, exhibited lower values for SL, SP and AUDPC and reasonable GY and its components, which are useful genetic resources for future CBB resistance breeding programs. Cultivar Nasir had a mean grain yield of 3.45 ton ha⁻¹ followed by VAX1 (2.86 ton ha⁻¹) and Hawassa Dume (2.83 ton ha⁻¹). Strong and negative correlation was found between AUDPC and GY ($r = -0.47$) at Mieso, and SL and GY ($r = -0.27$) at Melkassa revealing the effect of CBB. Hence, CBB resistant and high yielding genotypes making them ideal candidates for common bean breeding in Ethiopia or similar agro-ecologies emphasizing CBB resistance and enhanced agronomic performance

Keywords: Breeding for resistance, disease severity, grain yield, *Xanthomonas axonopodis* pv. *phaseoli*, *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans*

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3.1 Introduction

Common bean (*Phaseolus vulgaris* L., $2n=2x=22$) is a multi-purpose legume crop cultivated worldwide. It is an alternative and relatively cheap source of protein, complex carbohydrates, and other valuable micronutrients such as Ca, Cu, Fe, Mg, Mn and Zn, for more than 500 million people in the tropics (Broughton et al., 2003; Miklas et al., 2006a). In sub-Saharan Africa over 200 million people depend on common bean as a primary staple food (Broughton et al., 2003; FAO, 2017). In 2016, global production of common bean was 26.8 million tonnes (FAOSTAT, 2016) and over 80% of the global bean production is contributed by tropical countries. With a total annual production of 520,979 tonnes, Ethiopia ranks 10th in the world and fourth in Africa (FAOSTAT, 2017). Approximately 3.4 million smallholder farmers produce common bean in Ethiopia for household consumption and for export market. However, the recurrence of common bacterial blight has become a major yield and quality limiting factor of common bean production in Ethiopia (CSA, 2018).

Common bacterial blight (CBB) is one of the major diseases of common bean limiting production and productivity globally (Tar'lan et al., 2001; Miklas et al., 2003). The disease is caused by the bacterial pathogen *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. axonopodis* pv. *phaseoli* var. *fuscans* (*Xaf*) which are also widely found in Ethiopia (Fininsa, 2003). CBB is the most destructive and prevalent under the low to mid altitude with warm and moist environmental conditions. In these agro-ecologies the disease causes yield losses varying from 66 to 75% (Wortmann et al., 1998) depending on the growing season, cultivar susceptibility and crop stage. A yield loss of 22 to 40 % was recorded in Ethiopia (Fininsa, 2003; Fininsa and Tefera, 2011). CBB is seed-borne and the causative pathogen survives on or within infected seeds making its control difficult (Weller and Saettler, 1980).

Various methods are recommended to control CBB. These include cultural practices such as use of pathogen-free seed, good agronomic practices, and application of crop protection chemicals such as Kocide-101 (77 % copper hydroxide w/w). However, agronomic practices are relatively ineffective and the use of crop protection chemicals is limited due to increased cost of production, human safety and environmental concerns (Zanatta et al., 2007). An integrated disease management approach including cultural practices, use of crop protection chemicals and host resistance is key for effective control of CBB. The use of novel common bean varieties with durable resistance is believed to be the most effective, economic and environmentally friendly

approach to control CBB (Rodrigues, 1999; Miklaset al., 2003; Shi et al., 2011). Breeding for host resistance requires novel sources of resistance with farmer- and market-preferred traits. However, most CBB resistant sources have limited market-preferred traits and have specific agro-ecological adaptation (Tar'lan et al., 2001; Zanatta et al., 2007; Osdaghi et al., 2009). Thus, there is a need to screen diverse and new set of common bean genotypes under the target production environment to identify novel sources of CBB resistance for resistance breeding.

Previous studies identified CBB resistant common bean breeding lines for different market classes (Singh, 1999; Singh et al., 2001; Duncan et al., 2012). Notable sources of CBB resistance were reported in related species of common bean such as the scarlet runner bean (*P. coccineus*) (Welsh and Grafton, 2001; Yu et al., 1998) and tepary bean (*P. acutifolius*) (Marquez et al., 2007; Singh and Munoz, 1999) and interspecific crosses between *P. vulgaris* with *P. acutifolius* or *P. coccineus* (Tar'lan et al., 2001). However, the candidate lines that were bred as sources of resistance were poor performers under the diverse agro-ecologies in the tropics limiting their direct production (Silva et al., 1989). Nonetheless, these lines can be used as donor parents for gene introgression in tropical adapted lines for breeding population development and ideotype selection.

The International Center for Tropical Agriculture (CIAT) and various national breeding programs are developing promising CBB resistant common bean lines. These lines have been deployed in different countries in sub-Saharan Africa including Ethiopia for their CBB resistance and desirable agronomic traits. The introduced lines have to be rigorously evaluated under the prevailing environment and disease conditions to identify most adapted, best performing and CBB resistant lines for breeding or direct production. The presence of pathogenic variation in CBB causing pathogens were previously reported signaling variable disease response of candidate genotypes needing systematic selection and breeding (Mutlu et al., 2008; López et al., 2006; Mkandawire et al., 2004; Aggour et al., 1989).

Multi-environment evaluations of candidate common bean genotypes will allow selection of parents with CBB resistance and complementary agronomic traits. This will enable breeding population development for successful CBB resistance breeding and deployment of best adapted lines under variable disease pressure and pathotypes in the hotspot and target production areas (Osdaghi et al., 2009). Therefore, the objective of this study was to identify new sources of CBB

resistance from a diverse panel of genotypes from different sources for effective breeding. The selected lines will be used for developing CBB resistant varieties with desirable agronomic traits in line with farmers and market preferences.

3.2 Material and Methods

3.2.1 Description of the experimental sites

Three testing sites (Melkassa, Arsi Negelle and Mieso), representing major common bean growing regions, were selected for the study. The Melkassa site is situated at latitude of 8° 24' N and longitude of 39° 12' E with elevation of 1550 meters above sea level (masl). Whereas the Arsi Negelle site is found at latitude of 7° 25' N and longitude of 38° 31' E elevated at 1900 masl. The Mieso site is located at latitude of 9° 14' N and longitude of 40° 45' E with altitude of 1470 masl. The soil type of the Melkassa, Arsi Negelle and Mieso sites are sandy-loam, loam and clay, respectively. Arsi Negelle and Mieso are the testing sites of the Melkassa Agricultural Research Center of the Ethiopian Agricultural Research Institute (EIAR).

Figure 3.1A, 3.1B and 3.1C summarizes the mean weather data of the three sites during the two testing seasons (2017 and 2018). The Melkassa, Arsi Negelle and Mieso are known hotspots for CBB and representative of common bean production areas suitable for commercial bean production (Tumsa, 2007). Daily weather data were collected from the meteorological stations at each site and the mean values for each month in the growing season were calculated. The weather data indicated that the sites possessed suitable environmental conditions for CBB infection and disease development. High temperatures and relative humidity are key factors affecting CBB infection and disease development (Saettler, 1989; Harveson and Schwartz, 2007; Viteriet al., 2014). For instance, the Melkassa site had a mean relative humidity varying from 68.08 to 70.69 % and mean temperatures of 15.15 to 16.29 and 26.46 to 28.22 °C, respectively during the two testing seasons. Whereas, the mean relative humidity and the mean minimum and maximum temperatures were 70.28 to 72.16 % and 13.80 and 23.23 to 25.19 °C (Arsi Negelle site), and 62.65 to 65.78 % and 16.89 to 17.88 and 29.40 to 31.98 °C (Mieso site), in that order.

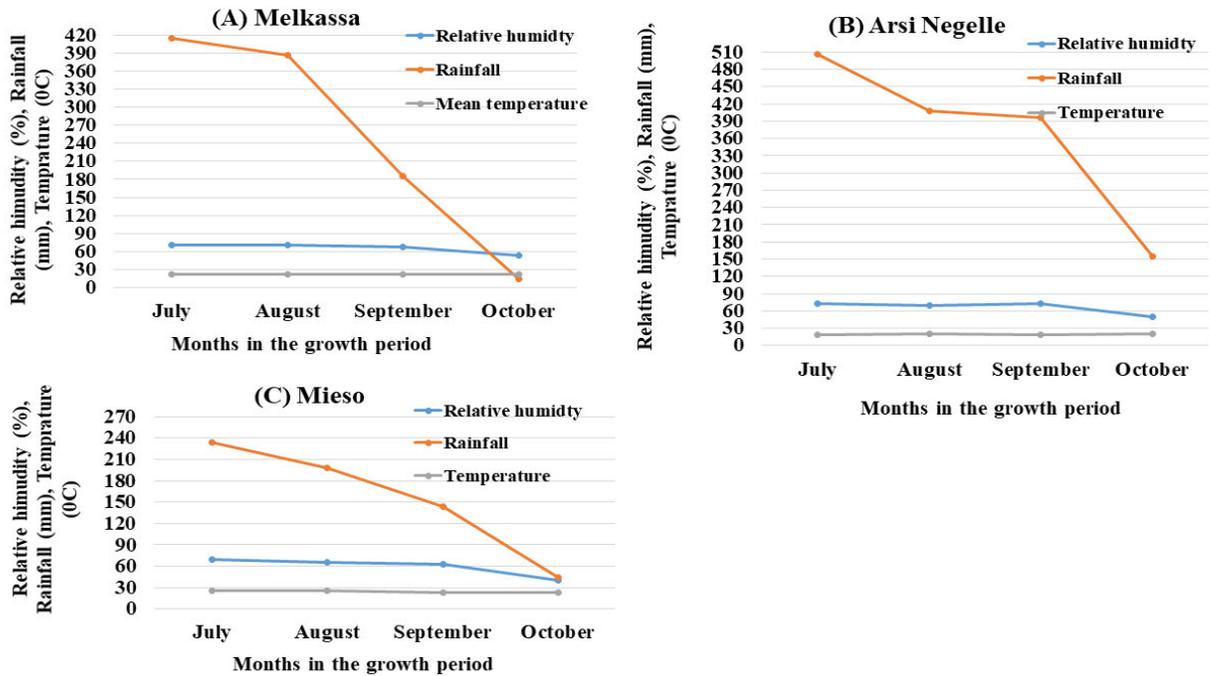


Figure 3.1 Mean monthly rainfall (mm), relative humidity (%), and mean temperatures (°C) of Melkassa (A), Arsi Negelle (B) and Mieso (C) sites during 2017 and 2018 in Ethiopia.

3.2.2 Genetic materials

One hundred and ten genotypes of common bean acquired from different sources were evaluated for CBB resistance and key agronomic traits. The list of genotypes, source and pedigree are provided in Table 3.1. The genotypes comprised of 36 accessions (ACC-lines) that obtained from the International Center for Tropical Agriculture (CIAT)/Uganda. ACC lines were tested for **CBB resistance** before they introduced to Ethiopia and found to be resistant (Divage, 2015). Further, genotypes with drought tolerance (coded as SEC-lines) and high zinc and iron content (coded as SMC lines) were sourced from CIAT/Colombia. Additionally, *Vulgaris acutifolius Xanthomonas* derived lines (coded as VAX-lines) acquired from CIAT/Uganda were included. The VAX-lines were bred for CBB resistance through interspecific crosses between *P. vulgaris* and *P. acutifolius* (Mejia-Jimenez et al., 1994; Singh and Munoz, 1999). Two genotypes: XAN159 and Teebus served as resistant and susceptible checks, respectively (Fourie, 2011). The two lines were obtained from the Agricultural Research Council (ARC) of South Africa and used as comparative controls. Also, 17 common bean landraces collected from Ethiopia and 32 varieties released by the EIAR were included.

Table 3.1 List, pedigree and source of common bean genotypes used in the study

Name/designation of genotypes	Number	Pedigree	Sources
ACC3	1	AFR298/(RMX2/BRB266)	CIAT-Uganda
ACC4	1	((RMA71/RMC65)/(RMX 2/BRB266)) /AFR298	CIAT-Uganda
ACC5	1	SAB659/(VAX6 x BRB264)	CIAT-Uganda
ACC6	1	((RMA71/RMC65)/(RMX 2/BRB266))/AFR298	CIAT-Uganda
ACC7	1	((RMA71/RMC65)/(RMX 2 /BRB266))/KATB1	CIAT-Uganda
ACC8	1	RMA52/SAB516	CIAT-Uganda
ACC9	1	RAA34/RMA60	CIAT-Uganda
ACC10	1	((RMC58/BRB263)/(RMC65/BRB265))/AND620	CIAT-Uganda
ACC11	1	AFR298/(RMA68/RMX19)	CIAT-Uganda
ACC12	1	((RMA71/RMC65)/(RMX 2/BRB266)) /AFR298	CIAT-Uganda
ACC13	1	((RMA71/RMC65)/(RMX 2/BRB266))/AFR298	CIAT-Uganda
ACC14	1	((RMA71/RMC65)/(RMX 2/BRB266))/AND620	CIAT-Uganda
ACC15	1	((RMA68/RMX 8)/(VAX6/CMB106))/LYAMUNGO85	CIAT-Uganda
ACC16	1	((RMA71/RMC65)/(RMX 2/BRB266))/AFR298	CIAT-Uganda
ACC17	1	RMA52 x SAB516	CIAT-Uganda
ACC18	1	LYAMUNGO85/RMA52/SAB516)	CIAT-Uganda
ACC19	1	RMA44/SAB514	CIAT-Uganda
ACC20	1	((RMA46/SAB514)/(RMC57/BRB263))/AFR298	CIAT-Uganda
ACC21	1	((RMA46/SAB514)/(RMC57/BRB263))/AFR298	CIAT-Uganda
ACC22	1	(BRB268 /RMC57)/SAB568	CIAT-Uganda
ACC23	1	AFR298/(RMA68/RMX19)	CIAT-Uganda
ACC24	1	((RMA46/SAB514)/(RMC57/BRB263))/AFR298	CIAT-Uganda
ACC25	1	AFR298/(RMX2/BRB266)	CIAT-Uganda
ACC26	1	((RMA71/RMC65)/(RMX 2/BRB266))/AFR298	CIAT-Uganda
ACC27	1	((RMA71/RMC65)/(RMX 2/BRB266))/AFR298	CIAT-Uganda
ACC28	1	LYAMUNGO85/(RMA52/SAB516)	CIAT-Uganda
ACC29	1	AFR298/(RMX2/BRB266)	CIAT-Uganda
ACC30	1	AFR298/(RMA68/RMX19)	CIAT-Uganda
ACC31	1	AFR298/(RMA68/RMX19)	CIAT-Uganda
ACC:109129/ID:PRO O 443_151, ACC:B11 625, ACC:182054/ID:PU EBLA 152, ACC:804554/ID:ZO RRO, ACC:110149/ID:VE RANO, ACC:108958/ID:ME DILIST and ACC:107212/ID:R 99	1	ACC:109129/ID:PRO 443_151, ACC:B11 625, ACC:182054/ID:PU EBLA 152, ACC:804554/ID:ZORRO, ACC:110149/ID:VERANO, ACC:108958/ID:MEDILIST and ACC:107212/ID:R 99	CIAT-Uganda
SEC12	1	MR 14215-6/MC-2P-MQ-MC	CIAT-Colombia
SEC20, SEC21, SEC22, SEC23, SEC24, SEC25 and SEC26	7	SX 15228-32/MC-17C-MQ-MC	CIAT-Colombia
SMC16, SMC21, SMC22, SMC23, SMC24 and SMC25	7	SDF216526-003/MC-4P-MQ-MC	CIAT-Colombia

Table 3.1 Continued

Name/designation of genotypes	Number	Pedigree	Sources
Acc No.2, Acc No.5, Acc No.7, Acc No.8, Acc No.10, Acc No.20, Acc No.37, Acc No.110, Acc No.130, Acc No.189, Acc No.191, Acc No.193, Acc No.211, Acc No.224, Acc No.305, Acc No.312, Acc No.321 and Acc No.325	17	na	Landraces from Ethiopia
SER125	1	SER125	EIAR
SER119	1	SER119	EIAR
Nasir	1	Dicta105	EIAR
Dimtu	1	DOR554	EIAR
Argene	1	AR-04-GY	EIAR
Nazareth-2	1	TA04 JI	EIAR
Melka Dima	1	XAN310	EIAR
Chore	1	STTT-165-92	EIAR
Awash-2	1	ICA Bunsu/SXB405	EIAR
Dinknesh	1	RAB 484	EIAR
KAT B1	1	KAT B1	EIAR
KAT B9	1	KAT B9	EIAR
SAB 632	1	SAB632	EIAR
SAB736	1	SAB736	EIAR
Biofort small seeded -15	1	Biofort small seeded -15	EIAR
Batu	1	A197/OM	EIAR
Biofort large – 50	1	Biofort large - 50	EIAR
Haramaya	1	G-843	HU
Chercher	1	STTT-165-96	HU
Dursitu	1	DOR-811	HU
IBADO	1	AFR 722	SARI
Gofta	1	G2816	EIAR
Ayeneu	1	GLP X92	EIAR
Hawassa Dume	1	SNNPR-120	SARI
Beshbesh	1	XAN76/BAT85	EIAR
Melke	1	CAL113/AND829	EIAR
Awash-1	1	ICA line Ex-Rico 23	EIAR
Awash Melka	1	PAN 182	EIAR
Roba-1	1	A176	EIAR
Tabor	1	A 788	EIAR
Red Wolaita	1	Local collection	EIAR
Mexican-142	1	G11239	EIAR
VAX1 and VAX2	2	A 769///A 775//ICA Pijao/G 40001	CIAT-Uganda
VAX3 and VAX6	1	A769///A 775//ICA Pijao/G 40001/XAN 309	CIAT-Uganda
VAX4 and VAX5	2	A769///A 775//ICA Pijao/G 40001/ XAN 263	CIAT-Uganda
USDK-CBB-15	1	K97305/3/SVM-2242//I9566–21–4-2/‘Montcalm’	CIAT-Uganda
G19833	1	Global collection	CIAT-Colombia
XAN159	1	UI-114/PI319441//PI319443/‘Masterpiece’	ARC-RSA
Teebus	1	na	ARC-RSA
Total	110		

na=not available; HU=Haramaya University; SARI=Southern Agricultural Research Institute/Ethiopia, EIAR=Ethiopian Agricultural Research Institute; CIAT= International Center for Tropical Agriculture; ARC=Agricultural Research Council/Republic of South Africa

3.2.3 Experimental design

Each trial was set up using an 11×10 alpha lattice design with two replications at each testing site. Each genotype was planted when the main season rain started (early July) on two 4 m rows which were 40 cm apart. The inter-row spacing between any two genotypes were 80 cm to minimize inter-plot interference. The net plot size for each genotype in each replication was 3.2 m². Two seeds were planted per hill separated by 10 cm and thinned to one plant per hill at 15 days after planting (DAP) to maintain a density of 40 plants per row. The experiments were conducted under rain-fed conditions and natural CBB infection and disease development. Di-ammonium phosphate (DAP) (46% P₂O₅) at 100 kg ha⁻¹, and urea (46% N) at 50 kg ha⁻¹ fertilizers were applied during plating. Weeds were manually removed.

3.2.4 Data collection

CBB severity was recorded four times at seven-day interval starting at the sixth reproductive stage (R6) when visible symptoms of CBB appeared on the leaves and pods. In each season, 30 plants per genotype were evaluated for SL and SP and the mean values of all assessed plants were calculated at each assessment event. CBB severity assessment was scored based on a scale of 1 to 9, where 1 = 0% of the leaves or pods show infection symptoms, 2 = 2%, 3 = 5%, 4 = 10%, 5 = 20%, 6 = 25%, 7 = 50%, 8 = 75% and 9 > 85% (CIAT, 1987; Opio, 1996). The severity scores were used to group the tested genotypes into three categories: resistant (for genotypes with scores of 1 to 3), moderately resistant (4 to 6) and susceptible (7 to 9). Disease expression over time was estimated by the area under disease progress curve (AUDPC) from the SL following the model presented by Vanderplank (1963).

$$\text{AUDPC} = \sum_{i=1}^{n-1} [0.5(x_i + x_{i+1}) (t_{i+1} - t_i)]$$

Where n is total number of CBB severity assessment events, t_i is the time of the ith assessment in days from the first assessment date, x_i is percentage of disease severity at ith assessment.

The following key agronomic traits were assessed: the numbers of pods per plant (PPP), the number of seeds per pod (SPP) and grain yield. The PPP was recorded as the average number of pods from 10 randomly sampled and tagged plants in a row. The SPP was the proportion of the total number of seeds to the number of pods from the 10 randomly selected plants per row. Grain yield (expressed in tonnes ha⁻¹) was estimated as the weight of seed from 30 randomly selected

and tagged plants and converted to tonnes ha⁻¹ for the plot size after adjusting at 12% moisture content using handheld moisture meter calibrated for common bean (Moisture meter G610i, Gehaka, Sao Paulo).

3.2.5 Data analysis

The mean values of epidemiological parameters such as SL, SP and AUDPC and agronomic traits (GY, PPP and SPP) were subjected to analysis of variance (ANOVA). A factorial ANOVA with the general linear model (GLM, PROC GLM) was used to compute a combined analyses across genotypes, seasons and sites using the following model:

$$Y_{ijk} = \mu + G_i + Y_j + S_k + (GY)_{ij} + (GS)_{ik} + (SY)_{jk} + (GYS)_{ijk} + e_{ijk}$$

Where Y_{ijk} is the overall genotype performance, μ is the overall mean, G_i is the effect of i^{th} genotype, Y_j is the effect of j^{th} season, S_k is the effect of k^{th} site, $(GYS)_{ijk}$ is the interaction effect and e_{ijk} is the error

When genotypes showed significant difference mean values were separated using Fisher's Unprotected Least Significant Difference test procedure ($P \leq 0.05$). Pearson correlation analysis was conducted to determine the degree of relationships among disease parameters and agronomic traits. Bi-plots and principal component analysis (PCA) were conducted to identify the genotypes with multiple desirable traits and to explore the extent of interrelationships among traits. All the analyses were conducted using SAS software (SAS, 2014).

3.3 Results

The seasonal weather conditions during the growing season had a fairly well rainfall distribution. These conditions facilitated disease infection and developments on different genotypes tested across the testing sites. Hence genotypes performances were measured appropriately to identify the resistant ones.

3.3.1 Analysis of variance for epidemiological and agronomic parameters

The seasonal effect was also significantly different for SP, AUDPC, PPP, SPP ($P \leq 0.01$) and GY ($P \leq 0.05$). The variable effect of season on SP and AUDPC indicated marked differences in climatic factors affecting CBB infection and disease development. Combined analysis of variance

across the test conditions/environments revealed a presence of three-way interaction of genotype x site x season. The genotype x site interaction had significant effect on all assessed traits, while the genotype x season interaction effect was significant for SL, SP and AUDPC. SL did not exhibit significant difference across seasons. The main effect of site and genotype were found to be significant for all traits ($P \leq 0.01$).

Table 3.2 Mean squares for CBB resistance and agronomic parameters of 110 common bean genotypes grown at three locations for two seasons in Ethiopia, 2017-2018.

Source of variation	Disease parameters			Agronomic parameters			
	DF	SL	SP	AUDPC	PPP	SPP	GY
Rep	1	1.99 ^{ns}	0.01 ^{ns}	474.56 ^{ns}	49.53 ^{ns}	1.36 ^{ns}	3131.49 ^{**}
Block(rep)	20	1.46 ^{ns}	1.61 [*]	289.96 ^{ns}	22.74 ^{ns}	1.06 [*]	21.60 ^{ns}
Site	2	15.90 ^{**}	145.74 ^{**}	18588.16 ^{**}	3240.71 ^{**}	158.63 ^{**}	8463.46 ^{**}
Season	1	1.38 ^{ns}	308.02 ^{**}	29620.56 ^{**}	1203.26 ^{**}	19.50 ^{**}	133.37 [*]
Genotype	109	10.34 ^{**}	5.62 ^{**}	2776.63 ^{**}	207.62 ^{**}	5.11 ^{**}	210.99 ^{**}
Sitexseason	2	25.21 ^{**}	85.69 ^{**}	2141.83 ^{**}	601.88 ^{**}	8.53 ^{**}	2681.26 ^{**}
Genotypexsite	218	2.09 ^{**}	0.90 [*]	290.66 ^{**}	29.91 [*]	0.87 ^{**}	47.94 ^{**}
Genotypexseason	109	1.82 [*]	2.83 ^{**}	595.52 ^{**}	20.60 ^{ns}	0.52 ^{ns}	25.64 ^{ns}
Genotypexsitexseason	218	1.68 [*]	0.78 ^{ns}	244.58 [*]	23.15 ^{ns}	0.54 ^{ns}	21.14 ^{ns}
Error	639	1.40	0.74	205.68	24.81	0.62	27.76
Trial statistics							
Mean		4.98	4.48	85.64	13.48	3.75	19.27
CV (%)		23.71	19.26	16.75	36.96	21.09	27.34
R ² (%)		72.92	82.09	81.84	74.99	77.29	79.41

DF = degrees of freedom; SL = CBB severity on leaf; SP = CBB severity on pod; PPP = pods per plant; SPP = seeds per pod; GY = grain yield; CV = Coefficient of variation; R² = R-square; * significant at $P < 0.05$; ** significant at $P < 0.01$; ns = non-significant

3.3.2 Genotypic performance based on disease parameters

Significant variation were recorded among the test genotypes for CBB severity on leaf. Fifteen genotypes (13.6 %) were categorized as CBB resistant based on low mean SL scores varying from 1 to 3 at all the three sites for two seasons (Table 3.3). Most of the resistant genotypes belong to VAX-, SEC- and SMC-lines and possessed mean SL values of ≤ 3.00 . About 54.5 % of the genotypes showed lower mean SL values compared to the resistant check (XAN159), which had a mean SL value of 5.18. The majority of the test genotypes (86.4%) had moderate resistance

with SL values ranging between 3.17 to 6.49 compared to the susceptible check, Teebus (6.53). Seven genotypes (Acc No.7, Chore, Acc No.5, Mexican-142, Acc No.8, Acc No.191 and Acc No.10) exhibited higher mean SL values varying from 6.53 to 6.87 compared with the susceptible check, Teebus.

Genotypes showed marked variation for pod severity. Most test genotypes that showed higher resistance to leaf infection also exhibited better resistance to pod infection. Only two genotypes (SMC22 and SMC25) showed moderate resistance for CBB based on pod infection albeit higher resistance based on lower leaf infection (Table 3.3). Similarly, VAX2 and SMC23 exhibited higher resistance to CBB based on lower pod infection and moderate leaf infection. Fifteen genotypes exhibited relatively higher resistance to pod infection (with mean SP values of < 3.00) and the remainder of the test genotypes had moderate resistance reaction. Compared to the CBB resistant check, XAN159 (with SP value of 4.39), 80 genotypes scored lower mean SP values, between 3.23 and 4.38. VAX6 with a mean SP value of 2.82, SEC25 (2.87), VAX2 (2.90), SEC21 (2.93), SEC23 (2.93) and SMC21 (2.96) showed the highest resistance to CBB for their lower pod infection compared to the resistant check (XAN159).

The area under disease progress curve showing the progression of severity of the CBB infection on test genotypes is presented on Figure 3.2. There were 50 genotypes that scored lower AUDPC values that ranged between 57.61 (SEC20) and 84.54 (Nazareth-2) compared to the resistant check XAN159 (84.79). Conversely, 59 genotypes had higher AUDPC values, between 84.95 and 116.62. Nine genotypes exhibited higher mean AUDPC values ranging from 108.89 to 116.62 compared to the susceptible check, Teebus (107.80). Most of the test genotypes that showed resistance to leaf and pod infection exhibited lower AUDPC values. The lowest AUDPC values were recorded for SEC20 (57.61), SEC22 (58.49), SEC20 (58.70), and SEC24 (58.77). Some genotypes such as VAX4, SMC24 and VAX3 scored low AUDPC values of 59.80, 62.07 and 62.04, in that order because of their moderate resistance to leaf and pod infection. The severity of CBB infection was the highest on the genotypes such as Acc No.5, Acc No.8, Acc No.10, Acc No. 21, and Acc No.191. Conversely, CBB severity was the lowest on some of the newly identified sources of resistance (SEC12, SEC20, SEC21, SEC24, SMC21 and SMC24) compared to the resistant check (XAN159) (Figure 3.2).

Table 3.3 Mean values for disease parameters and associated reaction types to CBB of 32 selected common bean genotypes when tested across two seasons at three sites in Ethiopia

Genotype	SL and reaction type	SP and reaction type	AUDPC
SEC21	2.82 (R)	2.93 (R)	64.02
SEC23	2.83 (R)	2.93 (R)	60.04
SMC21	2.83 (R)	2.96 (R)	64.83
VAX6	2.88 (R)	2.82 (R)	64.23
SEC12	2.91 (R)	3.00 (R)	58.70
SEC25	2.94 (R)	2.87 (R)	63.34
SMC22	2.95 (R)	5.48 (R)	63.06
VAX5	2.97 (R)	3.00 (R)	63.46
SEC20	3.00 (R)	3.00 (R)	57.61
SEC22	3.00 (R)	3.00 (R)	58.49
SEC24	3.00 (R)	3.00 (R)	58.77
SEC26	3.00 (R)	3.00 (R)	67.86
SMC16	3.00 (R)	3.00 (R)	72.12
SMC25	3.00 (R)	3.23 (MR)	62.98
VAX2	3.17 (MR)	2.90 (R)	61.78
SMC23	3.18 (MR)	2.98 (R)	71.26
VAX3	3.35 (MR)	3.20 (MR)	62.04
VAX1	3.37 (MR)	3.00 (R)	69.77
SMC24	3.48 (MR)	3.19 (MR)	62.07
USDK-CBB-15	5.05 (MR)	4.72 (MR)	94.97
XAN159	5.18 (MR)	4.39 (MR)	84.79
Nasir	5.78 (MR)	4.91 (MR)	91.15
Red Wolaita	5.86 (MR)	4.92 (MR)	90.70
Awash -1	6.16 (MR)	5.29 (MR)	96.81
Teebus	6.53 (MR)	6.00 (MR)	107.80
ACC No.7	6.59 (MR)	5.06 (MR)	115.73
Chore	6.76 (MR)	5.63 (MR)	102.58
ACC No.5	6.77 (MR)	5.74 (MR)	114.52
Mexican-142	6.78 (MR)	5.64 (MR)	107.18
ACC No.8	6.81 (MR)	5.81 (MR)	113.61
ACC No.191	6.85 (MR)	5.32 (MR)	114.47
ACC No.10	6.87 (MR)	5.60 (MR)	113.49
Mean	4.98	4.48	85.64
SE	0.04	0.04	0.65
CV (%)	23.71	19.26	16.75
R ² (%)	72.92	82.09	81.84
LSD (5%)	0.95	0.69	11.50
Significant level	**	**	**

SL = severity on leaf; SP = severity on pod; R = resistant; MR = Moderately resistant; SE = Standard error; CV = Coefficient of variation; R² = R-square; LSD = Least significant difference; ** significant at P < 0.01; * significant at P < 0.05

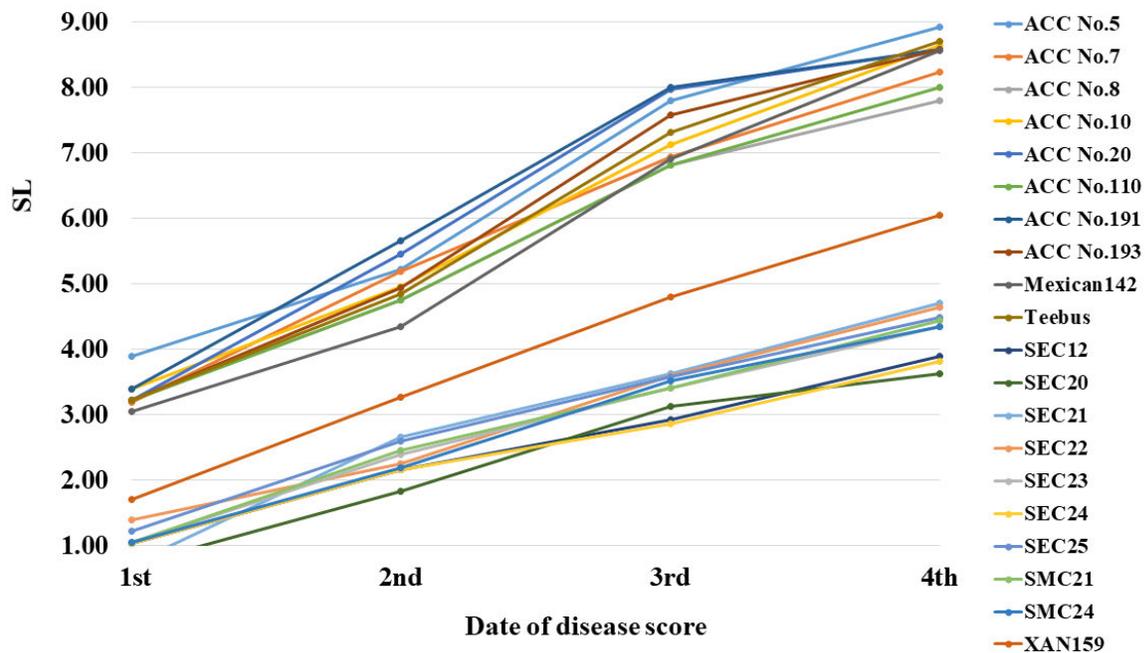


Figure 3.2 Disease progress curves of five resistant and five susceptible genotypes to leaf infection, when compared with the susceptible (Teebus) and resistant (XANA159) checks tested at three sites for two seasons in Ethiopia

3.3.3 Response of genotypes for selected agronomic traits

The overall assessment of the test genotypes showed that CBB susceptible check, Teebus showed better performance in agronomic traits under low disease pressure compared to the resistant check (Table 3.4). Forty nine genotypes scored high number of pod per plant (PPP) values compared with the CBB resistant check, XAN159. Overall genotypes SEC21 (with 20.71 PPP), SEC12 (20.15) and SEC20 (20.03) showed higher PPP values (Table 3.4). When compared with the resistant check, test genotypes such as SEC22, SEC23, VAX6, SEC24, SEC25, VAX1 and SMC21 had better PPP with values of 19.78, 19.56, 19.20, 18.92, 18.32, 17.31 and 14.88, in that order. The worst performing genotypes for PPP were ACC20 (with 6.77 PPP), ACC31 (6.90) and ACC21 (6.99) (Table 3.4).

Fifty-six genotypes scored higher number of seed per pod (SPP) ranging between 3.72 and 5.20 compared with Teebus (3.68 SPP). Most of the high performing genotypes for SPP belong to the Meso-American origin. The higher mean SPP were recorded for ACC:109129/ID:PRO 443_151 (5.20 SPP), followed by VAX1 (5.17) and Roba-1 (5.12), Beshbesh (4.95), VAX6 (4.88),

ACC:B11 625 (4.83), ACC No.5 (4.83) and SEC25 (4.82). The known CBB resistant genotypes such as VAX5 (with a mean SPP value of 5.54), SEC26 (5.53), SEC12 (4.40), VAX4 (4.40), SEC21 (4.31) and SMC24 (4.27) were the top performers. Genotypes with a relatively lower mean SPP values were ACC8 (2.71), ACC25 (2.68), ACC26 (2.61), ACC4 (2.50), ACC15 (2.3.8) and ACC9 (2.37), the majority of which belong to the Andean origin (Table 3.4).

There were 103 genotypes (94.0 %) which had relatively higher mean GY values varying from 1.42 to 3.05 ton ha⁻¹ when compared to Teebus with a grain yield of 1.41 ton ha⁻¹. Overall, Nasir (3.05 ton ha⁻¹) had the highest GY followed by VAX1 (2.86 ton ha⁻¹) and Hawassa Dume (2.83 ton ha⁻¹). There were 15 genotypes including the resistant check XAN159 with a low mean GY value of < 1.36 ton ha⁻¹ when compared to Teebus. The genotypes with relatively lower yield levels included Acc No.7 (0.94 ton ha⁻¹), Acc No.5 (1.02 ton ha⁻¹) and Acc No.8 (1.13) ton ha⁻¹). Some genotypes that had adequate resistance to leaf and pod infection also exhibited better GY that ranged between 2.38 to 2.86 ton ha⁻¹. These genotypes included VAX1, VAX6, VAX3, VAX2, SMC21, VAX5, SEC12, SEC21, SEC22, SEC24 and SEC20.

Table 3.4 Mean grain yield and yield component performance of selected 32 common bean genotypes of assessed across two seasons in three sites in Ethiopia

Genotype	PPP	SPP	GY (ton ha ⁻¹) ^a
Nasir	18.74	4.07	3.05
VAX1	17.31	5.17	2.86
Hawassa Dume	16.02	3.90	2.83
VAX6	19.20	4.88	2.78
Ayewew	14.80	3.61	2.76
Dinknesh	17.93	4.62	2.63
VAX3	17.43	4.12	2.61
ACC No.2	16.13	4.25	2.61
VAX2	16.48	4.19	2.58
Gofta	14.09	3.77	2.57
SMC21	14.88	3.63	2.25
VAX5	15.48	4.54	2.52
Melka Dima	9.55	3.53	2.47
Awash-2	18.05	3.90	2.47
SEC12	20.15	4.40	2.41
SEC22	19.78	4.23	2.39
SEC21	20.71	4.31	2.39
SEC24	18.92	4.14	2.36
Tabor	13.58	5.08	2.34
SMC24	14.14	4.27	2.03
Red Wolaita	13.25	4.10	1.90
Mexican-142	17.67	4.18	1.74
ACC18	10.67	3.29	1.74
USDK-CBB-15	8.61	3.31	1.71
ACC12	8.65	3.42	1.64
Teebus	20.03	3.68	1.41
XAN159	14.13	3.34	1.36
G19833	11.03	3.29	1.28
ACC:107212/ID:R 99	9.41	3.14	1.21
ACC No.8	11.87	4.33	1.13
ACC No.5	10.60	4.83	1.02
ACC No.7	11.34	3.84	0.94
Mean	13.48	3.75	1.93
SE	0.19	0.03	0.34
CV (%)	36.96	21.09	27.30
R ² (%)	74.99	77.29	79.10
LSD (5%)	3.99	0.63	0.47
Significant level	**	**	**

PPP = pods per plant; SPP = seeds per pod; GY = grain yield; SE = Standard error; CV = Coefficient of variation; R² = R-square; LSD = Least significant difference; ** significant at P < 0.01; * significant at P < 0.05, ^agenotypes are ranked based on GY response

3.3.4 Effect of test sites on disease expression and agronomic performance of common bean genotypes

There were significant differences for SL among the test sites (Table 3.5). Highest mean SL values were recorded at the Melkassa site (5.11) followed by Mieso (5.08) and Arsi Negelle sites (4.76). The mean SP values were the highest at the Mieso site (5.13) compared with Melkassa (4.26) and Arsi Negelle sites (4.04). The lowest mean AUDPC values were recorded at Arsi Negelle, while Melkassa had the higher AUDPC value (92.75). The higher mean PPP value was recorded at the Arsi Negelle (40.17) site. The highest mean GY was recorded at the Melkassa site (2.26 t ha⁻¹) compared to the Arsi Negelle (2.10 t ha⁻¹) and Mieso sites (1.43 t ha⁻¹).

Table 3.5 Mean values for disease and agronomic parameters of common bean genotypes evaluated in three sites and two seasons in Ethiopia

Sites	SL	SP	AUDPC	PPP	SPP	GY (t ha ⁻¹)
Melkassa	5.11 ^a	4.26 ^b	92.75 ^a	15.44 ^a	4.01 ^b	22.55 ^a
Arsi Negelle	4.76 ^b	4.04 ^c	80.02 ^c	14.61 ^b	4.17 ^a	20.98 ^b
Mieso	5.08 ^a	5.13 ^a	84.15 ^b	10.38 ^c	3.06 ^c	14.29 ^c
Mean	4.98	4.48	85.64	13.48	3.75	19.27
LSD (5%)	0.157	0.114	1.899	0.660	0.105	0.698

SL = severity on leaf; SP = severity on pod; PPP = pods per plant; SPP = seeds per pod; GY = grain yield, LSD = least significant difference, means in a column with similar letter (s) are not significantly different at $P < 0.05$

3.3.5 Association among disease and agronomic parameters

Table 3.6 presents the correlation coefficients and significant tests among disease and agronomic traits of the test genotypes over sites. Highly significant and positive correlations were observed among disease resistance trait measurements (SL, SP and AUDPC) across the test sites. Strong correlations were observed among SL and AUDPC ($r = 0.90$, $P < 0.01$) at the Melkassa site. A relatively low but significant correlation between SP and AUDPC ($r = 0.37$, $P < 0.01$) was recorded at the Mieso. All assessed agronomic traits exhibited moderate correlations among themselves in all the test sites. Relatively, strong correlation was recorded between PPP and GY ($r = 0.55$, $P < 0.01$) at Arsi Negelle

However, the correlation between SPP and PPP ($r = 0.14$, $P < 0.05$) was low at Mieso. Agronomic and disease traits exhibited negative associations at all the test sites except at Arsi

Negelle where PPP exhibited non-significant and positive associations with SL and SP. The strong negative association between disease parameters and agronomic traits was observed between AUDPC and GY ($r = -0.47$, $P < 0.01$) at Mieso, followed by SL and GY ($r = -0.27$, $P < 0.01$) at Melkassa. The low correlation among disease and agronomic parameters could be attributed to the high variability of weather conditions across the growing season at the Mieso site (Figure 3.1).

Table 3.6 Pearson correlation coefficients showing pairwise association of disease and agronomic parameters among 110 common bean genotypes assessed in two seasons and three sites in Ethiopia

Traits [§]	Melkassa						Arsi Negelle						Mieso					
	SL	SP	AUDPC	PPP	SPP	GY	SL	SP	AUDPC	PPP	SPP	GY	SL	SP	AUDPC	PPP	SPP	GY
SL	1.00						1.00						1.00					
SP	0.60**	1.00					0.84**	1.00					0.53**	1.00				
AUDPC	0.90**	0.73**	1.00				0.43**	0.50**	1.00				0.64**	0.37**	1.00			
PPP	-0.18*	-0.17*	0.14*	1.00			0.004 ^{ns}	0.09 ^{ns}	-0.09 ^{ns}	1.00			-0.22*	-0.11*	-0.24**	1.00		
SPP	-0.26**	-0.23*	-0.23*	0.25*	1.00		-0.17*	-0.16*	-0.13*	0.17*	1.00		-0.02 ^{ns}	-0.05 ^{ns}	-0.13*	0.14*	1.00	
GY	-0.28**	-0.19*	-0.27**	0.39**	0.23*	1.00	-0.01 ^{ns}	0.11*	-0.11*	0.55**	0.20*	1.00	-0.24**	-0.15*	-0.47**	0.47**	0.36**	1.00

[§] SL = severity on leaf; SP = severity on pod; AUDPC = area under disease progress curve; PPP = pods per plant; SPP = seeds per pod, GY = Grain Yield; * and ** denote significant correlations at P < 0.05 and at P < 0.01, respectively; ns=non-significant

3.3.6 Principal component and bi-plot analyses

The principal component analysis (PCA) identified two principal components (PC), that accounted for 84.48% of the total variation for reaction to diseases and agronomic performance among the test genotypes. The relationship among the PC and associated parameters are presented in Table 3.7. Principal component 1 (PC1) is positively correlated with disease parameters (SL, SP and AUDPC) each contributing to a loading score of > 0.45. Agronomic traits such as number of PPP and SPP showed higher loading scores of > 0.55 and were more correlated with principal component 2 (PC2).

Based on PCA bi-plot analysis the test genotypes were distributed in the four quadrants (Figure 3.3). The SEC-lines (e.g. SEC12, SEC20, SEC2, SEC22, SEC24, SEC25 and SEC26), SMC-lines (e.g. SMC21, SMC24, SMC16 and SMC25), VAX-lines (e.g. VAX2, VAX4 and VAX5) were plotted in Quadrant III. These genotypes had lower SL, SP and AUDPC values. Genotypes that combined low disease severity and better agronomic traits were allocated in Quadrant II. These genotypes included VAX1, VAX6, Awash Melka and Dinknesh. Most common bean collections of Ethiopia were plotted in Quadrant I. These genotypes were CBB susceptible associated with high values of SL, SP and AUDPC. Also these genotypes were poor performers for PPP and SPP traits. Lines introduced from CIAT/Uganda such as ACC5, ACC8, ACC11, ACC20, ACC21, ACC29 were allocated in Quadrant IV expressing intermediate values for disease and agronomic traits.

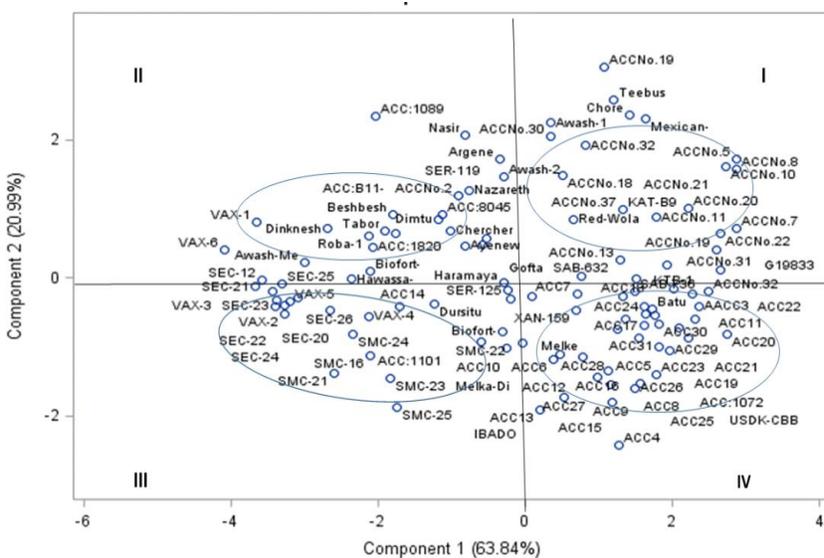


Figure 3.3 Bi-plot showing clustering of 110 common bean genotypes when tested at three sites and two seasons in Ethiopia

Table 3.7 Principal components for disease and agronomic parameters of 110 common bean genotypes tested at three sites and two seasons in Ethiopia

Parameters	PC1	PC2
Variance Explained	3.86	1.27
Proportion (%)	63.33	21.15
SL	0.45	0.38
SP	0.45	0.32
AUDPC	0.46	0.33
PPP	-0.34	0.55
SPP	-0.34	0.52
GY	-0.39	0.25

PC1 = Principal component 1; PC2 = Principal component 2; SL = severity on leaf; SP = severity on pod; AUDPC = area under disease progress curve; PPP = pods per plant; SPP = seeds per pod, GY = Grain Yield.

3.4 Discussion

Sustainable bean production and productivity is dependent on the availability of disease resistant common bean varieties with desirable farmer and market-preferred traits. Breeding for host resistance is an economic and environmentally friendly strategy to reduce losses incurred by the CBB (Miklas et al., 2003; Shiet al., 2011a). In the present study 110 genetically diverse common bean genotypes were screened for CBB resistance and for better agronomic traits in three sites and two seasons in Ethiopia. The sites represent the hotspot areas for CBB with favorable weather conditions allowing infection and disease development (Figure 3.1A to C).

Significant ($P < 0.05$) differences were found among the test genotypes for CBB reaction and agronomic traits (Tables 3.3 and 3.4). Previous reports indicated the presence of marked genetic variation among common bean genotypes with variable reaction to CBB and agronomic traits (Mutlu et al., 2008; López et al., 2006; Mkandawire et al., 2004). Opio et al. (2002) reported variable responses to CBB infection when assessing 118 common bean genotypes under field conditions in Uganda allowing selection of unique parents for breeding.

The mean disease severity on leaf and pod were reportedly key parameters used to identify CBB resistant common bean genotypes (Arnaud-Santanaet al., 1994; Ariyaratneet al., 1998). Based on low disease severity on leaf and pod the present study identified the following CBB resistant

genotypes: SEC21, SEC23, SMC21, VAX6, SEC12, SEC25, SMC22, VAX5, SEC20, SEC22, SEC24, SEC26, SMC16 SMC24,VAX6, SEC25, SEC21, SEC23, and SMC21 (Table 3.3). Arnaud-Santanal et al. (1994) reported that common bean lines with known CBB resistance such as XAN159, BAC6, and XAN112 exhibited the best combination of leaf and pod resistance agreeing with the present findings. However, XAN159 did not show CBB resistance in the present test environment attributable to the variable test conditions or physiological races of the pathogen (Lopez et al., 2006).

The common bean line SMC25 exhibited resistant reaction to the CBB based on low leaf infection but showed moderate resistance to pod infection. Furthermore, lines VAX1, VAX2 and SMC23 had resistance reaction based on pod infection but showed moderate resistance for leaf infection (Table 3.3). These findings agree with the report of Shiet al. (2011a) and Viteriet al. (2014a) who reported differential reaction of common genotypes to leaf and pod infections under the prevailing test conditions in Ontario (Canada) and Idaho (USA), respectively. In the current study the SEC-, SMC- and VAX-lines also displayed low AUDPC values in the three sites and two seasons suggesting their stable CBB resistance. These genotypes are ideal for future resistance breeding programs in Ethiopia. The SEC - and SMC -lines were initially bred for CBB resistance and drought tolerance (Amongi et al., 2019; LIL, 2016). These lines were also found to be resistant to CBB in Ethiopia based on preliminary findings by Tumsa et al. (2015). Yohannes et al. (2020) reported that some of the SMC- and SEC-lines performed better in terms of grain yield and resistance to the major bean diseases at the Areka site in Southern Ethiopia. The common bean lines which were selected for drought tolerance were reportedly resistant to the CBB. This was attributed to the related pedigree of the lines which had common parentage for drought tolerance and CBB resistance derived from the tepary bean (*P. acutifolius* A. Gray) (Rosas et al., 1991; Singh and Munoz, 1999; Yu et al., 2004; Liu et al., 2008; Beebe, 2012). Also, common bean genotypes selected for drought adaptation showed increased phenolic compounds inhibiting CBB infection and disease development (Blum, 1988; Blum, 2005; Sallam, 2011

The following genotypes: VAX1, VAX6, VAX3, VAX2, SMC21, VAX5, SEC12, SEC21, SEC22, SEC24 and SEC20 were selected for better agronomic performance (Table 3.4). The lines maintained higher values of PPP varying from 14.14 to 19.20, higher SPP (4.0 to 5.0) and enhanced GY (2.03 to 2.86 ton ha⁻¹). The selected complementary genotypes are promising candidate lines for direct production or CBB resistance breeding programs in Ethiopia or similar

agro-ecologies in the region. Despite their genetic values for CBB resistance, the VAX-lines do not have desirable seed color and shape preferred by end users in Ethiopia. This necessitates targeted crosses to develop breeding populations for selection of desirable sergeants with CBB resistance and suitable white seed color and round seed shape (Ibarra-Perez et al., 2005).

Positive and high correlations were detected among disease parameters across the test sites (Table 3.6). This suggests the high success rate of CBB infection and disease development for reliable selection of test genotypes was achieved. The positive correlations among SL, SP and AUDPC found in this study is in agreement with the report of Ravaet al. (1987) who indicated positive associations between leaf and pod infection. Significant genetic correlations were also reported between leaf and pod reaction types for bean genotypes assessed at Nebraska, USA (Arnaud-Santanal et al. 1994). **As expected negative correlations were observed among disease and agronomic traits in different environments** (Table 3.6). The negative correlations between disease parameters (SL, SP and AUDPC) and agronomic traits (PPP, SPP and GY) especially at the Melkassa and Mieso sites suggest the progress of the disease was higher in the susceptible genotypes leading to higher SL and decreased crop productivity. It was also reported that CBB severity was associated with a reduction of seed quality and quantity in common bean under a field condition (Donmez et al., 2013).

3.5 Conclusions

The study found significant variation for CBB reaction and agronomic traits among 110 common bean genotypes tested at three sites and two seasons in Ethiopia. This allowed selection of promising genotypes as new and potential sources for CBB resistance and grain yield influencing traits. The following genotypes: SEC21, SEC23, SMC21, SEC12, SEC25, SEC20, SEC22, SEC24 and SMC24 were identified as CBB resistant with low reaction types based SL, SP and AUDPC. These genotypes had combined CBB resistance and better PPP, SPP and GY performance. In Ethiopia SEC-lines are highly preferred for their white seed color and round seed shape for the market. Therefore, the above selected and complementary lines are recommended for direct production or common bean breeding population development to enhance CBB resistance and yield gains.

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CHAPTER FOUR: COMBINING ABILITY AND GENE ACTION CONTROLLING COMMON BACTERIAL BLIGHT RESISTANCE AND AGRONOMIC TRAITS IN COMMON BEAN

Abstract

The common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *Xanthomonas axonopodis* pv. *phaseoli* var *fuscans* (*Xaf*) is a major disease of the common bean (*Phaseolus vulgaris* L.). Developing high yielding, CBB resistant and market-preferred common bean varieties is the overriding consideration in bean breeding programs. This is dependent on the identification of promising parents and progenies through combining ability tests. The aim of this study was to select common bean parents and families with good combining ability effects and heritability for CBB resistance and agronomic traits. Eight CBB resistant common bean genotypes were crossed with four susceptible farmer-preferred common bean genotypes using a line x tester mating design. The F₂ families were evaluated at Melkassa and Arsi Negelle Agricultural Research stations in Ethiopia using an alpha lattice design with two replications. Disease **assessment traits** such as CBB severity on the leaf (SL), CBB severity on the pod (SP) and the area under disease progress curve (AUDPC) and agronomic traits such as the number of pod per plant (PPP), number of seeds per pod (SPP) and grain yield (GY) were recorded. The inheritance of all CBB resistance traits was largely attributed to additive gene effects. The H² values were moderately high and ranged between 0.55 (for PPP and GY) to 0.70 (for SPP), revealing the contributions of additive genes in the inheritance of these traits. Parents such as SEC12, SEC21, SEC20, SEC24 and SEC25 had negative and significant general combining ability (GCA) effects for CBB severity for leaf and pod infection, **revealing their contribution towards CBB resistance**. The F₂ population of the following crosses such as Nasir x SEC24, Red Wolaita x SMC21, Mexican142 x SMC21, Mexican142 x SEC25, Awash1 x SEC22, Red Wolaita x SEC12, Nasir x SEC22, Nasir x SEC20 and Awash1 x SEC12 were best specific combiners and therefore selected for CBB resistance breeding. These families displayed better agronomic attributes with significant and negative specific combining ability (SCA) effect for SL and AUDPC. The selected parents and families are useful genetic resources for future breeding of CBB resistant and agronomically superior transgressive segregants for common bean variety development in Ethiopia.

Keywords: Common bean, general combining ability effect, *Phaseolus vulgaris* L., specific combining ability effect, *Xanthomonas axonopodis*

4.1 Introduction

Common bean (*Phaseolus vulgaris* L., $2n=2x=22$) is highly valued and essential grain legume because of its high protein content (Gepts et al., 2008). In sub-Saharan Africa (SSA), the crop is a low-cost protein source to more than 300 million people (Broughton et al., 2003; CGIAR, 2014). Ethiopia is one of the leading common bean producers with global ranking of 10 and with a total annual production of 520,979 tonnes (FAO, 2017). Approximately 3.4 million smallholder farmers produce common bean in Ethiopia for household consumption, and for local and export markets (CSA, 2018). Nonetheless, the full potential of the common bean production sector in Ethiopia is constrained by a lack of improved varieties with biotic and abiotic stress tolerance. Amongst the biotic constraints, the common bacterial blight (CBB) disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. axonopodis* pv. *phaseoli* var. *fuscans* (*Xaf*) is the most damaging to common bean yields. The disease is wide spread in tropical and subtropical regions causing up to 50% yield losses worldwide (Viteri et al., 2014). Depending on the season and crop growth stages, a 1% increase in CBB severity causes a yield loss of about 10.5 -78.0 kg ha⁻¹ (Allen and Lenne, 1998). In Ethiopia, the disease causes up to 40 % yield loss (Fininsa and Tefera, 2001). Therefore, development of high yielding, CBB resistant and market-preferred common bean varieties is the overriding consideration in bean breeding programs.

Use of disease-free certified seed and bactericidal chemicals are effective strategies to minimize the yield losses due to CBB (Singh and Munoz, 1999). However, farmers in developing countries including Ethiopia have limited access to certified seeds and crop protection chemicals. Cultural control measures, such as intercropping and residue management, have been recommended as an alternative control method (Fininsa and Tefera, 2001). However, these control methods are not effective in CBB management. The use of host-plant resistance is considered the most economically feasible and sustainable approach for controlling the CBB in common bean production (Durham, 2011; Fourie, 2011; Fourie et al., 2011). Therefore, development and deployment of agronomically superior and CBB resistant common bean cultivars is an economic and sustainable approach to boost bean production and productivity. Subsequently, the International Centre of Tropical Agriculture (CIAT) and various national bean programs have developed breeding lines and elite common bean genotypes with suitable agronomic traits and considerable CBB resistance. These genetic resources are suitable sources of genetic variation for breeding locally adapted and market-preferred varieties.

Information on the combining ability of parents through their progenies for economic traits is essential in identifying productive and CBB resistant common bean cultivars (Parviz et al., 2016). The combining ability effects are quantified in terms of the estimates of general combining ability (GCA) and specific combining ability (SCA). The GCA is the average performance of a line in hybrid combinations and is due to additive genes action. The SCA refers to crosses that do relatively better or worse than would be expected based on the average performance of the lines involved and is due to non-additive gene action. In CBB resistance breeding, negative GCA and SCA effects of lines are desirable (Bokmeyer et al., 2009; Mukankusi et al., 2011). However, there are contradictory reports regarding the preponderant gene action conditioning CBB resistance. Both GCA and SCA effects have been reported in conditioning CBB resistance. For example, Rodrigues et al. (1999) reported that GCA effects were predominant over SCA for CBB resistance based on leaf severity assessment. Conversely, Trindade et al. (2015) reported that SCA effects were more important than the GCA in conditioning CBB resistance. Heritability for CBB resistance ranged between low (0-30%) and medium (30-60%) (Tar'an et al., 2001; Singh and Schwartz, 2010; Tryphone et al., 2013).

Estimates of genetic parameters are subject to the genetic composition of the test population and the environment (Falconer and Mackay, 1996). The Baker's ratio (BR) is widely used to test which type of genetic effects dominate trait inheritance. For self-pollinated crops like common bean, higher BR (near 1) implies the preponderance of additive gene effect conditioning trait inheritance. The line x tester mating design is the most commonly used method to estimate GCA and SCA effects and trait heritability (Kempthorne, 1957). It is a useful design for self-pollinated crop species high success rate (>70%) in beans with simple management conditions (Tai, 1976). It has been used in genetic analysis of traits of various legume crop species such as cowpea (Barro Antoine et al., 2017; Tchiagam et al., 2011; Romanus et al., 2008), soybean (Kurasch et al., 2017; Mebrahtu and Devine, 2009) and chickpea (Karami, 2011, Kumar et al., 2001).

To initiate common bean pre-breeding for CBB resistance and farmer-preferred agronomic traits, genetically diverse collections were characterized using agro- morphological traits (Tumsa et al., 2020). This enabled selection of potential and complementary parents for breeding. The combining ability effects of the selected parents and their progenies should be assessed to develop new breeding populations adapted to Ethiopia. Therefore, the objectives of this study were to

determine the combining ability effects and gene action controlling CBB resistance and agronomic traits in selected common bean genotypes to develop breeding populations.

4.2 Materials and methods

4.2.1 Developing breeding populations

Eight common bean lines (SEC12, SEC20, SEC21, SEC22, SEC25, SMC21 and SMC24) were selected following extensive screening experiments for CBB resistance and agronomic traits under field conditions in three environments during the 2017 and 2018. The lines were selected for their resistance reaction to CBB infection, better agronomic performance, and market preferred traits (Table 4.1). The eight lines were used as testers and crossed with four CBB susceptible but farmer-preferred varieties as lines (Mexican142, Awash1, Red Wolaita, and Nasir) using a line by tester mating design to generate 32 F₁ crosses. Hybridization were conducted using the ‘emasculation with a protected stigma method’ in which the floral buds of the female parent is emasculated, followed by pollination, while sepals are kept to protect the bud (Genchev, 2007). Crossing nursery was established under field conditions at Melkassa Agricultural Research Center. The crossing blocks was staggered based on flowering dates to create synchronization between male and female parents. Pollination was done during the early morning and late afternoon when the temperature was low. The 32 F₁ progenies were selfed to produce F₂ seeds for further evaluation of CBB resistance and agronomic traits.

Table 4.1 Pedigree, reaction to CBB, and agronomic traits of the selected parents

Parent (name /designation)	Pedigree	Reaction to CBB	Agronomic/market-Preferred traits	Role in the cross
Mexican142	G11239	Susceptible	Market value (white seed color and round seed shape)	Line
Awash1	PAN 182	Susceptible	Market value (white seed color and round seed shape)	Line
Nasir	Dicta105	Susceptible	Locally preferred for food, round seed shape (small red)	Line
Red Wolaita	Local collection	Susceptible	Locally preferred for food (good taste; small red)	Line
SEC12	MR 14215-6/MC-2P-MQ-MC	Resistant	Seed color and shape	Tester
SEC20	SX 15228-32/MC-17C-MQ-MC	Resistant	White seed color	Tester
SEC21	SX 15228-32/MC-17C-MQ-MC	Resistant	White seed color	Tester
SEC22	SX 15228-32/MC-17C-MQ-MC	Resistant	White seed color	Tester
SEC24	SX 15228-32/MC-17C-MQ-MC	Resistant	White seed color	Tester
SEC25	SX 15228-32/MC-17C-MQ-MC	Resistant	White seed color	Tester
SMC21	SDF216526-003/MC-4P-MQ-MC	Resistant	White seed color	Tester
SMC24	SDF216526-003/MC-4P-MQ-MC	Resistant	White seed color	Tester

4.2.2 Description of the test environments

The F₂ populations (32), together with the twelve parental genotypes, were evaluated at two sites situated in the Melkassa and Arsi Negelle research stations of the Ethiopian Institute of Agricultural Research (EIAR) during the 2019 cropping season. Melkassa Research Center is located in the semi-arid region of Central Rift Valley at 8° 24' N latitude and 39° 12' E longitude. The center is situated at an altitude of 1550 m above sea level (masl). It receives a mean annual rainfall of 763 mm with daily minimum and maximum temperatures of 14 °C and 28 °C, respectively. Arsi Negelle site is situated at a latitude of 7° 25'N and longitude of 38° 31'E with an altitude of 1900 masl. The soil at the Melkassa and Arsi Negelle sites are sandy-loam and clay-loam, respectively. [The main cropping season starts between late June and early July, based on the onset of the rainfall.](#) Both sites are hotspot areas for CBB disease, and hence natural disease initiation and development were used for genotype evaluation.

4.2.3 Experimental design and field management

The 44 test genotypes were evaluated using an 11 x 4 alpha lattice design with two replications at each site, [in 2019 main season.](#) An experimental unit consisted of two rows with 4 m length and the intra - and inter row spacing were 0.4 and 0.8 cm, respectively. Diammonium phosphate

(46 % P₂O₅) fertilizer was applied at 100 kg ha⁻¹. Urea (46% N) was applied at 50 kg ha⁻¹ of following the common bean's recommendations for the areas with split application (50% during planting and 50% at R3 stage). Recommended agronomic practices for common bean production were followed.

4.2.4 Data collection

Reaction to CBB infection was recorded based on the severity of disease symptoms on leaves and pods. Disease scoring started at the reproductive growth stage (R6) of the common bean using a scale of 1-9, where 1 = 0% of the leaves or pods show infection symptoms, 2 = 2%, 3 = 5%, 4 = 10%, 5 = 20%, 6 = 25%, 7 = 50%, 8 = 75% and 9 > 85% (CIAT, 1987; Opio et al., 1996). The recording was done four times at a seven-day interval when visible symptoms of CBB appeared on the leaves and pods. The CBB disease severity on leaves (SL) and disease severity on pods (SP) was assessed on 30 randomly selected and tagged plants in each plot. Disease rating was done based on a scale of 1-3 (resistant) 4-6 (moderately resistance) and 7-9 (susceptible). The sequential CBB rating scores of disease on leaf were used to calculate the AUDPC following Campbell and Madden (1990):

$$\text{AUDPC} = \sum_{i=1}^{n-1} [0.5(x_i + x_{i+1}) (t_{i+1} - t_i)]$$

Where n is the total number of CBB severity assessment events, t_i is the time of the ith assessment in days from the first assessment date, x_i is the percentage of disease severity at ith assessment.

Data on pods per plant (PPP), the number of seeds per pod (SPP), and grain yield was collected. PPP was recorded as the average number of pods from 10 randomly sampled and tagged plants in a row. The SPP was the proportion of the total number of seeds to the number of pods from ten randomly selected plants per row. Grain yield (expressed in tonnes ha⁻¹) was estimated as the weight of seed from 30 randomly selected and tagged plants and converted to t ha⁻¹ after adjusting to 12% moisture content.

4.2.5 Data analysis

Data from each site was analyzed separately followed by a combined analysis of variance (ANOVA) using the GLM procedure of SAS (SAS, 2014). The estimates of general and specific combining ability effects of the lines and testers were calculated following a line x tester procedure, according to Singh and Chaudhary (1979). The linear model on which the analysis

was based is: $Y_{ij} = \mu + E_i + g_j + g_k + s_{ijk} + r + b + \epsilon_{ijk}$, where Y_{ijk} is the mean of the cross at the i^{th} sites, j^{th} line with k^{th} tester; μ is the grand mean (trial mean), E_i is the site main effect, g_j is the line main effect (GCA for the lines), g_k the tester main effects (GCA for testers), s_{jk} is the specific combining ability effects resulting from the cross between the j^{th} line and the k^{th} tester, r is the number of replications, b is the incomplete blocks within replications, and ϵ_{ijk} is experimental error. Replications and incomplete blocks were considered as random effects. The sum of squares due to test crosses were partitioned into sites, testers, lines, and their interaction effects and were considered as fixed effects.

The GCA and SCA effects were calculated following Singh and Chaudhary (1979). GCA effects of testers were estimated as follows: $g_j = M_{jk} - OM$, where g_j is the GCA of the j^{th} line, M_{jk} is the mean of the j^{th} line across k testers, and OM is the overall mean. The GCA of lines is computed as: $g_k = M_{kj} - OM$, where g_k is the GCA of the k^{th} tester, M_{kj} is the mean of the k^{th} tester across j lines, and OM is the overall mean. The SCA effects were estimated as follows $s_{jk} = M_{jkk} - M_{jk} - M_{kj} + OM$, where M_{jkk} is the mean of the cross between j^{th} line and k^{th} tester, M_{jk} is the mean of the j^{th} line across k testers, M_{kj} is the mean of the j^{th} lines across k tester, and OM is the overall mean.

Variance components attributable to general and specific combining ability effects of lines and testers were computed according to Hallauer et al. (2010) using *SAS-proc varcomp* statements (SAS, 2014). The additive genetic variance (σ^2_a), and dominance variance (σ^2_d) were estimated assuming the value of inbreeding coefficient at 0 ($F=0$) for non-inbred populations as follows: $\sigma^2_a = 4 \sigma^2_m$; $\sigma^2_d = 4 \sigma^2_f / m - 4 \sigma^2_m$. The ratio of GCA and SCA variance ($\sigma^2_{gca} / \sigma^2_{sca}$) was used to test the relative importance of additive versus dominance gene action. Then Baker's ratio (BR) (Baker, 1978) was calculated as: $BR = (\sigma^2_{line} + \sigma^2_{tester}) / (\sigma^2_{line} + \sigma^2_{tester} + \sigma^2_{sca})$.

4.3 Results

4.3.1 Analysis of variance

Analysis of variance of test genotypes based on CBB parameters and agronomic traits is summarized in Table 4.2. The site main effect was significant ($P < 0.05$) for SL, SP, AUDPC, PPP and GY except for SPP. Combined analysis of variance showed that, except for grain yield, the main effects of genotypes were significant ($P < 0.05$) for CBB severity on leaf and pod, area

under disease progress curve, pod per plant and seed per pod. Site x genotype interaction effect was significant ($P < 0.01$) for SP and AUDPC.

Table 4.2 Mean squares and significant tests for CBB parameters and agronomic traits among 32 F_2 populations and 12 parents of common bean evaluated at Melkassa and Arsi Negelle sites in Ethiopia

Source of variation	DF	CBB parameters			Agronomic traits		
		SL	SP	AUDPC	PPP	SPP	GY
Replication in site	1	0.005	0.073	96.19	137.69	0.583	0.065
Block in replication	3	0.229	0.068	49.16	110.55	1.647	0.178
Genotype	43	5.091**	1.133**	531.32**	206.47**	1.233*	0.331
Site	1	0.545**	3.866**	2830.83**	4592.63**	2.035	4.316*
Genotype x Site	43	0.131	0.418**	95.12**	133.22	0.804	0.539
Error	84	0.138	0.056	44.30	98.03	0.724	0.401

SL = disease severity on leaf; SP = disease severity on pod; AUDPC = area under disease progress curve; PPP = pod per plant; SPP = seed per pod; grain yield; * significant at $P < 0.05$; ** significant at $P < 0.01$

4.3.2 Mean responses of test genotypes based on disease resistance traits

Table 4.3 summarizes the mean performances of the genotypes in terms of CBB resistance and agronomic traits. The SL ranged from a score of 2.59 to 7.25, with a mean of 3.33 at the Melkassa site. At the Arsi Negelle site, the SL values ranged from 2.35 and 7.04, with a mean of 2.90. Seventeen F_2 populations had SL values ranging from 2.59 to 2.99 at Melkassa suggesting that these genotypes were resistant to CBB. Twelve crosses showed resistance reaction with SL values between 2.35 and 2.94 at Arsi Negelle. The crosses with low SL values included Awash1/SEC21 (severity score of 2.59), Nasir/SEC20 (2.63), Nasir/SEC24 (2.68), Awash1/SEC20 (2.69) and Awash1/SEC12 (2.73) at Melkassa. Nasir/SEC24 (2.35), Nasir/SEC25 (2.42), Nasir/SEC21 (2.64) and Nasir/SEC20 (2.64) were among the crosses that showed the lowest SL values at the Arsi Negelle site. At the Melkassa site, five testers (SEC12, SEC20, SEC21, SEC22, and SMC24) had SL scores of less than 3.00, indicating their resistance to leaf infection. Similarly, five testers showed lower SL scores ranging between 2.49 (SEC12) and 2.93 (SMC21) at Arsi Negelle. However, all the lines showed higher SL values ranging from 6.09 (Red Wolaita) to 7.25 (Awash1) at both locations, confirming their susceptibility to leaf infection.

All the test-crosses progenies were resistant to CBB pod infection with mean SP values ranging between 2.74 and 2.94 at Melkassa and 1.85 and 2.84 at Arsi Negelle. Awash1/SEC21 (with SP

value of 2.74), Awash1/SEC20 (2.76), Awash1/SEC12 (2.77), and Nasir/SEC20 (2.77) were among the crosses with lower mean SP values. Also, all male testers had low mean SP values, ranging between 2.74 (SEC20) and 2.94 (SMC21), while all lines had high SP values ranging from 3.50 to 3.71. Nasir/SEC24 (with SP value of 1.85), Nasir/SEC25 (2.91), Nasir/SEC20 (2.09) and Nasir/SEC21(2.09) were amongst the crosses with the lower mean SP values at Arsi Negelle. The AUDPC values ranged from 43.71 to 93.30, with a mean of 51.62 for all tested genotypes at Melkassa. Awash1/SEC20 (with SP value of 43.71), Awash1/SEC21 (44.15), Red Wolaita/SEC21 (44.47), and Awash1/SEC12 (44.79) were among the crosses with low AUDPC values. In contrast, Awash1/SMC24 (55.66), Mexican142/SMC24 (55.40), and Red Wolaita/SMC24 (54.52) were among the crosses that had relatively higher AUDPC values at Melkassa. The tester parents showed low to moderate AUDPC values ranging from 43.71 (SEC12) to 55.66 (SMC21), while high AUDPC scores were recorded among the lines, with values ranging from 81.66 (Red Wolaita) to 93.30 (Awash1) at Melkassa. At the Arsi Negelle site, the lowest and highest AUDPC values were 37.64 and 100.96, respectively. Red Wolaita/SEC12 (with AUDPC value of 37.64), Nasir/SEC22 (44.49), Nasir/SEC20 (44.49), and Nasir/SEC25 (47.91) were among the crosses with the lowest AUDPC values at Arsi Negelle. The AUDPC values for the male parents ranged from 41.00 (SEC12) and 65.02 (SMC24), while female parents had AUDPC values ranging from 58.43 (Red Wolaita) to 100.96 (Mexican142) at Arsi Negelle.

4.3.3 Genotype performance for agronomic traits

The mean performances of the test genotypes for agronomic traits at the Melkassa and Arsi Negelle sites are presented in Table 4.3. At Melkassa, the number of pods per plant ranged from 22.50 to 62.80 with a mean of 34.71 among the test cross progenies. Mexican142/SEC22 (with mean PPP of 62.80), Mexican142 (47.32), Awash1/SMC24 (41.80), and Awash1/SEC25 (41.35) were amongst the progenies with the higher PPP counts. Testers such as SEC25 (with mean PPP of 60.65), SEC24 (58.95), and SEC22 (44.45) had the highest PPP counts. For lines (**recipient parents**), Mexican142 (with mean PPP of 47.52) and Awash1(37.59) scored the highest PPP. Mexican142/SEC21 (with mean PPP of 51.66), Mexican142/SEC25 (49.28), Mexican142/SEC20 (47.52) and Mexican142/SEC24 (46.29) were crosses with highest PPP values at the Arsi Negelle site. At the Arsi Negelle site, the number of PPP among testers ranged from 16.72 (SMC24) to 30.54 (SEC22), whereas that of lines ranged between 19.27 (Nasir) and 27.90 (Mexican142).

Families such as Nasir/SEC24 (5.27), Awash1/SMC21 (5.21), and Awash1/SEC20 (5.10) scored higher SPP values at Arsi Negelle. High SPP values were observed for the tester SEC20 (5.17) and SEC24 (4.98), for lines Nasir (4.61) and Mexican142 (4.29). Non-significant differences were observed for grain yield at both testing sites.

Table 4.3 Mean values for CBB parameters and agronomic traits among 32 F₂ populations and 12 parents of common beans evaluated at Melkassa and Arsi Negelle sites in Ethiopia

Sites	Melkassa						Arsi Negelle					
Genotypes	CBB			Agronomic traits			CBB scores			Agronomic traits		
	SL	SP	AUDPC	PPP	SPP	GY	SL	SP	AUDPC	PPP	SPP	GY
Crosses												
Awash1/SEC12	2	2.7	44.79	37.40	4.97	3.34	2.86	2.26	58.18	24.03	4.83	2.77
Awash1/SEC20	2	2.7	43.71	38.35	4.54	3.41	3.08	2.44	61.60	26.14	5.10	2.89
Awash1/SEC21	2	2.7	44.15	34.25	3.92	2.87	3.01	2.37	59.89	35.38	4.41	3.19
Awash1/SEC22	2	2.7	46.68	29.45	6.96	3.45	2.86	2.26	59.89	17.43	5.04	2.42
Awash1/SEC24	2	2.8	48.97	22.50	6.06	2.29	3.01	2.38	59.89	27.63	4.29	2.59
Awash1/SEC25	3	2.8	46.94	41.35	2.44	3.08	3.59	2.84	63.32	30.98	5.71	2.76
Awash1/SMC21	3	2.9	52.00	32.50	4.63	2.85	2.86	2.26	65.02	13.73	5.21	2.81
Awash1/SMC24	3	2.9	55.66	41.80	3.81	2.79	3.23	2.55	66.74	19.18	3.14	2.09
Mexican142/SEC12	2	2.7	46.76	38.35	4.23	2.83	3.08	2.44	63.31	37.84	4.15	3.01
Mexican142/SEC20	3	2.8	50.99	40.10	4.44	2.76	3.01	2.38	53.05	47.52	4.71	3.15
Mexican142/SEC21	2	2.8	48.18	41.15	4.48	3.36	2.86	2.26	54.76	51.66	4.29	3.15
Mexican142/SEC22	2	2.8	47.47	62.80	3.78	3.48	3.23	2.55	61.60	32.03	4.56	3.05
Mexican142/SEC24	3	2.8	48.61	27.65	4.45	2.32	3.23	2.55	65.02	46.29	3.97	2.96
Mexican142/SEC25	3	2.8	47.56	33.65	4.35	1.79	2.79	2.20	54.76	49.28	4.71	3.31
Mexican142/SMC21	2	2.8	49.61	44.90	4.25	3.52	3.08	2.44	56.47	20.42	3.83	3.19
Mexican142/SMC24	3	2.9	55.40	36.55	3.13	3.29	3.01	2.38	59.89	17.87	3.77	2.31
Nasir/SEC12	2	2.8	47.24	32.10	4.66	2.84	3.08	2.43	54.76	24.99	4.64	3.28
Nasir/SEC20	2	2.7	45.17	39.95	4.97	2.57	2.64	2.09	44.49	25.26	3.88	2.51
Nasir/SEC21	2	2.8	47.88	29.20	5.84	3.33	2.64	2.09	51.33	21.21	4.54	3.37
Nasir/SEC22	2	2.7	44.99	27.20	4.23	2.11	2.94	2.32	44.49	20.68	4.63	3.33
Nasir/SEC24	2	2.7	45.82	31.30	4.82	3.56	2.35	1.85	51.33	24.29	5.27	2.93
Nasir/SEC25	3	2.8	49.43	30.75	4.74	2.81	2.42	1.91	47.91	26.23	4.82	2.92
Nasir/SMC21	3	2.9	52.23	27.65	3.77	2.47	3.01	2.38	58.18	23.15	4.62	2.44
Nasir/SMC24	3	2.8	48.86	29.90	4.24	1.96	3.01	2.38	53.05	26.23	3.79	2.75
Red Wolaita/SEC12	3	2.8	47.39	31.80	5.41	3.02	3.08	2.44	37.64	20.77	4.81	2.88
Red Wolaita/SEC20	2	2.8	47.77	31.20	5.69	2.70	3.08	2.43	90.69	14.96	3.59	1.98
Red Wolaita/SEC21	2	2.7	44.47	38.85	5.23	3.22	2.94	2.32	56.47	15.93	5.45	2.56
Red Wolaita/SEC22	3	2.8	49.60	27.80	4.79	2.99	3.37	2.66	63.31	23.94	4.56	2.47
Red Wolaita/SEC24	2	2.7	44.89	29.10	5.79	3.16	3.23	2.55	59.89	17.25	4.76	2.69
Red Wolaita/SEC25	3	2.8	47.39	36.60	5.02	2.12	3.45	2.72	54.76	15.05	4.86	2.81
Red Wolaita/SMC21	3	2.8	51.82	27.75	4.48	2.76	2.93	2.32	66.73	19.80	4.24	2.82
Red Wolaita/SMC24	3	2.9	54.52	24.85	5.05	2.37	3.08	2.43	66.74	17.43	3.57	2.60

Table 4.4 Continued

Sites	Melkassa						Arsi Negelle					
Genotypes	CBB parameters			Agronomic traits			CBB parameters			Agronomic traits		
	SL	SP	AUDPC	PPP	SPP	GY	SL	SP	AUDPC	PPP	SPP	GY
Testers												
SEC12	3.02	2.77	42.22	39.30	5.29	3.71	2.49	1.97	41.00	20.86	4.86	2.16
SEC20	2.98	2.76	42.74	31.90	5.94	3.68	2.79	2.20	53.05	20.33	5.17	2.16
SEC21	2.85	2.77	42.99	27.75	3.94	3.52	3.01	2.38	49.62	20.07	4.39	2.03
SEC22	3.05	2.80	45.34	44.45	6.40	3.80	2.50	1.97	41.07	30.54	4.98	2.78
SEC24	2.80	2.75	43.40	58.95	4.38	3.56	2.86	2.26	54.76	23.41	4.59	2.41
SEC25	3.10	2.84	48.74	60.65	4.27	3.06	2.72	2.14	44.49	26.67	4.75	2.54
SMC21	3.61	2.97	54.77	23.25	4.97	2.65	2.93	2.32	51.33	18.13	4.02	2.75
SMC24	3.59	2.93	52.80	24.85	3.34	3.46	3.15	2.49	65.02	16.72	3.40	1.69
Lines												
Awash1	7.25	3.71	93.30	37.59	4.62	3.02	7.04	5.56	106.09	27.64	4.15	2.15
Mexican142	6.75	3.66	82.20	47.32	5.24	3.43	6.68	5.27	100.96	27.90	4.29	2.35
Nasir	6.31	3.51	82.19	32.57	4.49	3.48	6.60	5.21	83.85	19.27	4.61	2.96
Red Wolaita	6.18	3.50	81.66	32.54	4.41	2.77	6.09	4.81	58.43	25.35	3.94	1.77
Mean	6.62	3.60	84.84	37.51	4.69	3.18	6.60	5.21	87.33	25.04	4.25	2.31
CV (%)	8.34	1.62	4.96	22.51	22.13	25.97	11.39	11.39	13.88	34.22	12.43	16.78
LSD (0.05)	0.51	0.09	4.91	15.83	2.11	1.52	0.70	0.55	16.57	18.28	1.14	0.97
Significance	**	**	**	**	ns	ns	**	**	**	**	*	ns

SL = severity on leaf; SP = severity on pod; AUDPC = area under disease progress curve; PPP=pod per plant; SPP=seed per pod; GY = grain yield; LSD = least significant differences CV = coefficient of variation, ns = non-significant; * significant at P < 0.05; ** significant at P < 0.01

4.3.4 Combining ability analysis

Table 4.4 presents the mean squares and significant tests based on the general combining ability (GCA) and specific combining ability (SCA) effects. The GCA effects of the lines were significant (P < 0.05) for AUDPC and for the number of PPP, while the GCA effects of testers were highly significant (P < 0.05) for all CBB resistance traits and number of PPP. The SCA effects were significant for all CBB parameters (SL, SP, and AUDPC) but non-significant for all agronomic traits.

Table 4.5 Mean squares of female parents, male parents and their interaction (SCA) for 32 F₂ populations and 12 parents of commons beans evaluated at Melkassa and Arsi Negelle sites in Ethiopia, 2018.

Source of variation	DF	CBB scores			Agronomic traits		
		SL	SP	AUDPC	PPP	SPP	GY
Site	1	0.13	3.73**	2937.37**	4546.56**	1.98	4.36*
GCA _{Line}	3	0.46	0.20	351.96*	1190.19**	1.58	0.24
GCA _{Tester}	7	11.25**	2.69**	1375.31**	173.29*	2.98**	0.56
SCA	21	0.31*	0.09*	109.07**	105.54	0.70	0.27
Residual	94	0.87	0.36	128.10	114.48	0.78	0.44

SL = disease severity on leaf; SP = disease severity on pod; AUDPC = area under disease progress curve [what; NPPP = pod per plant; NSPP = seed per pod; grain yield; GCA_{Line} = general combining ability of line; GCA_{Tester} = general combining ability of tester; SCA = specific combining ability; * significant at P<0.05; ** significant at P<0.01

4.3.5 General combining ability (GCA) effects of CBB and agronomic traits

Table 4.5 summarizes the estimates of GCA effects for testers and lines for disease and agronomic traits. For SL, significant and negative GCA effects were observed by testers such as SEC21 (-0.17), SEC20 (-0.09), SEC24 (-0.06), and SEC12 (-0.05). Testers such as SMC24 (0.02), and SMC21 (0.08) showed positive GCA effects for SL which is not desirable. All the test lines showed positive GCA effects for SL, except Nasir (-0.16). Significant and negative GCA effects for SP were observed on SEC21 (-0.08), SEC20 (-0.03), SEC24 (-0.03) and SEC12 (-0.01). The GCA effects of lines were non-significant for SP. Negative GCA effects for AUDPC were observed among the testers such as SEC12 (-3.23), SEC21 (-1.72) and SEC25 (-1.73) making desirable parents for resistance breeding. The testers such as SMC24 (4.37) and (3.27) had positive GCA effects for AUDPC. All the test lines had positive GCA effects for AUDPC except Nasir (-3.41).

Common bean genotypes such as SEC21 (with GCA effect of 3.25), SEC25 (2.73), and SEC20 (2.73) were among the testers with significant and positive GCA values for PPP. Significant and positive GCA effects were observed only in the line Mexican 142 (9.05) for the number of PPP. Significant and positive GCA effects for SPP were observed in all the testers except for SMC21 and SMC24. Lines such as SEC21 (0.30), and SEC12 (0.17) had positive GCA effects for GY. In contrast, testers such as SMC24 (-0.31), SEC25 (-0.13) and SEC20 (-0.09) had negative GCA effects for GY.

Table 4.6 Estimates of general combining ability (GCA) effects of male and female parents for CBB parameters and agronomic traits for 12 common bean parents evaluated at Melkassa and Arsi Negelle sites in Ethiopia

Parents	CBB scores			Agronomic traits		
	SL	SP	AUDPC	PPP	SPP	GY
Male (testers)						
SEC12	-0.05**	-0.01	-3.23**	0.71*	0.14*	0.17*
SEC20	-0.09	-0.03	-0.43	2.73**	0.04	-0.09*
SEC21	-0.17	-0.08*	-1.72*	3.25**	0.19	0.30*
SEC22	0.00*	0.03*	-0.36	-0.04	0.24	0.09
SEC24	-0.06	-0.03	-0.18*	-1.96**	0.35	-0.01
SEC25	0.09*	0.03	-1.73	2.78**	0.01	-0.13*
SMC21	0.08	0.02	3.27**	-3.97**	-0.20*	0.03*
SMC24	0.20**	0.08*	4.37**	-3.48**	-0.77*	-0.31*
S.E.	0.03	0.01	0.62	0.78	0.09	0.05
Female (lines)						
Awash1	0.01*	-0.11	1.60*	-0.70	0.12	0.03
Mexican142	0.06	-0.11	0.73	9.05*	-0.38	0.14
Nasir	-0.16*	-0.23	-3.41	-2.70*	0.02	-0.01
Red Wolaita	0.10*	-0.07	1.08*	-5.64	0.26	-0.13
S.E.	0.03	0.06	0.72	1.59	0.11	0.05

SL = disease severity on leaf; SP = disease severity on pod; AUDPC = Area under disease progress curve; PPP=pod per plant; SPP=seed per pod; GY = grain yield, S.E. standard error; ns = non-significant; * significant at P<0.05; ** significant at P<0.01

4.3.6 Specific combining ability (SCA) effects of families for disease and agronomic traits

Significant SCA effects were recorded among populations for both disease and agronomic traits (Table 4.6). Sixteen F₂ populations (50%) showed negative SCA values for SL. The crosses such as Nasir/SEC24 (with SCA effect of -0.21), Red Wolaita/SMC21 (-0.14), Mexican142/SMC21 (-0.16), Mexican142/SEC25 (-0.14) and Awash1/SEC22 (-0.15) and Awash1/SEC12 (-0.15) were the best specific combiners based on lower CBB severity on leaf. Crosses such as Awash1/SEC25 (with SCA effect of 0.24), Nasir/SEC12 (0.28), and Nasir/SMC21 (0.30) contributed high positive GCA effects for SL and were regarded as poor combiners for resistance. Only two crosses showed negative SCA effects for SP, Nasir/SEC25 (-0.01), and Nasir/SEC24 (-0.02). Fifteen crosses showed negative SCA effects for AUDPC in a desirable direction. Significant and negative SCA effects were noted for families Red Wolaita/SEC12 (-8.42), Nasir/SEC22 (-4.57), Nasir/SEC20 (-4.41), and Mexican142/SMC21 (-4.04). The crosses such as Red Wolaita/SEC20 (8.00) Mexican142/SEC12 (4.46), and Nasir/SEC12 (3.28) showed high positive SCA effects for AUDPC.

Seventeen crosses showed positive SCA effects for number of pod per plant, and Mexican142/SEC22 (8.20 pods per plant), Awash1/SMC24 (4.47), Nasir/SMC24 (4.03), and Mexican142/SEC21 (3.90). These were selected as best combiners for PPP. Mexican142/SMC24 (-8.57), Awash1/SEC22 (-6.04), Nasir/SEC21 (-5.56), and Red Wolaita/SEC20 (-4.22) had negative GCA effects for PPP and were regarded as poor combiners. Awash1/SEC22 (1.07), Awash1/SMC21 (0.43), Nasir/SEC21 (0.41) and Mexican142/SEC20 (0.35) were among best combiners for SPP. Conversely, Awash1/SEC21 (-0.71), Awash1/SEC25 (-0.62), Awash1/SMC24 (-0.45), Nasir/SEC22 (-0.40), and Red Wolaita/SEC22 (-0.40) were poor combiners for SPP due to their negative SCA effects. Sixteen crosses had positive SCA effects for grain yield. Nasir/SEC24 (0.44), Awash1/SEC20 (0.38), Mexican142/SMC21 (0.35) and Red Wolaita/SEC24 (0.23) were the best combiners for GY and selected for further breeding.

Table 4.7 Estimates of specific combining ability (SCA) of 32 F₂ population of common bean evaluated at Melkassa and Arsi Negelle sites in Ethiopia

Cross	CBB scores			Agronomic traits		
	SL	SP	AUDPC	PPP	SPP	GY
Awash1/SEC12	-0.16*	0.04	0.03	0.50	0.08	0.03
Awash1/SEC20	-0.03	0.14	-1.60	0.01	0.10	0.38
Awash1/SEC21	-0.04	0.15	-0.94	2.06	-0.71*	-0.13
Awash1/SEC22	-0.15	0.02*	-1.04	-6.04**	1.07**	-0.01
Awash1/SEC24	0.05	0.15	-0.07	-2.49	0.14	-0.40
Awash1/SEC25	0.24*	0.32*	2.17	3.88	-0.62*	0.19
Awash1/SMC21	0.06	0.09	0.56	-2.43	0.43*	-0.06
Awash1/SMC24	0.03	0.19	2.15	4.47*	-0.45	-0.11
Mexican142/SEC12	-0.05	0.14	4.46**	-1.87	-0.13	-0.22
Mexican142/SEC20	0.13*	0.18	-1.37	1.82	0.35	0.07
Mexican142/SEC21	-0.03	0.13	-0.62	3.90	0.00	-0.02
Mexican142/SEC22	0.03	0.18	1.08	8.20**	-0.26	0.21
Mexican142/SEC24	0.22	0.25	3.19	-0.34	-0.33	-0.32
Mexican142/SEC25	-0.14	0.02	-0.92	-0.57	0.34	-0.30
Mexican142/SMC21	-0.16*	0.14	-4.04*	-2.63	0.05	0.35*
Mexican142/SMC24	-0.01	0.10	-0.53	-8.57**	0.02	0.14
Nasir/SEC12	0.28*	0.19	3.28*	-2.24	0.03	0.02
Nasir/SEC20	-0.06	0.09	-4.41**	2.37	-0.20	-0.21
Nasir/SEC21	0.19*	0.17	1.66	-5.56*	0.41	0.22
Nasir/SEC22	0.05	0.15	-4.57*	-3.53	-0.40	-0.19
Nasir/SEC24	-0.21	-0.02	-0.91	2.24	0.11	0.44*
Nasir/SEC25	-0.08*	-0.01	0.73*	-1.80	0.19	0.16
Nasir/SMC21	0.30*	0.26*	2.27*	1.86	-0.20	-0.41*
Nasir/SMC24	0.08	0.17	-3.08*	4.03*	0.19	-0.17
Red Wolaita/SEC12	0.00	0.08	-8.42*	1.01*	0.15*	0.09
Red Wolaita/SEC20	0.02	0.10	8.00*	-4.22	-0.23	-0.28
Red Wolaita/SEC21	-0.06*	0.07	-1.98	-0.43	0.32	-0.12
Red Wolaita/SEC22	0.13	0.18	2.65*	1.34	-0.40	-0.06
Red Wolaita/SEC24	0.01	0.14	-1.59	0.56	0.10	0.23
Red Wolaita/SEC25	0.05	0.19	-1.36*	-1.53*	0.11	-0.11
Red Wolaita/SMC21	-0.14	0.04	1.85	3.17*	-0.27	0.06
Red Wolaita/SMC24	-0.05*	0.06	2.10	0.05	0.25*	0.09
S.E.	0.02	0.02	0.64	0.78	0.06	0.05

SL = severity on leaf; SP = severity on pod; AUDPC = area under disease progress curve; PPP = pod per plant; SPP = seed per pod; GY = grain yield; S.E. Standard error; * significant at P<0.05; ** significant at P<0.01

4.3.7 Variance components based on disease parameters and agronomic traits

The traits the parents and progenies are summarized in Table 4.7. Higher line variance (0.0089) was obtained for SL followed by tester variance (0.007). The SP and AUDPC site variances were 0.1 and 48.7, respectively that contributed higher proportions followed by tester variances. Line

variance was high for PPP (38.3), while high variance was obtained from both line and tester (0.005). The SCA variance (0.02) was high for grain yield. Moderate to high narrow-sense heritability ($h^2=0.66$), and broad-sense heritability ($H^2=0.71$) estimates were computed for SL. The heritability of SP and AUDPC were relatively low. For GY, the h^2 estimates were low (0.22) and moderately high (0.60) for SPP. The H^2 values were moderately high and ranged between 0.55 (for PPP and GY) to 0.70 (for SPP). The Baker's ratios were closer to unity for SL (0.93), SP (0.83), SPP (0.79), NPPP (0.90) and SPP (0.85), and less than unity for AUDPC (0.41) and GY (0.40) suggesting the predominance of additive genes conditioning the inheritance of the agronomic and disease resistance traits. All were assessed traits and hybrid performance can be predicted based on the parents GCA effects.

Table 4.8 Variance components, narrow sense and broad sense heritability for CBB parameters and agronomic traits among 32 F₂ of common bean evaluated at Melkassa and Arsi Negelle sites in Ethiopia

Variance components	CBB parameters			Agronomic traits		
	SL	SP	AUDPC	PPP	SPP	GY
σ^2_{Site}	0.0014	0.1045	48.6636	32.7264	0.0017	0.0047
σ^2_{Lines}	0.0089	0.0036	5.1355	38.2874	0.0517	0.0033
σ^2_{Tester}	0.0070	0.0003	1.1166	3.6497	0.0517	0.0100
σ^2_{SCA}	0.0013	0.0008	8.9344	4.3678	0.0182	0.0204
σ^2_{Error}	0.0055	0.0025	3.1270	5.4775	0.0505	0.0230
h^2	0.6612	0.0355	0.0933	0.4962	0.5951	0.2173
H^2	0.7143	0.0428	0.2267	0.5479	0.6998	0.5495
BR	0.9257	0.8291	0.4117	0.9057	0.8503	0.3955

σ^2_{site} = variance due to site effect; σ^2_{Lines} = variance due to line; σ^2_{Tester} = variance due to tester; σ^2_{SCA} = variance due to cross; σ^2_{Error} = error variance ; h^2 = narrow sense heritability; H^2 = broad sense heritability; BR = Baker's ratio

4.4 Discussion

Development of common bean cultivars with CBB resistance, and good agronomic traits will improve common bean production and productivity in CBB hotspot areas in Ethiopia. In the current study, significant differences were observed among genotypes (32 F₂ and 12 parents) based on CBB resistance scores (SL, SP, and AUDPC) and agronomic traits (PPP and SPP) at two testing sites, suggesting the presence of considerable genetic variation for selection (Table

4.2). The difference among the common bean genotypes in leaf and pod resistance across the two testing sites could be attributed to the inherent genotype difference and favorable environmental conditions for CBB infection and disease development (Singh and Munoz, 1999; Mutlu et al., 2005). Genotypic differences exist for CBB resistance which will be the basis for CBB resistance breeding (Singh and Munoz, 1999; Duncan et al., 2011). Recently, considerable variation in CBB resistance was reported among common bean breeding lines in Brazil (Melo et al., 2019) and Ethiopia (Tumsa et al., 2020).

The GCA effects of the donor parents (testers) were highly significant for all CBB resistance traits. This suggests that CBB resistance genes' would be successfully integrated into the susceptible lines. This finding agreed with a study conducted in Uganda, that reported GCA effects were significant for leaf and pod infections (Alladassi et al., 2017). In the present study, the SCA effects for all disease resistance traits were significant but not for all agronomic traits. Alladassi et al., (2017) reported non-significant SCA effects for leaf and pod resistance. The preponderance of the GCA effects for CBB resistance traits implied the importance of additive gene action (Tryphon et al., 2012) suggesting the possibility of accumulating minor genes from desirable parents through recurrent selection method (Rodrigues et al., 1999). In disease resistance breeding, negative GCA and SCA effects are desirable (Bokmeyer et al., 2009; Mukankusi et al., 2011). In the current study, donor parents such as SEC21, SEC20, SEC24, and SEC12 showed negative GCA effects for SL, showing their contribution in reducing CBB infection on the leaf. GCA effects of donor parents such as SEC12, SEC21, and SEC25 were negative for AUDPC, confirming higher leaf resistance. The GCA effects of testers were significantly different for all agronomic traits, while those of lines were significant only for PPP (Table 4.5). The significant and positive GCA and SCA effects of testers observed in the study for PPP and SPP agreed with Atnaf et al. (2013). However, the current findings contradict those of Nienhuis and Singh (1988) who reported zero or negative GCA values for yield and its components. In this study, the GCA effect of testers was more important than the SCA effect for all agronomic traits. In contrast, Foolad and Bassiri (1983) reported that the SCA effect was more important than GCA effect for GY, PPP, and SPP in common bean.

The magnitude of negative SCA effects for disease resistance traits imply that the new families hinder CBB infection and disease development (Jeger and Viljanen-Rollinson, 2001) which was validated in several families (Trindade et al., 2015). In this study, the narrow sense (h^2) and broad

sense (H^2) heritability estimates were 0.66 and 0.71 for SL, respectively (Table 4.7). Medium to high heritability estimates have been reported for CBB resistance traits (Tar'an et al., 2001; Singh and Miklas, 2015). However, varied heritability estimates have been reported based on CBB leaf severity reaction in common bean (Arnaud-Santana et al., 1994; Ariyaratne et al., 1999). The low heritability value for SP in this study agrees with that of Aggour and Coyne (1989). Alladassi et al. (2017) reported moderately high h^2 value of 0.65 based on leaf severity and high value of 0.83 for pod severity of the CBB. **High heritability values imply the presence of higher phenotypic variation for breeding.**

Narrow sense heritability (h^2) estimates ranged from moderately low (0.22) to moderately high (0.60) for GY and SPP, respectively. H^2 was moderately high for PPP, GY, and SPP (Table 4.7). These findings agree with previous reports of Yohannes et al. (2020) who reported high H^2 for grain yield and its component traits. Ghimire and Mandal, (2019) reported high H^2 for PPP (0.93), SPP (0.96), and GY (0.84). Hence, for traits with high heritability values directed selection can be applied in varietal development. The Baker's ratio values for SL, SP, and AUDPC were 0.93, 0.83, and 0.41, respectively (Table 4.7). **This suggests that the hybrid performance can be predicted based on the parents GCA effects (Alladassi et al., 2017).**

4.5 Conclusions

Significant differences were observed among parents and progenies for CBB resistance and agronomic traits at both locations. The GCA effects of testers were highly significant for all CBB resistance traits, confirming their utility as donor parents and the many of additive gene action in conditioning CBB resistance in common bean. The SCA effects of families were significant for all CBB resistance traits allowing selection of unique crosses based on low leaf and pod severity for CBB resistance breeding. Parents such as SEC12, SEC21, SEC20, SEC24, and SEC25 had negative and significant general combining ability effects for CBB severity based on leaf and pod infection. The F_2 generations such as Nasir/SEC24, Red Wolaita/SMC21, Mexican142/SMC21, Mexican142/SEC25, Awash1/SEC22, Red Wolaita/SEC12, Nasir/SEC22, Nasir/SEC20, and Awash1/SEC12 exhibited low and negative SCA values and selected for further CBB resistance breeding. These families displayed better agronomic attributes and significant and negative specific combining ability effect for SL and AUDPC. The selected parents and families are useful genetic resources for selection of CBB resistant and agronomically superior common bean varieties.

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CHAPTER FIVE: INTROGRESSION OF COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) AIDED BY MARKER-ASSISTED SELECTION

Abstract

Common bean (*Phaseolus vulgaris* L., $2n = 2x = 22$) is one of the major food and cash crops globally. However, the productivity of the crop is low mainly due to common bacterial blight (CBB) disease incited by the *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and its variant *X. axonopodis* pv. *phaseoli* var. *fuscans* (*Xaf*). Host resistance is among the most economic and effective strategies to minimize yield losses caused by the CBB, hence, it is essential to introgress CBB resistance genes and quantitative trait loci (QTL) into susceptible genotypes from identified sources. The aim of this study was to introgress and track CBB resistance genes/QTL in selected commercial common bean genotypes through marker-assisted selection for cultivar development. Breeding developed from 16 crosses were field phenotyped at two CBB hotspot sites (Melkassa and Arsi Negelle) at F₂ generation stage in Ethiopia and genotyped using three selected and diagnostic single nucleotides polymorphism (SNP) markers (CBB_SAP6_801, CBB_06_TC_9138316 and CBB_SU91_g91004686) at Intertek in Sweden. The F₂ generations and parents involved in each cross were evaluated at both sites and data were collected on CBB severity on leaf (SL) and severity on pod (SP). Significant ($P < 0.001$) variations were recorded among test populations for CBB infection. Segregation analyses of crosses for SL and SP at the F₂ indicated a genetic ratio of 1:3:1 involving resistant: moderately resistant: susceptible individuals, respectively and suggesting that CBB resistance was conditioned by multiple genes. Significant genotype variation was observed when subjected to SNP analyses with 23% of the total variation was attributed to variation among the assessed populations. The SNP markers explained 22% (marker CBB_SAP6_801) and 87% (CBB_06_TC_9138316) of the total variations present in the test populations making them a marker of choice for future genetic analysis for CBB resistance. There existed significant ($P < 0.001$) negative correlation between phenotypic traits and the SNP markers ranging between -0.06 to -0.078. The two markers can be useful for developing breeding populations and for marker-assisted selection in common bean.

Key words: Quantitative trait loci, single nucleotide polymorphism, *Xanthomonas axonopodis* pv. *Phaseoli*

5.1 Introduction

Common bean (*Phaseolus vulgaris* L., $2n = 2x = 22$) is one of the major food and cash crops globally including in Ethiopia. Worldwide, it is cultivated across 30 million hectares per annum. About 7.6 million hectares is devoted to common bean production in Africa (Buruchara et al., 2011; FAOSTAT, 2014). Common bean is the major food staple to more than 100 million people in sub-Saharan Africa. It is rich in starch, protein, fiber, minerals, vitamins and folate contents (Mukankusi et al., 2019). Rwanda, Kenya and Uganda, with per capita consumption of 40–60 kg per person per year, are the leading consumers of common bean in the world (Broughton et al., 2003; Blair et al., 2013). Ethiopia is ranked 10th in the world and 4th in Africa with a total annual production of 520,979 tonnes (FAOSTAT, 2017). Approximately 3.4 million smallholder farmers produce common bean in Ethiopia for household consumption and income (CSA, 2018). However, the mean productivity of the crop under small-scale farmer condition is low (about 1.7 ton ha⁻¹) (Muthoni et al., 2017). The low productivity of the crop is attributed to different biotic and abiotic constraints among which diseases are the major impediments.

Common bacterial blight (CBB) disease of common bean incited by the *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and its variant *X. axonopodis* pv. *phaseoli* var. *fuscans* (*Xaf*) is one of the major production constraints in most production regions worldwide (Tar'lan et al., 2001; Perry and Pauls, 2012; Perry and Peter, 2016). The disease is cosmopolitan in occurrence and distribution leading to a significant yield loss (Perry and Pauls, 2012; Viteri et al., 2014). Yield losses vary depending on cultivar susceptibility, environmental condition, crop growth stage, among others. CBB causes up to 40% yield loss in Ethiopia (Fininsa and Tefera, 2001). Different disease management strategies, such as use of disease-free seed, bactericidal chemicals, crop rotation, and crop residue managements are recommended to reduced CBB epidemics and consequential yield losses. However, these strategies do not lead complete disease control and are not economical (Fininsa, 1996; Singh and Munoz, 1999; Coyne et al., 2003). Use of genetic resistance is among the most economic and effective strategies to minimize yield losses caused by CBB in common bean (O'Boyle et al., 2007; Chataika et al., 2011; Durham et al., 2013; Meziadi et al., 2015). Widely produced commercial cultivars such as Awash1, Mexican142, Nasir and Red Wolaita have increasingly become susceptible and succumbed to both to leaf and pod CBB infection in Ethiopia (Tumsa et al., 2020). Hence improving CBB resistance of commercial

cultivars and elite breeding lines could significantly benefit the common bean industry (O'Boyle et al., 2007)

Developing common bean genotypes with improved CBB resistance has been one of the main objectives in common bean breeding programs (Singh et al., 2001). Introgression of economically important traits, including CBB resistance, from genetically related species such as from tepary bean (*Phaseolus acutifolius* L.) and the scarlet runner bean (*P. coccineus* L.) into common bean were successfully achieved (Beaver and Osorno, 2009). Screening of large number of test genotypes and breeding populations under greenhouse or field conditions enables selection of individuals with desirable traits. However, phenotypic selection requires technical skill, material and financial resources (Witcombe and Virk, 2001). Use of molecular markers could significantly complement the competency and efficiency of plant breeding programs when combined with phenotypic selections (Gupta et al., 2010).

The inheritance of CBB resistance based on leaf and pod severity parameters is reportedly conditioned by few to several genes (Tar'an et al., 2001; Singh and Schwartz, 2010; Tryphone et al., 2013). Expression to CBB resistance is dependent on the genetic background of the source of resistance, environmental condition, disease pressure, crop growth stage and part of the plant infected, conditions that make CBB resistance breeding complex (Kelly et al., 2003; Santos et al., 2003; Singh and Schwartz, 2010; Durham et al., 2013). Thus far at least 24 quantitative trait loci (QTL) across all 11 the linkage groups has been reported adding to the complexity of gene tagging and tracking for resistance breeding against the CBB (Singh and Schwartz, 2010). Hence, use of phenotypic traits and diagnostic molecular markers can aid introgression and tracking of CBB resistance genes and QTL into susceptible genotypes.

Molecular markers are widely used in disease resistance breeding programs for genetic analysis and to fast track and pyramid candidate genes, among others (Kelly and Miklas, 1998; Miklas et al., 2006a; Mukankusi et al., 2019). The most commonly used marker systems in CBB resistance breeding include sequence characterized amplified region (SCAR), simple sequence repeats (SSR), and single nucleotides polymorphism (SNP) markers. SSR marker such as BC420 is reportedly associated with the linkage group (LG) B6 (Yu et al., 2000), while marker SU91 was linked to LG B8, and SAP6 on LG B10, all conferring CBB resistance in common bean (Miklas et al., 2000; Yu et al., 2004). Common bean lines possessing high level of CBB resistance have

been developed through phenotypic and marker-assisted selection. These included USDKCBB-15 (Miklas et al., 2006b), USWK-CBB-17 (Miklas et al., 2006c), USCR-CBB-20 (Miklas et al., 2011) and ABC-WeiHING (Mutlu et al., 2008).

Recently the SCAR and SRR markers were converted to single nucleotide polymorphism (SNP) markers. This allowed genotyping in gel-free systems through various service providers (Mukankusi et al., 2019). The SNP markers are widely used to genotype common bean populations for CBB resistance (Song et al., 2015). SNP markers are valuable for genotyping because of their abundance, stability and simplicity for genotyping (Shi et al., 2011). The SNP markers are codominant markers, and can distinguish homozygous and heterozygous individuals as early as the F₂ generation. Therefore, utilization of the known markers associated with disease resistance QTLs in a MAS strategy can improve the efficiency of CBB resistance breeding program in common bean. Therefore, the objective of this study was to introgress and track CBB resistance genes/QTL in selected commercial common bean genotypes through marker-assisted selection for cultivar development.

5.2 Materials and Methods

5.2.1 Description of the study sites

The study evaluated 16 selected F₂ populations and their eight parents at two testing sites namely Melkassa and Arsi Negelle during 2019 cropping season. The two sites belong to the Ethiopian Institute of Agricultural Research (EIAR) and are mostly used for screening common bean for the major diseases prevalent in the country. Melkassa is located in the semi-arid region of the Central Rift Valley at 8° 24' N latitude and 39° 12' E longitude. The center is situated at an altitude of 1550 m above sea level (masl). It receives a mean annual rainfall of 763 mm with daily minimum and maximum temperatures of 14 °C and 28 °C, respectively. [The main planting season at the testing sites starts between late June and early July based on the onset of rainfall. The distribution of the rain across testing sites was uniform throughout the cropping season.](#) Arsi Negelle site is situated at a latitude of 7° 25'N and longitude of 38° 31'E with an altitude of 1900 masl. The soil at the Melkassa and Arsi Negelle sites, respectively. Both sites are hotspot areas for CBB, and hence natural disease infection and development were used for genotype evaluation.

5.2.2 Field evaluation of the populations

Out of 32 crosses developed in this study (Chapter Four, Table 4.3), only 16 crosses were selected for the present study due to the overlapping pedigree of parents with known CBB resistance genes. The crosses used in the study included Awash1/SEC12, Awash1/SEC24, Awash1/SMC21, Awash1/SMC24, Mexican142/SEC12, Mexican142/SEC24, Mexican142/SMC21, Mexican142/SMC21, Nasir/SEC12, Nasir/SEC24, Nasir/SMC21, Nasir/SMC24, Red Wolaita/SEC12, Red Wolaita/SEC24, Red Wolaita/SMC21 and Red Wolaita/SMC24. [The seed from F₂ generations and their parents were grown in single row with a length of 10m and with a spacing between rows was 0.8m.](#) Each row had 100 plants. Seventy-five individual F₂ plants were randomly selected and tagged for disease assessment. CBB severity on leaf and pod was recorded three times at seven days interval starting at stage 6 (R6) during the reproductive phase when visible symptoms of CBB appeared on the leaf and pod. Each 75 plants per row were evaluated for CBB severity on leaf (SL) and severity on pod (SP) and the mean values of all assessment times were calculated. CBB severity assessment was scored based on a scale of 1 to 9, where 1 = 0% of the leaves or pods show infection symptoms, 2 = 2% of the leaves or pods show infection symptoms, 3 = 5%, 4 = 10%, 5 = 20%, 6 = 25%, 7 = 50%, 8 = 75% and 9 > 85% (CIAT, 1987; Opio et al., 1996). The severity scores were used to group the study genotypes into three categories: resistant (for genotypes with scores of 1 to 3), moderately resistant (4 to 6) and susceptible (7 to 9).

5.2.3 Genotypic screening for CBB resistance

5.2.3.1 Leaf sample preparation and genotyping

The remnant seed of the 16 F₂ populations were grown in the green house. Seventy-five F₂ plants in each population were randomly tagged used for genotyping. Genotyping data was used to establish the association with the phenotypic scores. Leaf samples from F₂ plants and parents that made a total of 1224 samples were collected at first trifoliate stages, dried and packed. Samples were prepared in 96-format plates leaf sampling kit based on the sampling instructions for SNP genotyping. Sampled leaves were dried in an oven at 45 °C for 12-24 hours before shipment. In general, 2 leaf disks of 5 to 6 mm diameter, to providing good quality and quantity DNA, were punched and shipped to SNP genotyping. Samples were genotyped based on single-locus genotyping procedure for common bean at Intertek Group PLC (Sweden) through the International Center for Tropical Agriculture (CIAT), Uganda Regional Office. Intertek Group PLC provides cost-effective genotyping service for breeders mainly in the Consultative Group of

International Agricultural Research (CGIAR) system (Mukankusi et al., 2019). All the samples were screened with three selected and diagnostic SNP markers namely CBB_06_TC_9138316, CBB_SAP6_801 and CBB_SU91_g91004686. The three markers were selected because of their high stability in terms of identifying resistant QTL in different association studies.

5.3.4 Data analysis

5.3.4.1 Phenotypic data analysis

The Chi-square test was used to determine the mode of inheritance for resistance to CBB among the F₂ plants using the Statistical Package for Social Science (SPSS) version 24 (SPSS , 2017). The results presented in Chapter Four indicated that the CBB resistance from all the donor parents were inherited quantitatively, hence the progenies are heterogeneous. Therefore, progenies from each cross segregated differently although the parents were considered to be homozygous for the genes controlling resistance to CBB. The sample plants from the 16 F₂ generations were tested for disease resistance traits ratios of 1:3:1. The PROC CORR function of SAS (SAS, 2014) was used to analyze the correlation between phenotypic traits and markers and Pearson correlation (r) values were used to test the magnitude of the relationship.

5.2.4.2 Genotypic data analysis

Allele frequency, heterozygosity and the fixation index were calculated as follows based on Hartl and Jones (1997):

$$H_o = \frac{\text{Number of heterozygosity}}{N}$$

Where H_o is the observed heterozygosity, i.e. the proportion of N samples that are heterozygous at a given locus.

$$H_e = 1 - \sum P_i^2$$

Where H_e is the expected heterozygosity, i.e. the proportion of heterozygosity expected under random mating, and P_i is the allele frequency of the i^{th} allele

$$F = \frac{H_e - H_o}{H_e}$$

The analysis of molecular variance (AMOVA) was conducted following (Peakall et al., 1995) to allocate genetic variations among and within populations using GenALEX 6.5. F-statistics (Fst) of AMOVA was used to test the magnitude of genetic variation among and within populations (Meirmans, 2006). To determine whether the observed value is significantly greater than expected, the observed values of the Fst were tested against the outcomes of the permutations at 5% level of significance. If the observed values are greater than the computed, then it was declared that the results were significant.

5.3 Results

5.3.1 Inheritance of CBB resistance

Significant variations in CBB resistance among the F₂ generations were observed in Chapter Four, Table 4.2. In this Chapter only a Chi square test was conducted to test the ratio of the number of plants sampled from each cross in their reaction to leaf and pod infection. Table 5.1 summarizes the frequencies of progenies from 16 crosses showing variable reactions to leaf and pod infection at Melkassa and Arsi Negelle testing sites. The Chi square test revealed variation ($P < 0.001$) among populations for their reaction to CBB on leaf and pod at both sites.

The F₂ progenies of all 16 crosses segregated into expected ratio of 1:3:1 ($X^2 = 96.65$; $P < 0.001$) for resistant: moderately resistant: susceptible individuals, in that order for SL at the Melkassa site except for Red Wolaita/SMC21 that showed a segregation ratio of 3:2:1. Different segregation ratios were computed for severity on pod (SP) at the Melkassa site. For instance, progenies from three crosses segregated into ratio of 3:2:1 with resistant: moderately resistant: susceptible individuals, in that order, progenies from five crosses segregated into a ratio of 2:3:1, whereas progenies from the remaining eight crosses segregated into a ratio of 1:3:1 ($X^2 = 104.09$; $P < 0.001$). The segregation ratios for SL and SP among the F₂ progenies at the Arsi Negelle site were different from that of the Melkassa site. Progenies from five families segregated into a ratio of 1:3:1 ($X^2 = 83.22$; $P < 0.001$) for SL at the Melkassa site. Progenies from two crosses segregated into a ratio of 1:3:1 ($X^2 = 89.83$; $P < 0.001$) for SL and the remaining had variable segregation patterns.

Table 5.1 Proportion of F₂ plants from 16 crosses and 8 parents based on their CBB reaction on leaf and pod evaluated at two testing sites in Ethiopia.

Populations	Melkassa								Arsi Negelle							
	SL				SP				SL				SP			
	R	MR	S	Ratio	R	MR	S	Ratio	R	MR	S	Ratio	R	MR	S	Ratio
Crosses																
Awash1/SEC12	15	36	24	1:3:2	36	27	12	3:2:1	18	49	8	2:3:1	22	39	13	2:3:1
Awash1/SEC24	15	43	17	1:3:1	32	31	12	2:2:1	33	23	19	3:2:2	33	25	17	3:2:1
Awash1/SMC21	18	40	17	1:3:1	21	44	10	2:4:1	14	46	15	1:4:1	19	41	15	2:3:1
Awash1/SMC24	10	51	14	1:4:1	18	47	10	2:4:1	21	43	11	2:3:1	21	45	9	2:3:1
Mexican142/SEC12	13	47	15	1:3:1	32	34	9	2:2:1	33	28	14	2:2:1	33	28	14	2:2:1
Mexican142/SEC24	16	44	15	1:3:1	21	40	16	2:4:2	27	34	14	2:2:1	27	25	22	2:3:2
Mexican142/SMC21	18	43	14	1:3:1	15	45	15	1:3:1	23	38	14	2:3:1	30	30	15	2:2:1
Mexican142/SMC21	13	49	13	1:4:2	14	49	12	1:3:1	32	29	14	2:2:1	32	30	13	2:2:1
Nasir/SEC12	20	45	10	2:3:1	20	35	20	1:2:1	36	29	10	3:2:1	27	38	10	3:2:1
Nasir/SEC24	19	49	7	2:4:1	15	53	7	1:4:1	27	40	8	2:3:1	25	40	10	3:2:1
Nasir/SMC21	12	47	16	1:4:2	20	39	16	2:3:2	18	41	16	2:4:2	18	45	12	3:2:1
Nasir/SMC24	16	51	8	2:4:1	13	51	11	1:4:1	19	43	13	2:3:1	24	42	9	3:2:1
Red Wolaita/SEC12	14	52	9	1:3:2	11	55	9	1:4:1	21	39	15	2:3:1	22	37	16	2:3:1
Red Wolaita/SEC24	16	56	3	2:4:1	21	44	10	2:3:1	28	36	11	2:3:1	36	28	11	3:2:1
Red Wolaita/SMC21	38	27	10	3:2:1	18	47	10	2:3:1	20	39	16	2:3:2	27	39	9	2:3:1
Red Wolaita/SMC24	22	34	19	2:3:2	18	43	14	2:3:1	21	35	19	2:3:2	25	30	20	2:2:1
Parents																
SEC12	75	-	-	-	75	-	-	-	75	-	-	-	75	-	-	-
SEC24	75	-	-	-	75	-	-	-	75	-	-	-	75	-	-	-
SMC21	75	-	-	-	75	-	-	-	75	-	-	-	75	-	-	-
SMC24	75	-	-	-	75	-	-	-	75	-	-	-	75	-	-	-
Nasir	-	-	75	-	-	-	75	-	-	-	75	-	-	-	75	-
Red Wolaita	-	-	75	-	-	-	75	-	-	-	75	-	-	-	75	-
Awash1	-	-	75	-	-	-	75	-	-	-	75	-	-	-	75	-
Mexican142	-	-	75	-	-	-	75	-	-	-	75	-	-	-	75	-
Chi square test	$X^2 = 96.65$ P < 0.001				$X^2 = 104.09$ P < 0.001				$X^2 = 83.22$ P < 0.001				$X^2 = 89.83$ P < 0.001			

CBB= common bacterial blight ; SL = Severity on leaf; SP = Severity on pod; R = Resistant; MR = Moderately resistant; S = Susceptible and X^2 = Chi square

5.3.2 Genotypic evaluation

5.3.2.1 SNP analysis, heterozygosity and fixation indices

Among the three SNPs used for genotyping, markers CBB_06_TC_9138316 and CBB_SAP6_801 were polymorphic, while marker CBB_SU91_g91004686 was monomorphic and removed from the analysis. The marker CBB_SAP6_801 called 96.98% of the samples with a 3.02% missing value, while CBB_06_TC_9138316 successfully called 97.14% of the tested samples with a 2.86% missing value. Out of the total tested populations, 667 (54.50 %) showed positive, 352 (28.64%) homozygosity and 170 (13.89%) heterozygosity values based on the SNP

marker CBB_SAP6_801. But 928 samples (75.81%) showed positive response, 141 (11.47%) negative response and 120 (9.80%) heterozygosity based on the marker CBB_06_TC_9138316.

Awash1/SEC12, Awash1/SEC24, Mexican142/SEC12 and Red Wolaita/SEC12 showed lower leaf and pod infection levels at both sites which were confirmed by the presence of both markers. Similar to the donor parents, some populations showed 100.00% positive response to either of the two markers. Awash1/SMC21 was 100.00% positive to the markers CBB_SAP6_801 and CBB_06_TC_9138316 identified CBB resistance present in 7 crosses. Two donor parents (SEC12 and SEC24) were positive to the two markers, but SMC21 and SMC24 were positive only to CBB_06_TC_9138316. The three recipient commercial parents (Awash1, Mexican142, Nasir and Red Wolaita) were negative and undetected via the markers CBB_SAP6_801 and CBB_06_TC_9138316.

Significant genetic differences were detected among the test populations based on markers CBB_06_TC_9138316 and CBB_06_TC_9138316 (Table 5.2). The observed heterozygosity (H_o) values were lower (0.02-0.276), compared to expected heterozygosity (H_e) (0.040-0.489). Higher heterozygosity values were recorded for the cross Red Wolaita/SMC24, with H_o of 0.267 followed by Red Wolaita/SMC21 with a value of 0.264. The genetic diversity (H_e) of the two crosses were 0.489 and 0.444, respectively. The fixation indices (F) ranged between 0.408 and 0.799.

The SNP marker CBB_SAP6_801 was polymorphic across all populations except Awash1/SMC21, while marker CBB_06_TC_9138316 was polymorphic across seven populations only (Table 5.2). The H_o value ranged between 0.05 to 0.30 and H_e varied between 0.03 to 0.50 when assayed with CBB_SAP6_801. Lower H_o value (0.05) was recorded from Awash1/SEC24 and higher H_o was noted from Red Wolaita x SMC21 (0.30) followed by Nasir/SEC24 (0.25) when assayed with CBB_SAP6_801. Higher genetic diversity (0.50) was detected in Red Wolaita/SEC24 when analyzed with CBB_SAP6_801. The mean H_o was the highest in Red Wolaita/SMC24 (0.36) and the lowest in Mexican142/SMC21 (0.11). The two crosses had mean diversity values of 0.05 and 0.04, in that order when tested by the marker CBB_06_TC_9138316. The two parental lines which are known sources of CBB resistance (SMC21 and SMC24) possessed the marker CBB_SAP6_801 but the other two resistant parental lines (SEC12 and SEC24) possessed both SNP markers (Raatz et al., 2019). The recipient parents,

which had moderate susceptibility to leaf and pod infection at both sites were not revealed by the two markers except Mexican142 that was diagnosed with CBB_SAP6_801.

Table 5.2 Frequencies of favorable alleles (%), observed heterozygosity, expected heterozygosity and fixation index of 16 F₂ common bean populations based on SNP markers

Population	CBB_SAP6_801				CBB_06_TC_9138316				Total			
	AF	Ho	He	F	AF	Ho	He	F	Ho	He	F	
Awash1/SEC12	92.57	0.04	0.14	0.71	100.00	0.00	0.00	Na	0.02	0.07	0.71	
Awash1/SEC24	97.30	0.05	0.05	-0.03	98.65	0.00	0.03	1.00	0.03	0.04	0.49	
Awash1/SMC21	100.00	0.00	0.00	Na	69.33	0.16	0.43	0.62	0.08	0.21	0.62	
Awash1/SMC24	93.84	0.04	0.12	0.65	46.62	0.34	0.50	0.32	0.19	0.31	0.48	
Mexican142/SEC12	63.04	0.25	0.47	0.47	100.00	0.00	0.00	Na	0.06	0.25	0.75	
Mexican142/SEC24	56.08	0.12	0.49	0.75	100.00	0.00	0.00	Na	0.12	0.23	0.47	
Mexican142/SMC21	25.74	0.16	0.38	0.58	85.51	0.06	0.25	0.77	0.11	0.32	0.67	
Mexican142/SMC21	30.28	0.18	0.42	0.57	72.60	0.11	0.40	0.73	0.15	0.41	0.65	
Nasir/SEC12	63.33	0.09	0.46	0.80	100.00	0.00	0.00	Na	0.05	0.23	0.80	
Nasir/SEC24	47.33	0.25	0.50	0.49	100.00	0.00	0.00	Na	0.13	0.25	0.49	
Nasir/SMC21	59.23	0.17	0.48	0.65	71.32	0.13	0.41	0.68	0.15	0.45	0.66	
Nasir/SMC24	59.46	0.11	0.48	0.78	56.08	0.26	0.49	0.48	0.18	0.49	0.63	
Red Wolaita/SEC12	71.23	0.14	0.41	0.67	100.00	0.00	0.00	Na	0.07	0.21	0.67	
Red Wolaita/SEC24	54.11	0.21	0.50	0.59	100.00	0.00	0.00	Na	0.10	0.25	0.59	
Red Wolaita/SMC21	58.11	0.30	0.49	0.39	72.30	0.23	0.40	0.43	0.26	0.44	0.41	
Red Wolaita/SMC24	39.71	0.18	0.48	0.63	52.24	0.36	0.50	0.28	0.27	0.49	0.46	

Ho= Observed heterozygosity; He = Expected heterozygosity; F = Fixation index; Na = Not available

5.3.2.2 Analysis of molecular variance

Analysis of molecular variance partitioned the total genetic variation to different groups (Table 5.3). The AMOVA revealed the presence of significant variation ($F_{st} > 0$) for total genetic variance. Hierarchical partitioning of total genetic variation among populations indicated that the two SNP markers attributed to the presence of a total genetic variation of 23% among populations, 45% among individuals and 32% within individuals (Figure 5.1A). The SNP marker SAP6_801 explained the variations of 22%, 48% and 32% among populations, among individuals and within individuals, respectively (Figure 5.1B). The SNP marker CBB_06_TC_9138316 attributed to a variation of 87% (among population), 7% (among individual samples) and 6% (within individual samples) (Figure 5.1C).

Table 5.3 Mean squares and significance level of total variances, variances due to SNP markers CBB-SAP6_801 and CBB_06_TC_9138316 among populations, among individuals and within individual genotype

Source of variations	DF	MS of CBB_SAP6_801	MS of CBB_06_TC_9138316	MS Total
Among populations	23	5.203	3.91	9.114
Among individuals	1165	0.296	0.160	0.456
Within individuals	1188	0.069	0.050	0.119
F-Statistics				
Fst		0.236	0.217	0.870

DF = Degree of freedom; MS = Mean square; Fst = F-statistics ; Indicate significance and probability level

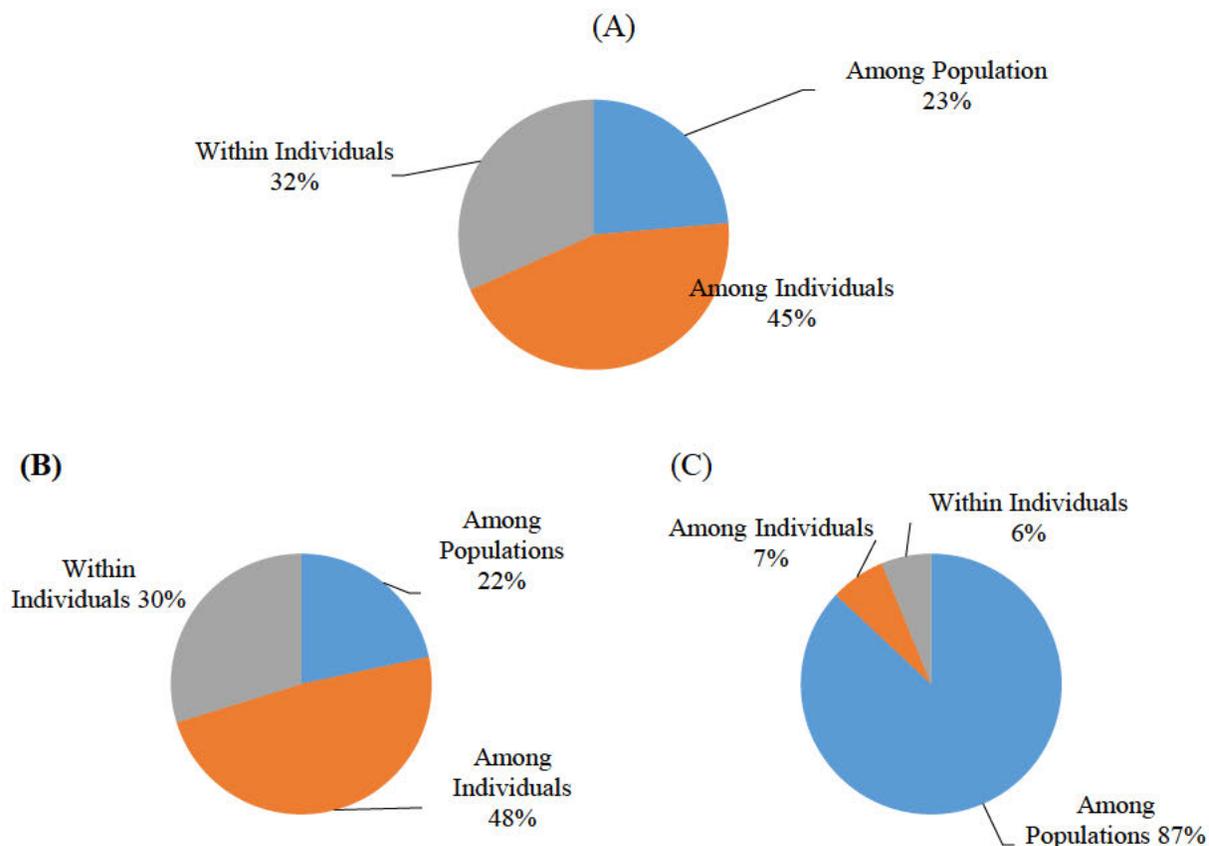


Figure 5.1 Percentages of total molecular variance (A), due to marker CBB_SAP6_801 (B) and due to marker CBB_06_TC_9138316(C) among populations, among individuals and within individual samples tested

5.3.3 Correlation between CBB resistance traits and SNP markers

Table 5.4 presented the correlation coefficient (r) between phenotypic reaction to CBB and SNP outputs. Low but significant correlations were recorded between severity on leaf (SL) and severity on pod (SP) ($P < 0.05$) with the marker CBB_SAP6_801 at the Melkassa and Arsi Negelle sites. Non-significant correlations were detected between marker CBB_06_TC9138316 and CBB resistance traits at both sites. Marker CBB_SAP6_801 negatively correlated with SL ($r = -0.074$) and SP ($r = -0.060$) at Melkassa. At the Arsi Negelle site, negative correlations were observed between marker CBB_SAP6_801 and SL ($r = -0.068$) and SP ($r = -0.067$).

Table 5.4 Summary phenotypic correlation coefficients between SNP markers and leaf and pod reaction to CBB infection at Melkassa and Arsi Negelle sites in Ethiopia.

SNP markers	Melkassa		Arsi Negelle	
	SL	SP	SL	SP
CBB_SAP6_801	-0.074**	-0.060*	-0.068*	-0.067*
CBB_06_TC9138316	0.009	-0.013	0.033	0.032

SL= Severity on leaf; SP = Severity on pod; ** = Significant at $P < 0.01$; * = Significant at $P < 0.05$; SNP= Single nucleotide polymorphism

5.3.4 Marker-assisted selection (MAS) for CBB resistance

Table 5.5 summarized the number of plants in each cross that showed positive response to the SNP markers, CBB_SAP6_801 and CBB_06_TC_9138316 and mean CBB severity on leaf and on pod assessed at the Melkassa and Arsi Negelle sites. Significant variation ($P < 0.001$) was observed between populations for CBB resistance at both sites. About 25.74% (18 plants out of 68) was a minimum frequency that showed positive response to CBB_SAP6_801. But all tested sample plants showed positive response to CBB_SAP6_801, and individuals from crosses such as Awash1/SEC24 and Awash1/SMC24 responded 97.3 and 93.8%, respectively to the same marker. The proportions of plants that showed positive response to CBB_06_TC9138316 ranged from 46.62% (Awash1/SMC24) and 100.00% (Awash1/SMC21). Families with higher number of individual plants that showed positive response to both SNP markers included Awash1/SEC12, Awash1/SEC24, Mexican142/SEC12 and Red Wolaita/SEC12. The frequencies of plants in these populations ranged between 71.23 to 100.00% for CBB_SAP6_801 and from 69.33 to 100.00% for CBB_06_TC9138316.

The mean severity on leaf values of these families ranged between 2.97 (Awash1/SEC12) and 3.41 (Awash1/SMC21) at Melkassa and between 2.79 (Awash1/SEC12) and 3.02 (Awash1/SMC21) at the Arsi Negelle site. All sample plants from Awash1/SMC21 showed positive response to the marker CBB_SAP6_801, where 69.33% of samples from this cross responded positively to CBB_06_TC_9138316. All sample plants belonging to Awash1/SMC21 had moderate resistance to CBB at both sites. Out of the 16 crosses, 7 showed 100.00% positive response to CBB_06_TC_9138316. The SL values of the 7 crosses ranged from 2.91 (Nasir/SMC24) and 3.03 (Nasir/SEC12) at Melkassa. The SL values ranged from 2.75 (Mexican142/SEC24) and 2.83 (Red Wolaita/SEC24 and Red Wolaita/SMC21) at the Arsi Negelle site. Individual plants that showed positive response to both markers possessed favorable alleles conditioning CBB resistance. Furthermore, these plants displayed lower levels of leaf and pod infections hence can be advanced through marker-assisted selection as promising and new sources of CBB resistance.

The CBB resistant parental lines, SEC12 and SEC24, expressed higher levels of CBB resistance based on lower leaf and pod infections at both sites [and possess the two markers](#). Other sources of CBB resistance (SMC21 and SMC24) that carry CBB_SAP6_801 showed resistance to leaf and pod infection except SMC21 that had relatively higher SL value of 3.50 at the Melkassa site. The recipient parents showed moderate susceptibility to leaf and pod infection at both sites and carried neither of the two markers except Mexican142 that had positive response to CBB_SAP6_801.

Table 5.5 The number and proportion (%) of individual plants that carried the SNP markers CBB_SAP6_801 and CBB_06_TC9138316, and CBB severity reaction assessed at Melkassa and Arsi Negelle sites in Ethiopia

Population	Number of plants positive to CBB_SAP6_801			Number of plants positive to CBB_06_TC9138316		Disease parameters				
	N	n	%	n	%	Melkassa		Arsi Negelle		
						SL	SP	SL	SP	
Crosses										
Awash1/SEC12	74	69	92.57	74	100.00	2.97	2.51	2.79	2.44	
Awash1/SEC24	74	72	97.30	73	98.65	3.00	2.50	2.84	2.48	
Awash1/SMC21	75	75	100.00	52	69.33	3.41	2.62	3.02	2.66	
Awash1/SMC24	73	69	93.84	34	46.62	3.04	2.52	2.66	2.32	
Mexican142/SEC12	69	43	63.04	69	100.00	2.96	2.49	2.81	2.45	
Mexican142/SEC24	74	41	56.08	74	100.00	2.98	2.51	2.75	2.41	
Mexican142/SMC21	68	18	25.74	58	85.51	2.93	2.50	2.73	2.38	
Mexican142/SMC24	71	21	30.28	52	72.60	2.97	2.50	2.84	2.48	
Nasir/SEC12	75	47	63.33	75	100.00	3.03	2.52	2.82	2.47	
Nasir/SEC24	75	35	47.33	75	100.00	2.91	2.48	2.81	2.45	
Nasir/SMC21	65	38	59.23	46	71.32	3.02	2.51	2.77	2.42	
Nasir/SMC24	74	44	59.46	41	56.08	2.91	2.48	2.80	2.45	
Red Wolaita/SEC12	73	52	71.23	73	100.00	2.99	2.50	2.80	2.45	
Red Wolaita/SEC24	73	40	54.11	73	100.00	3.02	2.52	2.83	2.48	
Red Wolaita/SMC21	74	43	58.11	54	72.30	2.95	2.50	2.83	2.48	
Red Wolaita/SMC24	68	27	39.71	36	52.24	3.01	2.50	2.72	2.38	
Parents										
SEC12	4	4	100.00	4	100.00	2.79	2.27	2.57	2.24	
SEC24	4	4	100.00	4	100.00	2.62	2.65	2.73	2.38	
SMC21	2	2	100.00	0	0	3.50	2.87	2.71	2.37	
SMC24	2	2	100.00	0	0	2.89	2.42	2.67	2.33	
Nasir	2	0	0	0	0	6.74	4.64	6.38	4.61	
Red Wolaita	2	0	0	0	0	6.75	4.96	6.03	5.12	
Awash1	2	0	0	0	0	7.55	4.79	6.58	5.20	
Mexican142	4	0	0	0	100.00	7.03	5.22	4.58	3.76	

N = total number of sampled plants; n = number of plants showing positive response to a SNP marker; SL= Severity on leaf; SP = Severity on pod, CBB=Common bacterial blight

5.4 Discussion

Common bean is one of the most important legume crops in Ethiopia. However, production and productivity of the crop is constrained by different diseases including the common bacterial blight (CBB). Host resistance is the most effective and environmentally friendly strategy to control major diseases of the common bean including CBB worldwide (Vandemark et al., 2008). In order to develop CBB resistant variety, screening of a large number of populations under controlled environment and field conditions is paramount importance using phenotypic traits and high

throughput molecular markers (Witcombe and Virk, 2001). Use of molecular markers could significantly improve the efficiency of plant breeding programs when integrated with conventional selection methods (Gupta et al., 2010). Genomics tool have been successfully implemented in different common bean improvement programs (O'Boyle et al., 2007; Beaver and Osorno, 2009; Blair et al., 2013; Raatz et al., 2019). Correlation estimates between presence of markers linked to resistance genes can contribute to the MAS (Yu et al., 2000). Once correlation is validated, use of molecular markers allow distinctions between homozygous and heterozygous individuals with the gene of interest at the F₂ or advanced generations such as in recombinant inbred lines. This will enable early generation selection of novel segregants (Gupta et al., 2010).

In the current study phenotypic variations, genotypic variation and their correlations were explored for CBB resistance breeding involving various crosses (Tables 5.1, 5.2 and 5.3). A Chi-square test analysis revealed different level of resistance among individual plants in the assessed crosses based on mean severity scores on leaf and pod (Table 5.1). The findings indicated that most crosses were consistent with expected genetic ratio of 1:3:1 involving resistant, moderately resistant and susceptible individuals, in that order. This suggested that the inheritance of CBB resistance is quantitative. Previous studies reported the presence of two major and many minor genes involved in CBB resistance in common bean breeding populations assessed in Malawi (Chataika et al., 2011). A study on CBB resistance in tepary bean at the F₂ generation indicated the presence of one or more loci involved in controlling resistance to CBB (Urrea et al., 1999). The present study contradicts with the report by (Tryphone et al., 2012) who indicated that CBB resistance in a parental common bean line VAX4 was conditioned by dominant genes only. Singh and Miklas (2015) reported that CBB resistance is controlled by major or minor genes based on the sources of the resistance in agreement to the present findings.

In the present study families with higher number of individuals carried both the SNP markers (Awash1/SEC12, Awash1/SEC24, Mexican142/SEC12 and Red Wolaita/SEC12). These plants had lower leaf and pod CBB severity values at both the Melkassa and Arsi Negelle sites (Table 5.5). The total variations due to the two SNP markers were 23% among population and 45% among individuals and 32% within individuals (Figure 5.1A). The marker CBB_SAP6_801 was unique and was responsible for the higher total variations of 22%, 48% and 32% among populations, among individuals and within individual plants, respectively (Figure 5.1B). The SNP marker CBB_06_TC_9138316 attributed to a variation of 87% among populations, 7% among

individuals and 6% within individuals (Figure 5.1B). Previous reports indicated the presence of > 75% of the total phenotypic variation for CBB resistance on leaves based on QTL analysis in an F₂ population involving a cross of BAT 93 x Jalo EEP 558 (Nodari et al., 1993). Other studies reported that markers linked to QTL on B8 explained 20% of the total variation, while markers linked to QTL on B6 explained 22% of the phenotypic variation for CBB reaction on leaf (Yu et al., 2000). Other reports identified QTL on B10 that explained 9.5% of the variation in CBB reaction (Mutlu et al., 2005). Resistance due to CBB QTL accounted for only 14% to 29% of the phenotypic variation on leaf and pod respectively (Jung et al., 1996). The presence of QTL on linkage group B6 associated with marker BC420 explained 63% of the phenotypic variation (Yu et al., 2004). Durham et al. (2013) argued that the presence of QTLs on B6 and B8 accounted for 37 to 46% of phenotypic variation for CBB resistance under field condition.

There existed a significant difference among the number of individual plants amongst the assessed crosses assayed by the SNP markers. The number of individual plants ranged between 26 to 100% detected by the marker CBB_SAP6_801 and from 46 to 100% by CBB_06_TC9138316 (Table 5.2). Mean SL values of these families ranged from 2.97 (Awash1/SEC12) to 3.41 (Awash1/SMC21) at Melkassa, and from 2.79 (Awash1/SEC12) to 3.02 (Awash1/SMC21) at the Arsi Negelle site. Individual plants from Awash1/SMC21 showed 100% positive response to marker CBB_SAP6_801 and 69.33% of samples from this cross responded positively to CBB_06_TC_9138316, but exhibited moderate resistance to CBB at both testing sites. Durham et al., (2013) reported that lines that showed positive response to the marker revealed lower disease severity scores than the lines with negative response for the marker implying the trait and marker are associated). Other reports substantiated that frequencies of favorable alleles ranged between 10 and 49% and were associated with leaf and pod resistance in the field (Shi et al., 2011).

In this study, variations were observed among crosses in the observed heterozygosity (H_o), and genetic diversity (H_e). The H_o values were lower (0.02 - 0.276) compared to H_e values (0.040 - 0.489). Higher H_o was observed in the cross Red Wolaita/SMC24, with a value of 0.267 followed by Red Wolaita/SMC21 with 0.264. The H_e of the two crosses were 0.489 and 0.444 in that order. The H_o values attributed to CBB_SAP6_801 ranged between 0.05 to 0.30, while the H_e values ranged between -0.03 to 0.50. Shi et al. (2011) reported H_o values ranging from 0 to 0.62 with one SNP providing a value of 0.30 in common bean. There were low negative but significant

correlations among CBB resistance traits and SNP marker, CBB_06_TC_9138316 (Table 5.4) suggesting that QTL identified in this study conditioned CBB resistance. The presence of favorable markers is related to low infection on leaf at both the Melkassa and Arsi Negelle sites. These findings agreed with previous report that indicated the significant negative correlation between SU91 and BC420 on leaf and pod resistance in field and greenhouse experiments (OBoyle and Kelly, 2004). It was also reported that the presence of SU91 revealed direct relationship with lower leaf disease rating (4.67) compared to 6.56 for plants lacking the marker. In the same study it was reported that lower mean pod disease severity value of 0.53 was observed in the presence of SU91 compared to a severity value 1.05 when absent (Yu et al., 2000).

Effective markers would identify homozygous and heterozygous individuals at the F₂ generation which could assist to undertake early generation selection of progenies with QTLs responsible with CBB resistance (Gupta et al., 2010). In CBB resistance breeding in common bean two markers that explained 22% of the total variation were reportedly effective to select resistant progenies (Tar'an et al., 1998). In the present study 23% of the total variation among populations and 45% among population were explained by the two SNP markers. Tar'an, (2001) reported that markers that explained 10.2 to 42.2 % of phenotypic variation were ideal to be used for MAS. In the current study there were lines that showed resistance to leaf or pod infection but did not possess either of the two SNP markers. Similar responses were also reported when the test genotypes were phenotypically resistant to CBB despite a lack of the expression of a marker used (Kachulu et al., 2011). Resistant progenies lacking the markers under study provide evidence for the need to combining phenotypic and MAS when selecting for traits of quantitative inheritance. Once specific target allele associated with the resistance QTL is identified, use of a marker is appropriate to select resistance individuals especially in the larger breeding population (Tryphone et al., 2012). Breeding for CBB resistance requires diagnostic markers with high level of correlation with disease parameters and/or linkage should exist between CBB resistance and molecular markers (Yu et al., 2004).

5.5 Conclusions

The present study found significant genotypic variation for CBB resistance among the new breeding populations when assayed with SNP markers and based on field evaluations across two sites. The SNP markers explained 22% (marker CBB_SAP6_801) and 87%

(CBB_06_TC_9138316) of the total variations present in the test populations making them a marker of choice for future genetic analysis of CBB resistance. There existed significant correlation ($P < 0.001$) between data on phenotypic traits and the SNP analyses. The study found that the above two SNP markers are correlated with phenotypic CBB resistance. which can be used for MAS and breeding population development in common bean. Amongst the new crosses individual plants were selected with CBB resistance showing positive association with the SNP markers. The selections are recommended for genetic advancement through marker-assisted selection.

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General overview and implications of the study

6.1 Introduction and objectives of the study

The common bean (*Phaseolus vulgaris* L.) is an important legume crop in Ethiopia, providing food and income security to millions of smallholder farmers. In terms of quantity of common bean production, Ethiopia is ranked 10th in the world and 4th in sub-Saharan Africa (SSA). In the country common bean is cultivated by about 3.6 million smallholder farmers on an estimated area of 306,187 ha with a net annual production of 520,979 tons. Despite the increased area of production and economic value of common bean, the mean productivity of the crop is low (1.7 tons ha⁻¹) in Ethiopia, far below the potential yields reaching up to 4.5 tones ha⁻¹ elsewhere. The low productivity of the crop is caused by an array of abiotic, biotic and socio-economic constraints. Use of low yielding varieties, drought stress, fungal and bacterial diseases, insect pests, poor soil health are among the major constraints to common bean production and productivity in the country. In the past more than 50 common bean varieties were released to enhance yield gains under optimal growing conditions. However, most of the released varieties succumbed to the common bacterial blight (CBB) disease caused by *Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans*. Hence, there is a need for development and deployment of new varieties with durable resistance and farmer-preferred traits. Developing common bean cultivars that are high yielding, locally adapted, farmer-preferred with durable CBB resistance is an overriding consideration for successful disease management and yield gains.

This overview summarizes the research objectives and highlights the fundamental findings and implications of the study.

The objectives of the study were:

- 1 To identify constraints affecting common bean production and productivity and to identify farmers' perception on the constraints, and their trait preferences for inclusion in common bean breeding programs, specifically for disease resistance breeding.
- 2 To identify new sources of CBB resistance from a diverse panel of genotypes to develop CBB resistant common bean varieties.
- 3 To select common bean parents and families with good combining ability effects and heritability for CBB resistance and agronomic traits for variety development.

- 4 To introgress and track CBB resistance genes/QTL in selected susceptible commercial common bean genotypes through marker-assisted selection for cultivar development.

6.2 Summary of the research findings

6.2.1 Farmers' perceptions on production, production constraints, trait preference and disease management options in two major common bean growing regions of Ethiopia: implications for common bacterial blight disease resistance breeding

A participatory rural appraisal (PRA) was conducted in two major common bean growing regions, Oromia and Southern Nations, Nationalities and Peoples' Region (SNNPR) in Ethiopia. Data were collected using semi-structured questionnaires and focused group discussions with 255 farmers during 2017. Key inferences were made based on quantitative and qualitative statistical analyses. The following were the key findings:

- ❖ Drought stress (reported by 46.3% of the respondent farmers), diseases (24.4%), insect pests (12.6%) and lack of seeds of improved varieties (12.2%) were identified as the most severe constraints to common bean production across the study areas.
- ❖ Among the identified biotic constraints of common bean, CBB was ranked as the most devastating disease reported by 63.5% the respondents. Only 9.8% of the respondents reported using introduced common bean varieties with disease resistance and better agronomic traits.
- ❖ A significant proportion of the respondent farmers (28.6%) did not use any disease control methods. Yield loss due to common bean disease was reported to reaching up to 70% in the study areas.
- ❖ CBB resistance, high yield and farmer-preferred traits are the main drivers of common bean improvement in Ethiopia.

6.2.2 Identification of sources of resistance to common bacterial blight in common bean in Ethiopia

One hundred and ten diverse accessions were evaluated for CBB resistance at three hotspot sites (Melkassa, Arsi Negelle and Mieso) for two seasons (2017 and 2018) in Ethiopia. Data on mean disease severity on leaf (SL) and mean disease severity on pod (SP), the area under disease progress curve (AUDPC), number of pods per plant (PPP), number of seeds per pod (SPP) and

grain yield (GY) were collected. Data were subjected to standard analysis of variance and principal component analysis. The core findings of the study were:

- ❖ The genotype × site interaction (G × E) had significant effect on all assessed traits. This indicated the presence of marked variation among tested genotypes in CBB resistance across the testing sites.
- ❖ Genotypes including SEC21, SEC23, SMC21, VAX6, SEC12, SEC25, SMC22, VAX5, SEC20, SEC22, SEC24, SEC26, SMC16 SMC24, VAX6, SEC25, SEC21, SEC23 and SMC21 exhibited lower values of SL, SP and AUDPC suggesting that they can be useful genetic resources for future CBB resistance breeding programs.
- ❖ The best yielding variety was cv. Nasir, which produced a grain yield of 3.45 ton/ha, followed by VAX1 (2.86 ton/ha) and Hawassa Dume (2.83 ton/ha).
- ❖ CBB-resistant and high yielding genotypes had the higher PP and SPP making them ideal candidates for common bean breeding in Ethiopia or similar agro-ecologies emphasizing CBB resistance and enhanced agronomic traits.

6.2.3 Combining ability and gene action controlling common bacterial blight resistance and agronomic traits in common bean

Eight CBB resistant common bean genotypes were crossed with four susceptible farmer-preferred common bean genotypes using a line x tester mating design. The F₂ populations were evaluated at Melkassa and Arsi Negelle Agricultural Research stations in Ethiopia using an alpha lattice design with two replications. Disease parameters such as SL, SP and AUDPC and agronomic traits such as the number of pod per plant (PPP), number of seed per pod (SPP) and grain yield (GY) were recorded. The key findings of the study were:

- ❖ The inheritance of all CBB resistance parameters is largely attributed to additive gene effects.
- ❖ There were high heritability values for yield and yield components revealing the contributions of additive genes in conditioning trait inheritance.
- ❖ Parents such as SEC12, SEC21, SEC20, SEC24 and SEC25 had negative and significant general combining ability (GCA) effects for CBB severity for leaf and pod infection.
- ❖ The F₂ families such as Nasir/SEC24, Red Wolaita/SMC21, Mexican142/SMC21, Mexican142/SEC25, Awash1/SEC22, Red Wolaita/SEC12, Nasir/SEC22, Nasir/SEC20 and

Awash1/SEC12 were best specific combiners and selected for CBB resistance breeding. These families displayed better agronomic attributes with significant and negative specific combining ability effect for SL and AUDPC.

- ❖ The selected parents and families are useful genetic resources for future breeding of CBB resistant and agronomically superior transgressive segregants for common bean variety development in Ethiopia.

Introgression of common bacterial blight resistance in common bean aided by marker-assisted selection

Common bacterial blight resistant and agronomically promising common bean breeding populations were developed involving 16 cross combinations. The new populations were field phenotyped at two CBB hotspot sites (Melkassa and Arsi Negelle) in Ethiopia and genotyped using three selected and diagnostic single nucleotides polymorphism (SNP) markers (CBB_SAP6_801, CBB_06_TC_9138316 and CBB_SU91_g91004686) at Intertek in Sweden. Progenies and parents involved in each cross were evaluated at both sites and data were collected on CBB severity on leaf (SL) and severity on pod (SP). The main findings of the study were:

- ❖ Significant ($P < 0.001$) variations were recorded among test genotypes for CBB severity.
- ❖ Segregation analyses of crosses for SL and SP at the F₂ indicated a genetic ratio of 1:3:1 involving resistant: moderately resistant: susceptible individuals, respectively and suggesting that CBB resistance was conditioned by multiple genes.
- ❖ Significant genotype variation was observed based on SNP analyses with 23% of the total variation was attributed to among the assessed populations.
- ❖ The SNP markers explained 22% (marker CBB_SAP6_801) and 87% (CBB_06_TC_9138316) of the total variations present in the test populations making them a marker of choice for future genetic analysis of CBB resistance.
- ❖ There existed significant correlation ($P < 0.001$) between data on phenotypic traits and the SNP analyses. The study has selected CBB resistant individuals with QTL associated with the two SNP markers useful for marker-assisted selected and breeding population development in common bean.

6.3 Implications of the research findings to CBB resistance breeding

- ❖ The PRA study revealed that new common bean cultivars must possess CBB resistance, high yields and farmer-preferred traits to meet the needs and preferences of producers and the market.
- ❖ The selected genotypes with high CBB resistance and better grain yields are important genetic resources for CBB resistance breeding programs for variety development and release.
- ❖ The existence of both additive and non-additive gene effects for CBB resistance and agronomic traits suggest that breeding gain can be realized through targeted hybridization and selection.
- ❖ The selected SNP markers are useful for marker-assisted breeding targeting CBB resistance and desirable agronomic traits.
- ❖ Overall, the present study identified valuable genetic resources of common bean parents and new families with high combining ability for CBB resistance and desirable agronomic traits. The selections are recommended for genetic advancement and to identify transgressive segregants based on CBB resistance, agronomic traits and SNP markers to develop pure lines for cultivar release and deployment in Ethiopia.