SCREENING SELECTED COMMON BEAN GENOTYPES FOR RESISTANCE TO *Xanthomonas axonopodis pv. phaseoli* CONSTRAINING BEAN PRODUCTION IN KAKAMEGA COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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To my loving and caring husband Joseph and children Mercy, Klein, Kelvin and Liance.

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ACRONYMS AND ABBREVIATIONS

BCMNV	Bean Common Mosaic Necrotic Virus
BCMV	Bean Common Mosaic Virus
BRR	Bean Root Rot
CBB	Common Bacterial Blight
CFU	Colony Forming Units
HR	Hypersensitive Response
KALRO	Kenya Agricultural and Livestock Research Organization
NA	Nutrient Agar
PDA	Potato Dextrose Agar
PR	Pathogenesis-Related
RCBD	Randomized Complete Block Design
Xap	*Xanthomonas axonopodis pv. phaseoli*

DEFINITION OF TERMS USED

Culture : To artificially grow micro-organisms on a prepared food material. Immune: Cannot be infected by a given pathogen.

Epiphytic : Growing externally on a plant without parasitizing it.

Exudates : Material that has passed from within a plant structure to the outer surface or into the surrounding medium; as in leaf exudate, root exudate,

Inoculation: The arrival or transfer of a pathogen onto a host**.**

Necrosis : Death of tissue, cells or organ

Pathogenicity: The capacity of a pathogen to cause disease

Plant Disease: A physiological disorder or structural abnormality that is harmful to the plant or only it's parts or products that reduced the economic value.

Resistance: The ability of an organism to exclude or overcome, completely or in some degree, the effect of a pathogen or other damaging factor.

Resistant : Possessing qualities that hinder the development of a given pathogen.

Sterilization: The elimination of pathogens and other living organisms from soil, containers, etc. by means of heat, chemicals, or radiation

Susceptible: Lacking the inherent ability to resist disease or attack by a given pathogen; non-immune.

Symptom: The external or internal reactions or alterations of a plant as a result of a disease.

Virulence: The degree of pathogenicity of a given pathogen.

Tolerance: The ability of a plant to sustain the effects of a disease without dying or suffering serious injury or crop loss.

Transmission: The transfer or spread of a pathogen from one plant to another.

ABSTRACT

Beans play a significant role in food security owing to its nutritional value and generation of income. However, output of beans in Western Kenya is hindered by diseases, pests, soil infertility and unfavorable weather resulting to low productivity. Of the many diseases of beans, common bacterial blight (CBB) caused by *Xanthomonas axonopodis pv. phaseoli* (*Xap*) is a disease of economic importance in common beans (*Phaseolus vulgaris* L.). Due to the fact that chemicals have not been effective against CBB, the use of resistant genotypes is a central management strategy. The current study was carried out in the field and in the green house of KALRO-Kakamega in 2013 and 2014 to screen nine bean genotypes for resistance to common bacterial blight disease. Experiments were conducted in randomized complete block design with three replications in a 9×2×2 factorial factor during the greenhouse and field screening and 4×2×2×2 field experiment when assessing the role of soil amendments and method of cropping on disease incidence and severity. During growth, data on plant height, number of pods/plant, length of pods and size and number of CBB spots was taken. Yield parameters were also assessed. During the study, the isolates that were recovered from leaf samples were categorized as *Xanthomonas* like, with regard to their yellow pigment and convex mucoid morphology. Reaction to *Xap* was assessed as the number of spots on the leaves and diseased leaf area (DLA). The findings from the experiment revealed a significant variation ($P<0.05$) on the entire traits studied among the nine bean genotypes. The experiment revealed that the mean CBB disease severity was significantly lower in bean plants that were not inoculated compared to those that were inoculated. Disease incidence, distribution and severity differed significantly ($P<0.05$) among the different bean genotypes. The CBB was significant ($P<0.05$) in the bean genotypes and was influenced by the soil amendments applied and the method of cropping used either monocropping or intercropping. Data from the field and greenhouse experiments were in conformity. None of the evaluated genotype was immune to CBB. CAL77 and Cal 156A genotypes exhibited high level of resistance to CBB, thus a better variety to use. Seven genotypes namely Cal 285, Cal 256, CAL271A, Cal274, KK 8 and Cal 87 showed moderate resistance. In the green house, it was observed that disease symptoms were severe in beans planted in non-sterile soil and inoculated with *Xap* compared to those planted in sterile soil and non-inoculated respectively. In the field trials, it was noted that bean plants grown with DAP were significantly ($P<0.05$) taller, had more number of pods per plant and significantly ($P<0.05$) higher yield per plot than those grown on soils with chicken manure. Monocropped beans had significantly ($P<0.05$) higher growth and yield parameters that were studied. This study therefore recommends that further evaluation and screening be done, susceptible genotypes be tried in other locations; establish the factors that confer high levels of tolerance in Cal 77 and Cal 156A and advice farmers on the correct farming methods.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Legumes belong to *Fabaceae* family and are grown agriculturally for food, animal feeds and act as source of income (Considine *et al*., 2017). Common bean (*Phaseolus vulgaris* L.) is rich in nutritional content and economically potent food crop which is grown globally (Leitich *et al*., 2016 Tugce *et al*., 2018). The annual global production of common bean is approximately 12 million metric tons, with 5.5 million metric tons alone in Latin America Caribbean (LAC) and 2.5 million metric tons in Africa (Broughton *et al*., 2003; Popovic *et al*., 2012). Common bean represents 65% and 32% of total protein consumed and energy respectively and therefore, it is a vital nutrient resource to approximately 500 million people in parts of Africa and Latin America (Blair *et al*., 2010; Tugce *et al*., 2018). Minerals and nutrients that the common beans contain make a balanced healthy diet (Agriculture and Agri-Food Canada, 2007).

Bean production in Africa has been declining despite the fact that they are the most affordable plant protein source to the many rural population. This is due to both biotic and abiotic factors, poor agricultural practices and high cost of certified and improved seed varieties (Katungi *et al*., 2009; Kajumula and Muhamba 2012). In Kenya, declining bean production is associated with soil infertility, pest and diseases as indicated in table 1.1.

Table 1.1. Losses (tyr^{-1}) to bean production in Africa, ranked in descending order by constraint

Biotic Constraint	East and Central Africa	South Africa	Sub-Sahara Africa
Angular leaf spot	281,300	93,500	384,200
Anthracnose	247,400	69,800	328,000
Bean stem maggot	194,400	96,400	297,100
Root rot	179,800	31,000	221,100
Bruchids	163,000	77,600	245,600
CBB#	145,900	69,800	220,400
Aphids	136,300	58,900	196,900
Rust	118,700	72,400	191,400
Bean common mosaic virus	144,600	29,800	184,200

Source: Akibode (2011)
CBB-Common Bacterial Blight

Bean yields declined from 600kg ha^{-1} to 400kg ha^{-1} in 2004 due to diseases (MOARD, 2004). The common bean (*Phaseolus vulgaris* L., Fabaceae) is one of the most essential food crop in Kenya (GOK, 2006; Otsyula and Wambulwa, 2010). It is suitable for enhancing food security due to its adaptability to different cropping systems, short growing cycle, high yields and rich nutrient composition (Mwaniki, 2002; Otsyula and Wambulwa, 2010). In Kenya, common bean is mainly cultivated in Western, Nyanza and Eastern regions (Katungi *et al*., 2009; FAOSTAT 2017). In Western region, these areas fall in the counties of Kisii, Vihiga, Kakamega and Trans-Nzoia, which have a potential production of over 20,000 tons per year (Otsyula and Wambulwa, 2010). Consumption of beans in Kenya is above 60 kg per capita per year (Katungi *et al*., 2009).Yields of beans on farmer's fields are lower (400 kg/ha) than the yields attained at KALRO research centers

(1500 and 2500 kg/ha) (Nekesa *et al*., 1998; Otsyula, 2010) due to a gradual fall in soil fertility and rising levels of pests and diseases. Diseases are estimated to be the second largest constraint after low soil fertility (Wagara and kimani, 2007, Leitich *et al*., 2016).

In Kenya, food legumes are the second most grown grain after maize (Muthomi *et al*., 2007, One Acre Fund, 2013). In most cases, they are intercropped with cereals like maize, cassava, sorghum and millet, where the legumes are the minority crops while the cereals are the majority (Tsubo *et al*., 2003).

In Western Kenya, food legumes are grown mainly as a source of income and food by the small scale farmers (Ojiem, 2006, One Acre Fund, 2013). Legumes are rich sources of proteins, which is approximately 18–25%, with soy bean having the highest percentage of protein content about 35-43% (Tharanathan and Mahadevamma, 2003).Common beans contain nutrients and minerals such as calcium, folate (B vitamin), iron, magnesium, phosphorus, potassium and zinc and thus contribute to a balanced healthy diet (Cortes *et al*., 2013). Beans are grown because of their high commercial value, nutritional value and its most affordable and cheapest plant protein source (FAOSTAT, 2014). The polyphenolic compounds (phenolic acids, tannins and flavonoids) found in beans may contribute to a wide range of health benefits, being potent antioxidants (Marathe *et al*., 2011). Bean also replaces meat and other animal products leading to a reduction in the exploitation of economically important animals, with enhanced animal wellbeing (Friel *et al*., 2009).

The use of legumes as intercrops has been shown to have numerous benefits to the soil including controlling erosion and weeds, lowering nutrient loss and water, and increasing nutrient availability to the plants including fixing nitrogen by symbiotic soil bacteria Rhizobia (Giller, 2001; Shapiro and Sanders, 2002; De Groote *et al*., 2010).

Bean diseases include those caused by fungi, bacteria and viruses (Mureithi, 2005; Mwangombe *et al*., 2007; Mangeni *et al*., 2014). Some of these diseases include, angular leaf spot (*Phaeoisariopsis griseola*), bean common mosaic virus, bean common mosaic necrotic virus, powdery mildew (*Erysipe polygoni*), bean rust (*Uromyces phaseoli typica*), bean root rots, anthracnose and common bacterial blight caused by *Xanthomonas axonopodis pv*. *Phaseoli* (Kimani *et al*., 2005; Leitich *et al*., 2016). Using resistant beans varieties is one of the most resourceful, cost effective and ecologically compatible strategy in the control and management of diseases (Otsyula, 2010; Otsyula, 2016).

Kenya Agricultural and Livestock Research Organization (KALRO) have been playing a key role in the advancement of bean genotypes that are resistant to various diseases. Up to 300 bean lines have been bred for resistance against root rot, bean common mosaic necrotic (BCMNV) and bean common mosaic virus (BCMC) (Otsyula, 2010; Otsyula, 2016). Results in the field have been variable and therefore there is need to subject the varieties to more diseases like anthracnose, halo blight and common bacterial blight. This study was purposed to determining the reactions of selected bean genotypes to CBB and also evaluated the role of cultural practices on CBB severity and incidence.

1.2 Statement of the research problem

The common bean (*Phaseolus vulgaris*) is ranked the second important source of dietary protein after meat and third most important source of calories therefore a major stable food in Eastern and South Africa (Kimani *et al*., 2005; Larochelle *et al*.,2013). In Kenya, beans are the third most important food crop (Food and Agriculture Organization, 2017). Beans are a crucial aspect in food security with regard to its nutritional value, short growing period and adaptation to different cropping systems (Mwaniki, 2002; One Acre Fund, 2013). Of importance is its role both in nutrition and generation of income (Mwang'ombe *et al*., 2007). In Western Kenya, output of beans is hindered by pests and diseases together with reduced soil fertility resulting from successive cropping with lack of soil amendments (Okalebo *et al*., 2006; Rubyogo *et al*., 2007; Chirwa *et al*., 2006) and unfavorable weather conditions. The results are poor rural livelihood, increase poverty level and low productivity of beans (Giller *et al*., 2011).

Among the diseases of economic importance affecting common bean worldwide is common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (Gillard *et al*., 2009; Cabi.org, 2015). *Xanthomonas axonopodis* pv. *phaseoli* is distributed in all bean growing regions worldwide including Africa and is favored by high humidity and warm to high temperatures (Abiy *et al*., 2006; Chen *et al*., 2012). Secondary host to CBB are the other different legume crops like; alfalfa, clover, peas, chickpeas, lentils, lupins, mesquite, carob, soybeans, peanuts, and tamarind, (de Carvalho *et al*., 2011). When there is early plant infection, greater damage occurs as a result of premature defoliation, which reduces the surface area available for photosynthesis, and also interferes with the translocation of organic

substances. The quality of seeds and pods is reduced by the lesions formed while the quantity is decreased due to reduced seed number and size (Fourie, 2002). It has been shown that, depending on the season, for every 1% rise in the incidence of CBB for the period of reproductive growth, yields reduction of 3.5 kg/ha to 11.5 kg/ha results (Fourie, 2011). Clean certified seeds are important since most of the pathogens are seed-borne (Fourie, 2011). Seed-borne diseases result in poor crop establishment and consequently huge crop losses (Dawson and Bateman, 2001; Islam *et al*., 2004).

Although most people rely on beans as the main source of plant protein (Atillal *et al*., 2002), continuous cropping and poor crop nutrition has led to build up of soil pathogens (Otsyula *et al*., 1998a). the results are declining bean yield in farmers' field to 400 $kgha^{-1}$ on average compared to 1500 $kgha^{-1}$-2500 $kgha^{-1}$ in the research centers (Otsyula, 2010). There is a need to urgently develop a more affordable and effective means of controlling bean diseases, based on improving plant health nutrition using locally available material such as organic soil amendments, for example chicken manure, as this method has been extensively used in greenhouses conditions and very little has been done on field (Patel, 1996). Screening bean varieties for resistance to CBB will therefore improve food security, nutrition, income and living standards of the people in Kakamega County.

1.3 Justification of the study

Legumes are the second most cultivated grains after maize in Kenya (Muthomi *et al*., 2007 Kimiti *et al*., 2009). Beans play an important role in alleviating malnutrition in resource-poor households, since they are not only a cheap source of concentrated protein, but also slow release vitamins, carbohydrates and minerals (Tharanathan and Mahadevamma,; 2003 Katungi *et al*., 2009; Katungi *et al*., 201. The most significant

solution to the food insecure population in Kenya, which rates 67%, is beans (Fantom, 2014).

Low production of beans is a result of many diseases of beans including common bacterial blight. Currently there are no studies that have been conducted on testing for virulence of *Xap* and no documentation of this has been made in Kakamega county resulting in a great decline on bean production. Due to the fact that there is very scanty information on screening for resistance and the lack of evidence for availability of bean genotypes that are resistant to *Xap* in the study area, there is an urgent need to curry out this study. The losses resulting from CBB can be reduced using clean seeds and improving soil fertility but this has not been practiced in the developing countries (Otsyula, 2016). Some practices like crop rotation have been limited because the land available per person in Kenya has dropped from 7.2 hectares in 1960s to just 1.7 hectares in 2005 due to the rapid population growth Otsyula, 2010). Besides, the use of soil amendments to control the CBB disease has not been reported therefore a need to use it as an alternative disease management strategy.

Screening for disease resistance is therefore important because it results in resistant varieties of beans which can then be grown in areas prone to CBB thus improving the bean yield in the farmer's field. The net production of beans in Western Kenya will increase and apart from beans being a source of income to the local communities, it will ensure sufficient food supply, improved nutrition and health geared towards achieving vision 2030. Results from this study will be used to advice both the farmers and researchers on the importance of screening for resistance to bean diseases and the role of soil amendment and method of cropping in improving bean quality and quantity.

1.4 Hypotheses

i. The inoculum collected and formulated from infected bean seeds is not virulent.

ii. The available bean genotypes in Kakamega County are not resistant to Common bacterial blight disease.

iii. There is no significant effect of soil amendment and method of cropping on incidences and severity of Common bacterial blight disease on bean genotypes.

1.5 Objectives

1.5.1 General objective

The main objective was to screen selected bean genotypes for resistance to common bacterial blight disease in Western Kenya and its management through selected cultural practices.

1.5.2 Specific objectives

The specific objectives of this study were:

i. To assess the virulence of *Xanthomonas axonopodis pv. phaseoli* on bean genotypes in Kakamega.

ii. To screen nine available bean genotypes for resistance to common bacterial blight disease under screen house and field conditions.

iii. To determine the effect of soil amendment and method of cropping on incidences and severity of common bacterial blight disease on the available bean genotypes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bean production practices in Western Kenya

Beans are grown in Western Kenya mainly for food and as an income generating activity (Nekesa *et al*., 1998; Mwang'ombe *et al*., 2007; Mhango *et al*., 2013). They can be intercropped with maize or other cereals, a cropping practice common with small-scale farmers in the tropics. Typically, cereal crops including sorghum (*Sorghum bicolor*), maize (*Zea mays*) and millet (*Pennisetum glaucum*) are the main crops, whereas legume crops such as pigeon pea (*Cajanuscajan*), beans (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea*), cowpea (*Vigna unguiculata*), and soybean (*Glycine max*) are the associated crop types (Tsubo *et al*., 2001, Buruchara *et al*., 2011). The choice of grain legumes by farmers is mainly influenced by factors like cash generation ability, palatability and high yield levels (Brooker *et al*., 2015: Considine *et al.,* 2015). Intercropping has many advantages to the farmer including reduced farm inputs and labor cost, diet diversification, addition of cash crops and reduced risk of crop failure (Otsyula, 2016).

Most farmers in the Western region of Kenya plant beans twice annually within the long rains and short rain period (Jaetzold *et al*., 2009). Majority of them use their own kept seeds from the previous seasons during planting (Buruchara, 2010; Scott *et al*., 2003; Rubyogo *et al*., 2007). Few farmers use cattle manure amendments during planting while most lack the inputs (Kimiti *et al*., 2009). Cattle manure is not sufficient since it is only available to 50% of households in smallholder farming systems and is limited to cattle owners (Garrity *et al*., 2012). For control of diseases, most farmers rely on cultural

methods such as crop rotation, ploughing of the debris deeply, controlling weeds, and minimizing of movement when the fields are wet (Allen *et al*., 1998; Nyankanga *et al*., 2012).

2.2 Economic importance of *Xanthomonas axonopodis pv. phaseoli*

The main threat to common bean (*Phaseolus vulgaris* L.) production all over the world is common bacterial blight disease (CBB) caused by *X*. *axonopodis pv*. *phaseoli* (*Xap*) (Belete and Bastas 2017). In different parts of the world, for instance in South Africa, a big losses has been seen in bean farming both on an industrial range and on the seeds production have been caused by CBB (Rodriguez *et al*., 2014) and is the main restraining factor for exportation. In Kenya also, Percentage crop losses related to CBB disease of between 10% and 75% have been reported ((Buruchara *et al*., 2011)). The disease causes both qualitative and quantitative losses in yields ranging from 10% to 40%, (Belete and Bastas 2017), depending on condition of the environment, the cultivar of the bean, and its defenselessness (Sintayehu, and Amare, 2016.). The disease is destructive during high rainfall, humidity and temperature (25-35°C). High quality and yield losses are experienced when the temperature is at its optimum (28°C) (Akhavan *et al*., 2013). It also makes reasonable losses on the other different legume crop like, peas, chickpeas, lentils, it attacks as a secondary host (De Carvalho *et al*., 2011).

Common beans are consumed as an alternative to animal protein in developing countries because they are rich in proteins (6-22%), (Blair *et al*., 2010), as not fully formed green pod or as dry pulses. CBB causing bacteria is seed-borne (Belete and Bastas 2017) and as long as the seed remains viable, it survives (Fetene and Ayalew, 2016)). The pathogen is

therefore disseminated through contaminated seeds (Belete and Bastas 2017) and the disease is spread through infected germplasm (MOARD 2014)). Most economically disadvantaged farmers store and reuse the bean seed during planting (Otsyula 2016). This coupled with poor storage facilities cause a threat to bean farming and bean production (He, 2010).

2.3 Taxonomy and Morphology of *Xanthomonas axonopodis pv. phaseoli*

The genus *Xanthomonas* comprise of 27 species that are pathogenic to approximately 400 host plants. It is within the family *Xanthomonadacea* and order *Xanthomonadales*. The pathogenic strains show high levels of host specificity (Kassahun 2008)). Brown pigment is produced in culture media by both *Xanthomonas campestris pv. phaseoli* and its fuscous variant, *Xanthomonas phaseoli* var. *fuscans*, which causes common bacterial blight (Rodríguez and Creamer, 2014).

Today, the pathogens are commonly called *Xanthomonas axonopodis pv. phaseoli* var. *fuscans* and *Xanthomonas axonopodis pv. phaseoli*. They are reported to occur in many bean production regions of the world and are frequently found in association (Rodríguez and Creamer, 2014). Generally, *X. axonopodis pv. phaseoli* bacterium is anaerobic, gram negative, rod-shaped (0.4-0.9 × 0.6-2.6 μm) with a single polar flagellum which don't form spores (Mahuku *et al*., 2006). The extracellular polysaccharide slime (EPS) surrounds the polar flagella (Alavi *et al*., 2008). Colonies are yellow, convex and slimy on media containing glucose. If high levels of tyrosine are added to the culture media, they release limited levels of diffusible brown pigments (Schaad *et al*., 2005).

2.4 Biology of *Xanthomonas axonopodis pv. phaseoli*

The bacterium enters the seeds via the xylem and phloem system of the pedicel and funicular (Kaur *et al*., 2005). It penetrates the leaves through the wounds or stomata and invades the spaces between the cells, causing a steady disintegration of the middle lamella. The stomata of the epicotyls and hypocotyls, from infected cotyledons or the vascular system of the leaf act as entry points to the stem (Karavina *et al*., 2011).

2.5 Disease symptoms of *Xanthomonas axonopodis pv. phaseoli*

At the start, leaf symptoms appear as water-soaked spots which then enlarges and recurrently band together with adjoining lesions (Chen *et al*., 2012). Infected tissues appear flabby and lesions are often surrounded by a narrow zone of lemon-yellow tissues. The widespread development of necrosis may cause defoliation or stem girdle (Osdaghi and Zademohamad, 2016). The symptoms on the foliar parts are small, light green, angular, water-soaked, or translucent spots (Belachew *et al*., 2015). During warm, wet condition, the lesions speedily broaden and fuse. The center becomes dry, brown and surrounded by a distinct, narrow zone of yellow tissue as the lesion extend. Lesion varies in size depending on the plant stage. In highly prone genotypes, the lesions enlarge until the leaves appear parched or sun scalded (Chen *et al*., 2012). When wind whipped, heavily infected leaves may become tattered and later, dry up and fall off. Stomatal openings are the exit point through which bacteria ooze out and provide inoculums for secondary spread.

Infected pods have a central cream or yellow colored bacterial colony seen as water-soaked lesions that drip as yellow lots of bacterial , which with time, become shallow and dark reddish-brown blotches (Chen *et al*., 2012). Pod infection often result in streak,

deformation and infection of the seeds by the bacterial although some seed may be seen to be healthy. Common blight bacteria continue to exist between bean crops in relationship with weeds, bean remains, and seeds (Mkandawire *et al*., 2004).

Infected seed may rot or shrivel if contamination occurs during the period of pod and seed development, but when the infection is severe, the whole pod may be badly shrunken producing seeds that may fail to develop or are deformed (Balaz *et al*., 2008; Buruchara *et al*., 2010). Seeds grow and become symptomless to the disease and may appear healthy in less severely affected pods while others may become slightly wrinkled (Buruchara *et al*., 2010). When infected seeds are planted, some may totally fail to germinate or the resulting seedlings develop blight lesions on the cotyledons, first true leaves and stems (Harveson, 2009). In humid weather, bacterial trickles that are yellow in color are seen in infected pods and leaves, and later dry to form a coating (OMAFRA, 2009). At the point of attachment of the cotyledons in first node, the stem may disintegrate causing the plant to break.

2.6 Life Cycle of *Xanthomonas axonopodis pv. phaseoli*

For optimum growth of the bacteria, a minimum temperatures range of 5 °C and 9 °C and maximum temperatures range of 30 °C to 39 °C is required (Belete and Bastas 2017). The three main growth phases of the bacteria are: pathogenic, epiphytic and survival (Karavina; *et al*., 2011). The life cycle of the bacteria involve endurance on natural material or tools (survival), to sluggish growth on host tissue without penetration under good conditions (epiphytic), followed by tissue penetration and exponential growth (pathogenic) and back again. Typically, *Xanthomonas* are host specific and only cause disease in the host species they were originally isolated from and grow more slowly in

host tissue compared to other bacterial species (De Carvalho *et al*., 2011). Host resistance, plant and tissue age, and vigor influence bacterial cell multiplication and disease reaction.

Xanthomonas can grow epiphytically outside the plant without invading inside tissues for long. In addition to weather and host physiology, foliar age and other micro flora affect the growth of the bacteria (Gilbertson, and Hagedorn 1990). The EPS slime of the *Xanthomonads* acts as a hydrophilic barrier and helps them tolerate harsh conditions like sunlight and dehydration. The bacteria can survive in association with volunteer host plants, weeds, soil, and on or in the seed itself (Karavina *et al*., 2011).

Common bacterial blight disease causing pathogen can overwinter in dead decomposing bean plant tissue or in previously infected debris in old bean field (Chen *et al*., 2012). It can stay alive for six to eighteen months on the soil surface in plant residue. Seeds contaminated and infected are a major source of inoculums (Akhavan *et al*., 2013). The bacteria can spread from field to field and plant to plant through farm machinery, surface-drainage water, stormy and splashing rains and overhead irrigation (Belete and Bastas 2017). The bacteria can also spread when leaves come in contact with other wet leaves, animals, people and insects such as whiteflies and leaf miners, infested soil, and plant debris (Harveson, 2009).

2.7 Transmission of *Xanthomonas axonopodis pv. phaseoli*

Common bacterial blight disease is spread from one field to another through the seeds (Chen *et al*., 2012). Plants developing from infected seeds usually form lesions on the primary nodes or cotyledons (Belete and Bastas 2017).

Under humid conditions, the lesions enlarge and greasy bacterial masses build up on the surface of the leaf and latter extend to healthy plants. The minimum bacteria per seed considered necessary to generate infected plants under field conditions are approximately 1000 to 10,000 cfu (Cafati, and Saettler, 1980: Buruchara *et al*., 2010).

2.8 Infection and host range of *Xanthomonas axonopodis pv. phaseoli*

Severe infection occurs following storms with wind-blown rain as it causes wounds on the plant leaves, this forces the bacteria to pass through natural openings for example stomata, into the intercellular spaces (Belete and Bastas 2017). During feeding, insect can cause wounds which are good points for infectivity (Jacques *et al*., 2004). After penetration, *Xap* can take a period of 10-14 days from preliminary infection to multi)ply rapidly in the intercellular spaces and for secondary spread to occur (Karavina 2011). For infection to occur and the disease to develop, an optimum temperature ranges of 28 °C to 32 °C is required (Buruchara, 2010).

The bacteria can invade the xylem and phloem system of the bean plant through natural openings and then spread systemically in the plant resulting in wilting, or can also penetrate the developing pods and cause infection of the seed coats (Marques *et al*., 2005). In addition to common bean (*Phaseolus vulgaris*), *Xanthomonas axonopodis pv. phaseoli* affects and cause a disease on other leguminous plants including lima beans (*Phaseolus lunatus* cv.*christmas*) and peas (He, 2010; Belete and Bastas 2017).

2.9 Role of soil amendments on *Xanthomonas axonopodis pv. phaseoli*

The composition of carbon in the dry weight of plant tissues is roughly 40-50% (Namugwanya *et al*., 2014). Organic manure feeds the soil with vital organic matter which plays a significant role in improving qualitative and quality beans (Kanitka, 2006). The nutrients are released by the activities of soil microbes which include bacteria, fungi, protozoa, nematodes and mites. The soil fauna initiates combination and mixing of residues into the soil (Mugwira, 1997). Soil bacteria and fungi play a role in nutrient immobilization and mineralization, (Sintayehu, and Amare, 2016). It increases the nutrient and water holding capacity, encourages root development, improve aggregation and prevent erosion (Kanitka, 2006).

The soil microbes play an important role in plant defense against infection by producing inhibitory antimicrobial secondary metabolites (Raaijmakers and Mazzola, 2012,) inducing plant tissues resistance (Conrath et al., 2015), competition for nutrients and space, directly interacting with the pathogen through hyperparasitism or antibiosis (Ghorbanpour et al., 2018). Plant-parasitic nematodes and pathogenic bacteria have been shown to form a complex associations leading to the development of plant diseases by; predisposing agent, modifying physiology of host tissues so as to increase its susceptibility, breaking host resistance to bacterial pathogens, acting as vectors of bacterial pathogens and changing the rhizosphere micro flora. (Tollenaere *et al*., 2016)

2.10 Effect of maize legume intercropping on *Xanthomonas axonopodis pv. phaseoli*

This is advanced agricultural method were two or more food crops are planted at the same time in the same space (Moshira *et al*., 2015). It is commonly practiced worldwide and is most popular in the tropics (Hassen *et al*., 2017). There are so many considerations put in place during intercropping such as selection of compatibility of crops, time of planting, quantity of planting, maturity of the crop and socio-economic status of the farmers (Ashenafi, 2015).

Intercropping increases productivity via reduced weed competition, improving resource utilization, reducing the risks, and ensuring that the yields are stable (Shrikrishnah *et al*., 2008; Workayehu and Wortmann, 2011). Intercropped legumes do not compete with the maize for nitrogen source as they fix nitrogen from the atmosphere (Adu-Gyamfi *et al*., 2007). Intercropping is an ecological method used for management of pests, diseases and weeds via natural competitive principle (Ayeni, *et al*., 2012). It is also a practice used culturally to reduce CBB infestation because the maize or sorghum used as an intercrop provides physical barriers that restrict the movement of *Xap* between bean plants (Zhu *et al*., 2016). Intercropping bean with maize reduces incidence and severity of the disease, delays epidemics onset, and retards the rate at which the disease progresses (Tsubo, *et al*., 2005).

2.11 Management practices of CBB

Mechanism that lessens the primary inoculum such as destroying weeds and volunteer beans plants, field sanitation, using chemicals such as foliar copper bactericide and practicing crop rotation where possible, are some CBB management options (Sallam, 2011; Belete and Bastas 2017).

In addition, burning or early integration of bean residues into soil, planting healthy seed and developing resistant cultivars lowers the CBB inoculum (Osdaghi and Zademohamad, 2016). Effective bacterial blight disease management involves use of genetic resistance (Miklas *et al*., 2003; Fourier *et al*., 2011) together with crop rotation, certified seed, and field sanitation (Sintayehu, and Amare, 2016.).

Seed-borne nature of the bacteria that causes CBB makes its control very difficult and therefore this disease remains as one of the leading diseases of bean globally (Mabagala, 1997). Germplasm exchange and international seed trade spreads the bacterial pathogen between nations worldwide (Muimui *et al*., 2011). The use of seeds that are free from the pathogen is one of the most efficient management approaches for this disease (Fininsa, 2003; ISTA. 2010; Balachew *et al*., 2015). Beans can be grown in areas which are unfavorable for pathogen development if such seeds are to be obtained (Oshone *et al*., 2014). Inspection should be done and the resulting seeds should be tested for freedom from the pathogen causing CBB (Mahuku *et al*., 2006). Rapid degradation of exposed debris through deep plowing prevents survival of CBB associated with debris (Karavina *et al*., 2011). Losses caused by CBB can be minimized by practicing crop rotation of beans with other plants like millet and intercropping of beans with maize or sorghum rather than a monoculture (Sintayehu and Amare, 2016.). Also, avoiding the use of sprinkler irrigation system as it favors the dispersion of bacteria compared with other irrigation systems (Osdaghi *et al*., 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area and Study materials

3.1.1 Study area

The study was carried out during the short rain season of 2013 and both long and short rains of 2014 at KALRO-Kakamega research station. The study area is located in a high potential agro-ecological zone within Kakamega municipality, 1.5 km South-East of the town center (Appendix IV). It is situated at $00^{o}28^{o}$North latitude and $34^{o}75^{o}$ East longitude at an altitude of 1524 meters above sea level. The area receives an annual rainfall of about 1850 mm. The mean daily minimum and maximum temperatures are 14^{o}C and 27^{o}C, respectively (Jaetzold *et al*., 2009). Kakamega has a bimodal rainfall pattern: the long rainy season in March to June and the short rainy season in September to November. The relative humidity varies between 30% and 100%; while average day length is 12 hours (Otsyula, 2016).

3.1.2 Study materials

Bean seeds used were obtained from KALRO-Kakamega Germplasm Collection Centre. Characteristics of genotypes of beans used during this study are described in Table 3.1. In this study the bean genotypes used are advanced lines that have been developed for disease resistance by bean breeding program at KALRO-Kakamega for different disease constraints and are in the national performance trials pending release to the farmers. In addition, these genotypes are of preferred market classes (seed coat color and seed size) which give them an advantage of release and high adoption rate by the farmers (Table 3.1). The bean genotype KK8 was used as a positive check because its reaction to other

pathogens is well known and is widely grown by farmers in Kakamega County. Viable seeds of the 9 bean genotypes used in this study were obtained from bean breeding program of KALRO-Kakamega. The source of CBB inocula was KALRO farms and from farmers' fields around KALRO-Kakamega Research Centre.

Table 3.1. Characteristics of bean genotypes used in greenhouse evaluations at KARLO-Kakamega in 2013

Bean genotype	Colour	Size	Reaction to *Pythium* root rot	Reaction to BCMV	Reaction to BCMNV
CAL 181	White Calima	Medium	RR	Unknown	RR
CAL271A	White Calima	Medium	RR	RR	Unknown
CAL 274	Red Calima	Small	RR	RR	Unknown
KK 8	Red Calima	Medium	RR	RR	RR
CAL 285	Red Calima	Medium	RR	Unknown	Unknown
CAL 87	Red Calima	Small	RR	Unknown	Unknown
CAL156A	Red Calima	Medium	RR	Unknown	RR
CAL 256	Red Calima	Medium	RR	Unknown	RR
CAL 77	Red Calima	Small	MR	Unknown	Unknown

RR = Resistant reaction, MR = Moderately resistant reaction, Medium = 35- 40 gm/100seeds, Small = 15-25 gm/100 seeds according to Otsyula (2010).
BCMNV (bean common mosaic necrotic virus) BCMV **(**bean common mosaic virus)
CAL (Calima) KK (Kakamega)

3.2 Experimental design and plot size

This study was carried out in KALRO-Kakamega research station under greenhouse and field conditions. The greenhouse experiment comprised a 9 × 2 × 2 factorial design of nine bean genotypes (9 factors), with or without the CBB pathogen (2 factors) and grown in non-sterile or sterile soil (2 factors), resulting in 36 treatment combinations (Table 3.2). For each of the 36 treatment combinations, 2 plants were grown in the same pot

with 4 pots per treatment (that is, n=16) replicated three times, giving a total of 216 bean plants (N) (Table 3.2).

Table3.2. Greenhouse experimental design

Bean genotypes		KK 8	CAL 181	CAL 77	CAL 271A	CAL 274	CAL 285	CAL 87	CAL 156	CAL 256
Inoculum	**Soil**									
Non-inoculated	Sterile	X	X	X	X	X	X	X	X	X
	Non-sterile	X	X	X	X	X	X	X	X	X
Inoculated	Sterile	X	X	X	X	X	X	X	X	X
	Non-sterile	X	X	X	X	X	X	X	X	X
Treatments (X) =36		Replicates = 3			n = 2				N = 216	

CAL (Calima) KK(Kakamega)

The pots were laid out in a randomized block design (RCBD) under greenhouse conditions of 12:12 hr photoperiods', temperature and humidity. A random blocking design field trial comprising of 9 × 2 factorial factor based on the bean variety used (9 factors) inoculated or non-inoculated (2 factors) was used. The measurement of each block was 12m × 7.3m. Each block had 18 plots and each plot had 2 rows each with 31 plants inoculated or non-inoculated and replicated 3 times. In between each block was a 1M pathway.

3.3 Leaf sample collection and bacteria isolations

The strains of *Xap* bacteria were isolated from the leaves of common beans that were collected during surveys in the KALRO-Kakamega and from farmers around the research station. These samples were grouped into two leaf sources as; farmers' field leaves and research trial leaves.

During the surveys, representative common bean farms were selected at random and sampled for CBB symptoms. Leaves with characteristic CBB symptoms (irregular necrotic lesions with yellow borders and water-soaked spots) were collected and dried between paper towels. For each leaf sample, tissues (16 mm^2) were cut along the lesion border, placed in a drop of distilled water on a microscope slide and macerated. A 10-fold serial and sequential dilution (to 10^{-5}) was used to reduce the dense culture of bacterial cells obtained from the leaf extract by transferring aliquots to diluents (Oztuk *et al* . ,2018). This was achieved by methodically agitating the suspension and transferring 1 ml of solution from tube #1 into tube #2. Mix gently , transfer 1 ml of solution from tube #2 into tube #3.Continue to transfer and mix through tube 5. A 0.1 ml of each undiluted and diluted extracts were streaked (Plate 3.1) on three plates of semi-selective media, *Xanthomonas axonopodis pv. phaseoli* (XAP1). 72 hours plates incubated at 25 °C were observed. All the bacterial colonies showing that showed yellow pigmentation, convex margins with mucoid colonies were examined and counted to quantify how many bacteria exist in a solution units (cfu) per ml of leaf extract from the plate which has more than 30 of these colonies but less than 300. The suspected colonies of the pathogen were sub-cultured yeast extract-dextrose-calcium carbonate agar (YDCA) to

purify them and one colony of the purified samples were maintained on NA and YDCA slant at 4 °C for further test.

Plate 3.1. Streaked plate showing *Xanthomonas axonopodis pv. Phaseoli*

3.4. Pathogen Identification

3.4.1. Gram Stain Reaction**.**

During Gram reaction, a drop of water was placed on the middle of the slide followed by a small amount of yellow pigmented colonies which was removed from the culture using a sterile wire loop. The two were mixed and the resulting bacterial smear was fixed on the microscope slide by flaming it several times. After cooling, crystal violet was pipetted onto the surface and gently flooded onto the smear for 1 minute. The slide was then tilted and then gently rinsed with distilled water using a wash bottle. It was again flooded with Gram's iodine and let to stand for another 1 minute, then, tilted gently and rinsed with distilled water using a wash bottle. Decolonization was done using 95% ethyl alcohol were the slide was tilted slightly and alcohol applied drop by drop for 10 seconds until the alcohol runs almost clear. The last step involve flooding with safranin to counter-stain and let stand for 45 seconds, rinse with distilled water using a wash bottle and dried using

blotting paper. The mounted specimen was examined under a compound microscope with 100× lens using oil immersion with no cover slip.

3.4.2. Pathogenicity Test

The pathogenicity test experiment was performed in a greenhouse using four plastic pots of 25cm diameter filled with sterilized clay soil. The sterilization was done by autoclaving the soil for 3 h in metal buckets at 121 °C. Seeds of GLP 2 were surface sterilized by immersing in 95 % (V/V) ethanol for 10 seconds, followed by a 4-min treatment with 0.5 % (V/V) sodium hypochlorite and then rinsed four times with sterilized water. Five seeds were planted per pot and at emergence the number of plants was reduced to two per pot. Isolates of *X. axonopodis* pv. *phaseoli* were inoculated onto 21 day old GLP 2 common bean variety. Leaves were sprayed with fresh pure culture (24–36 h) of the suspected bacterium as a water suspension at a concentration 10^6– 10^7 CFU/ml using a hand-held laboratory sprayer. Plants were sprayed with sterilized water and covered by a transparent lid to maintain high humidity for one day in order to facilitate infection. Inoculated plants were examined daily for development of symptoms. Types of symptoms induced by inoculation were recorded seven days after inoculation. Bacteria with the same characteristics as those inoculated were re-isolated from the leaves showing symptoms as per the criteria that were established by Robert Koch to identify the causative agent of a particular disease (Byrd, and Segre, 2016), these include: the microorganism or other pathogen must be present in all cases of the disease, the pathogen can be isolated from the diseased host and grown in pure culture, the pathogen from the pure culture must cause the disease when inoculated into a healthy, susceptible

laboratory plant, the pathogen must be re isolated from the new host and shown to be the same as the originally inoculated pathogen (Walker *et al*., 2006).

3.5. Preparation of growth medium

Top soil from Kakamega forest, chicken manure and sand used to fill the pots were mixed in the ratio of 3:2:1 by volume respectively. Part of the mixture was steam sterilized at 121^{o}C for 3 hours and allowed to cool before being packed in half of the pots. The same mixture but of non-sterilized soils was used in control pots.

3.6 Planting and inoculation

3.6.1 Greenhouse trials

Greenhouse trial experiments were conducted at the KALRO-Kakamega, during the short rainy season from September to November 2013. For five minutes, 20% sodium hypochlorite was used to sterilize the seeds from nine different bean genotypes followed by washing twice with distilled water. Two bean seeds were planted in each pot at a depth of 5 cm and a spacing of 5 cm between the plants. Pots were arranged on a greenhouse bench in a randomized complete block design (Plate 3.2) where the temperature was maintained at 25 °C ± 2 throughout, with 12 h light and 12 h dark. The suspension of 10^{8} cfu ml^{1} *Xap* isolate in distilled water, was prepared from 48 hour-old cultures grown on NA (Mkandawire *et al*., 2004).

Plate 3.2. Nine days old common beans in the greenhouse during the 2013 short rainy season

The suspension of the bacteria was sprayed onto the aerial parts of the fully expanded trifoliate leaves of the plant (fifteen days old plants), using a high-pressure hand sprayer. After inoculation, transparent nylon paper was used to cover the plants for four days (Dursun *et al*., 2002). The control plants were sprayed with sterile water. To ensure high humidity, plants were watered heavily and covered with perforated transparent polythene sheets for four days (Mwesigwa, 2009). Disease incidence and severity in form of CBB spots on the leaves and pods was scored (Hira *et al*., 2016). Growth parameters of the genotypes including mean height at flowering was also assessed.

3.6.2 Reaction of nine common bean genotypes to *Xap* isolates in the field conditions

Field experiments were conducted at the KALRO-Kakamega field, during the long rainy season from March to June 2014 to investigate the reaction of nine different common bean genotypes to *Xap* isolates. Preparation of plots that were 1 m apart was done and planted according to standard commercial practices. A complete randomized block

design with 9×2 factorial design of nine bean genotypes (9 factors), with or without the pathogen *Xap* (2 factors) and replicated three times (Plate 3). The application of DAP fertilizer was at a rate of 50 kg ha^{-1}. In the experiment, the treatments applied were; inoculation with *Xap* and control (distilled water) by spraying onto the aerial parts of plants using a high-pressure hand sprayer. After inoculation, transparent nylon paper was used to cover the plants for four days so as to manage the drift effect (Dursun *et al*., 2002). From 20–25 days after inoculation, plants were scored for disease reaction and CBB symptoms according to Lak *et al*. (2002b).

Disease incidence and severity in the form of CBB spots on the leaves and pods was scored (Hira *et al*., 2016). In addition, growth parameters of the genotypes like the mean height at flowering, mean number of leaves and number of days to maturity were assessed (Nkalubo, 2007; Mwesigwa, 2009). Yield parameters including the mean number and weight of pods per plant and mean seed weight per plant were also assessed (Nkalubo, 2007; Mwesigwa, 2009).

Plate 3.3. Field experiment during the long rainy season of 2014

3.7 Effect of soil amendment and method of cropping on CBB disease

The experiment was done at the KALRO-Kakamega field, during the short rainy season in September to November 2014 to investigate the effect of soil amendment and method of cropping on incidences and severity of CBB in the bean genotypes. The spacing between the seeds of common bean genotypes sown was 45 cm while the plots were 1 m apart. Preparation and planting was done according to standard commercial practices. A RCBD of 4×2×2×2 monocrop of beans or intercrop of maize and beans with DAP (50 $kgha^{-1}$) (Appendix I) or chicken (Appendix II) manure added as needed in the same amount for all bean lines and inoculated with *Xap* or sprayed with distilled water(control) and replicated three times. A 2-day-old culture of *Xap* grown on NA at 28 °C was the source of inoculum. Plants were sprayed using a high-pressure sprayer at two weeks and at the time of flowering with a 50 ml of suspension per plant containing 10^8 cfu ml^{-1}. Prior to inoculation, favorable microclimate for bacterial infection was created by spraying the plants with water. Response to CBB symptoms and disease were rated from 20–25 days after inoculation.

3.8 Data collection and disease score

Disease incidence for CBB was measured by counting the number of infected pods and leaves (Plate 3.4). On the other hand, the disease severity was assessed by estimating the proportion of total photosynthetic area that was diseased using a disease assessment key (Hira *et al*., 2016), showing different disease severity in the form of CBB spots on the leaves and pods (Appendix iii). Plants were scored from 20–25 days after inoculation using the CIAT 1-9 scale as; no symptom=1, slight=3, moderate=5, severe=7 and complete discoloration=9 (Buruchara *et al*., 2010). Resistance (R) was assigned to plants

with no or limited symptoms (score 1-3), tolerance (T) was assigned to plants with 4-6 disease score, whereas plants with a score of 7-9 were considered to be susceptible (S) (Plate 3.4).

Growth parameters of the genotypes that were assessed were mean height at flowering, (Nkalubo, 2007; Mwesigwa, 2009).Yield parameters assessed were, mean number and weight of pods per plant and mean seed weight per plant (Nkalubo, 2007; Mwesigwa, 2009).

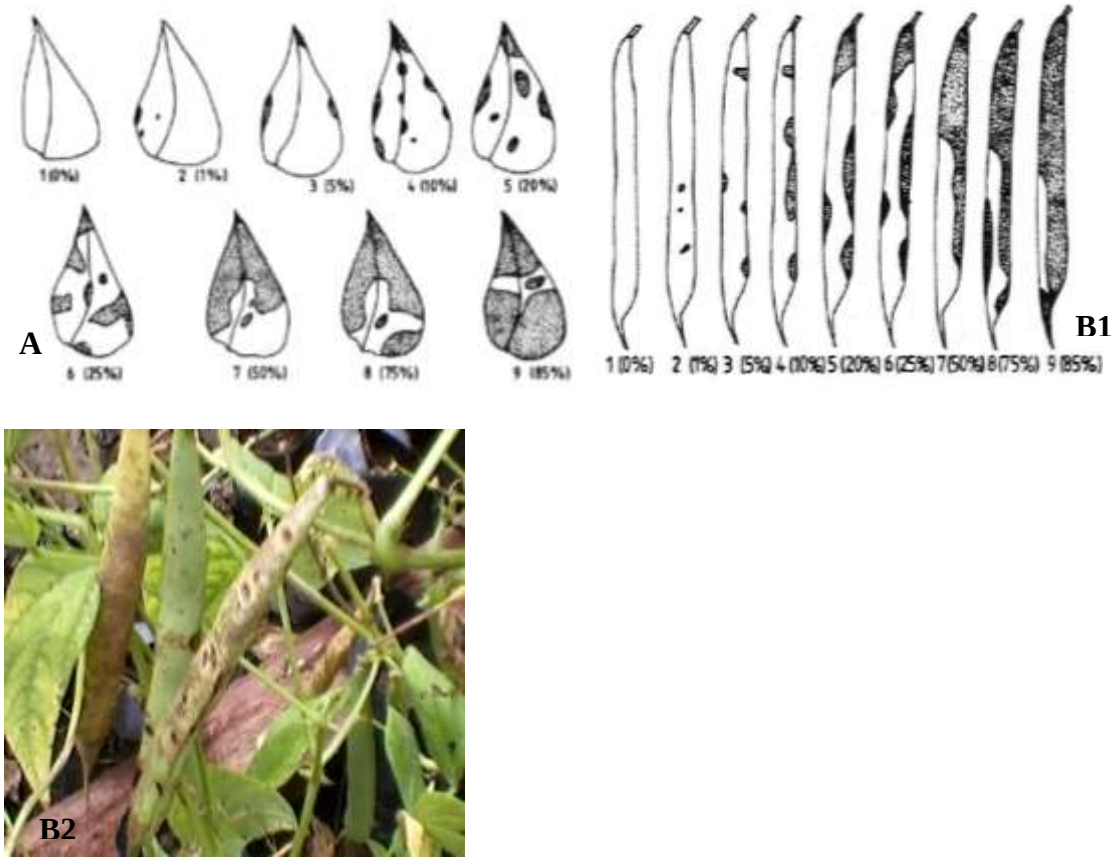

Plate 3.4: Disease assessment on leaves (A) and on the pods (B1-2)
(Source of diagram A and B1 Manandhar, *et al*., 2016 ,B2 Chepkemboi)

3.9 Data analysis

Data was analyzed using SAS portable software for scientific data analysis. For each of the greenhouse and field experiments, disease severity index was statistically analyzed using analysis of variance (ANOVA; P=0.05). Tukey's HSD at 95 % probability level tests were used to separate significant treatment means. Categorical data for CBB disease incidence and symptoms was summarized by CIAT scale 1-9 and log transformed to normalize (van Schoonhoven and Pastor-Corrales, 1987) and applied to differentiate between susceptible, tolerant and resistant bean genotypes. Summary statistics including means and standard errors for numerical data such as plant height, pod count and pod weights were generated using proc means. Analysis of variance between treatments for these parameters was conducted.

CHAPTER FOUR

RESULTS

4.1 Virulence of *Xanthomonas axonopodis pv. phaseoli*

The result from isolation of the bacteria from the leaves showed that *Xap* was recovered from 13 out of the 24 leaf samples that were under investigation. This indicated that there was high pathogen prevalence (54.17 %) in the leaf samples collected (Table 4.1). Low incidence of *Xap* (25 %) was shown in leaf collected from KARLO-Research trial site compared to the highest occurrence that was recorded in leaves collected from farmers' field leaf lots (75 %).

Table 4.1: Occurrence of *Xap* in bean leaf samples from different seed sources

Seed source	No. of sample collected	No of *Xap* positive sample	% *Xap* prevalence
Research trial sites	4	1	25
Research trial sites	4	1	25
Research trial sites	4	1	25
Farmers' field	4	3	75
Farmers' field	4	3	75
Farmers' field	4	4	100
Total	24	13	54.167

Results of *Xap* populace established that *Xap* colony populations were very high for all leaves that were positive for the common bacterial blight disease ranging from 1.23×10^5 to 6.53×10^6 cfu/ml from the leaf extracts. The *Xap* colony population from leaf lots obtained from KALRO research trial farms was significantly lower (1.23×10^5 cfu/ml) compared to those from leaves obtained from farmers' field (6.53×10^6 cfu/ml).

Well separated single colonies showing yellow, mucoid, xanthomonad-like colonies (Plate 4.1) indicated that the inoculum that was cultured and sub-cultured on fresh XAP1 media plates was *Xap* since these growth and morphological characteristics are typical of the bacteria.

Plate 4.1: Yellow, mucoid colonies of *Xap* on XAP1 media

4.1.1 Pathogen characterization

The bacterial isolates recovered from leaf samples were categorized as *Xanthomonas* like, basing on their yellow pigment and convex mucoid morphology as indicated in Plate 4.1. The colonies of *Xap* on XAP1 medium were smooth, convex, mucoid, bright yellow, round with entire margins. Biochemical test results also confirm that the colonies were rod shaped and gram negative.

4.1.2 Pathogenicity test

All the bacteria isolated and recovered from the extract of leaves led to development of symptoms ten days after inoculation during the pathogenicity test on bean plant (Plate 4.2). The symptoms were first seen as small water soaked spots around growing tips on the underside leaves. Lesions slowly increased in size and merged developing into large asymmetrical shape which becomes dry, brown and enclosed by a thin yellow edge.

Plate 4.2: Necrotic spots and yellowing of bean leaves caused by common bacterial blight disease

4.2 Screening selected bean genotypes for resistance to common bacterial blight disease in the greenhouse and field conditions

4.2.1 Evaluation of selected bean genotypes for resistance to common bacterial blight in the green house experiment

The findings from the greenhouse experiment revealed a significant variation ($P<0.05$) on the entire traits studied among the nine bean genotypes (Table 4.2). There was a significant variation between *Xap* inoculated and non-inoculated (control) plants in disease severity rating (CBB score) indicating that all the bean genotypes were infected by CBB (Table 4.2). The bean genotypes inoculated with *Xap* recorded a significantly ($P<0.05$) higher CBB score compared with the bean genotypes that were not inoculated but grown in non-sterile soil (Table 4.2 and Figure 4.1).

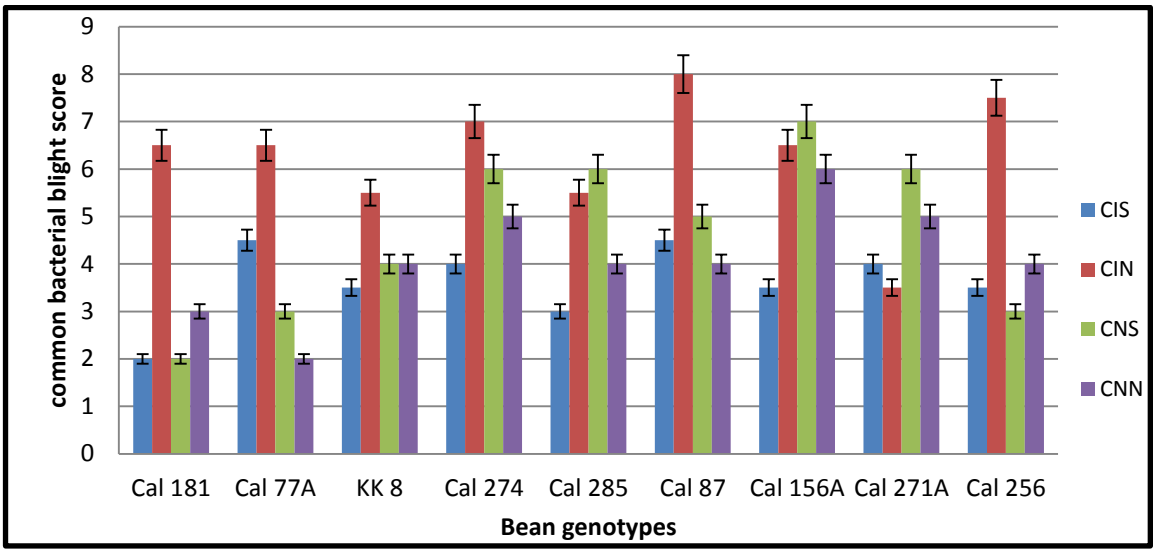

Figure 4.1: CBB score in the greenhouse for the nine bean genotypes in different soil treatments.

Cal (Calima), KK (Kakamega), CIS-CBB score in *Xap* inoculated plants in sterile soil, CIN-CBB score in inoculated plants in non-sterile soil, CNS-CBB score in non-inoculated plants in sterile soil, CNN-CBB score in non-inoculated plants in non-sterile soil

CBB was scored on a scale of 1-9 based the CIAT scale (Buruchara *et al*., 2010).

Table 4.2. Effect of the *Xap* inoculum on CBB severity and plant growth parameters on nine bean genotypes in the greenhouse

	**CBB score	Plant field weight (g)	Plant length (cm)	Yield/ Plot (g)	No. Seed / pod	No. Pod / plant	Pod length (cm)
Genotype*							
CAL 156A	3.50±0.22ab	6.35±0.64ab	5.61±0.04f	4.62±0.53b	3.00±0.13bcd	2.00±0.18c	5.35±0.36b
CAL 181	2.50±0.22c	6.76 ±1.19ab	8.73±0.25a	4.01±0.58c	4.28±0.52a	3.00±0.18ab	8.00 ±0.76a
CAL 256	3.41±0.20ab	5.72±0.84ab	6.66±0.2d	3.97± 0.39c	3.36 ±0.38b	2.50±0.28abc	7.63±0.49ab
CAL 271A	3.33±0.17ab	6.35±0.51ab	6.06±0.24e	5.22 ±0.49a	3.67±0.17ab	2.00 ±0.28c	5.01±0.47b
CAL 274	3.50±0.22ab	6.36±1.02ab	4.10±0.23g	4.12±0.46c	2.50 ±0.32cd	3.25±0.38a	7.56±0.72ab
CAL 285	3.17±0.17b	5.17 ±0.92b	8.90±0.32a	4.18±0.46c	2.42±0.2d	2.00±0.28c	7.46±0.56ab
CAL 77	3.75 ±0.35a	6.81 ±1.22a	5.40±0.09f	4.14±0.47c	3.37±0.22b	2.00 ±0.28c	5.16 ±0.45b
CAL 87	3.75±0.35a	6.54±0.86ab	8.42±0.38b	3.88±0.60c	3.25±0.38bc	2.50±0.28abc	7.88±0.82ab
KK8	3.25±0.17ab	5.91±0.82ab	7.12±0.23c	4.03±0.49c	3.45±0.36b	2.25±0.38bc	7.86±0.48ab
Treatment							
Xap Inoculated	3.70±0.15	4.39±0.09	6.76±0.32	3.13±0.09	3.00±0.14	2.33±0.17	6.76±0.34
Non-inoculated	3.00±0.0	8.06±0.28	6.80±0.33	5.35±0.09	3.51±0.19	2.44±0.14	7.01±0.36
P values							
Genotype	<.0001	0.0429	<.0001	<.0001	<.0001	0.0002	0.0012
Treatment	<.0001	<.0001	0.3180	<.0001	<.0001	0.4196	0.5454
Genotype × treatment	<.0001	0.0030	<.0001	0.0345	<.0001	<.0001	0.8875

*Values (Mean ± SE) followed by different letters in the same column are significantly different based on Tukey's HSD at 95 % probability level.

** Transformed CBB scored on a scale of 1-9 based the CIAT scale as; no symptom=1, slight=3, moderate=5, severe=7 and complete discoloration=9 (Buruchara *et al*., 2010). Resistance (R) was assigned to plants with no or limited symptoms (score 1-3), tolerance (T) was assigned to plants with 4-6 disease score, whereas plants with a score of 7-9 were considered to be susceptible (S).

There was a significant interaction ($P<0.05$) in CBB severity between the inoculated and non-inoculated bean genotypes (Table 4.4). Except for Cal 285 and Cal 156A, the CBB score was significantly higher on *Xap* inoculated bean genotypes grown on non-sterile soils (CIN) compared with the *Xap* inoculated and non-inoculated genotypes grown on both sterile and non-sterile soils (Figure 4.1). All the bean genotypes were tolerant to *Xap* infection except Cal 181 that was slightly resistant based on the CBB score and resistance rating score on the CIAT scale (Table 4.2; Figure 4.1).

The plant weight for the various bean genotypes varied significantly ($P<0.05$) with CAL285 recording significantly higher field weight (Table 4.2). All the other genotypes did not differ significantly with each other. The *Xap* inoculated bean genotypes recorded significantly ($P<0.05$) lower plant weight compared to non-inoculated (Table 4.2). In addition, the interaction between the treatments and genotypes varied significantly ($P<0.05$) as indicated in Table 4.2.

Although the plant length of *Xap* inoculated and non-inoculated beans did not differ significantly between the treatments, there was a significant difference ($P<0.05$) established among the genotypes with Cal 285 being the tallest. A significant interaction between the treatments and genotypes was established (Table 4.2).

The yields per plot in the greenhouse differed significantly ($P<0.05$) among the genotypes and between the treatments with *Xap* inoculated genotypes recording significantly ($P<0.05$) lower yields compared to the non-inoculated bean genotypes (Table 4.2 and Figure 4.2). In *Xap* inoculated plots, genotype Cal 156A had significantly higher yield in sterile and non- sterile soil compared to Cal 181 and Cal 274 which

recorded the lowest in non-sterile soil. Cal 181 and Cal 256 had significantly lower yield in sterile soil. On the other hand, Cal 156A, Cal 271A and Cal 285 produced significantly ($P<0.05$) higher yield in both sterile and non-sterile soil in non-inoculated plots. It was established that there was significant interaction between the treatments and genotypes (Table 4.2).

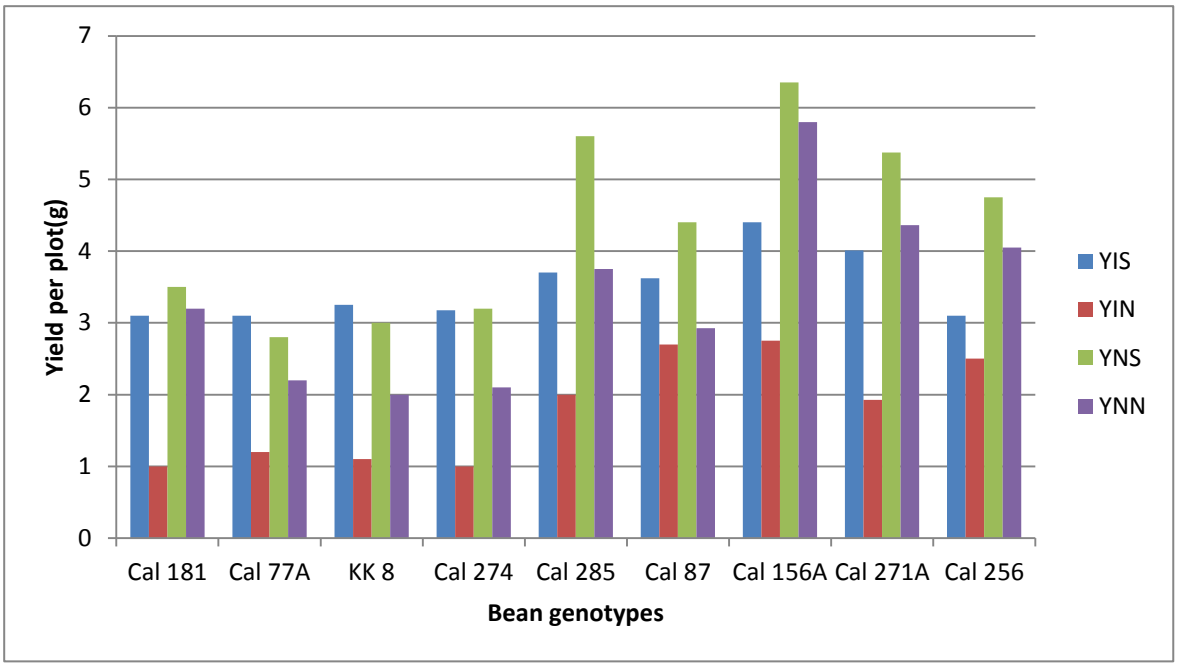

Figure 4.2: Average yield per plot among the nine bean genotypes in the greenhouse

YIS-Field weight per plot in inoculated plants in sterile soil, YIN- Field weight per plot in inoculated plants in non-sterile soil, YNS- Field weight per plot in non- inoculated plants in sterile soil

Although the number of seeds per pod, pods per plant and pod length varied significantly ($P<0.05$) among the genotypes, there was no significant difference ($P<0.05$) that was established between the treatment on the pods per plant and pod length (Table 4.2 and Figure 4.3). The interaction between the genotype and treatment was established on seeds per pod and pods per plant (Table 4.2).

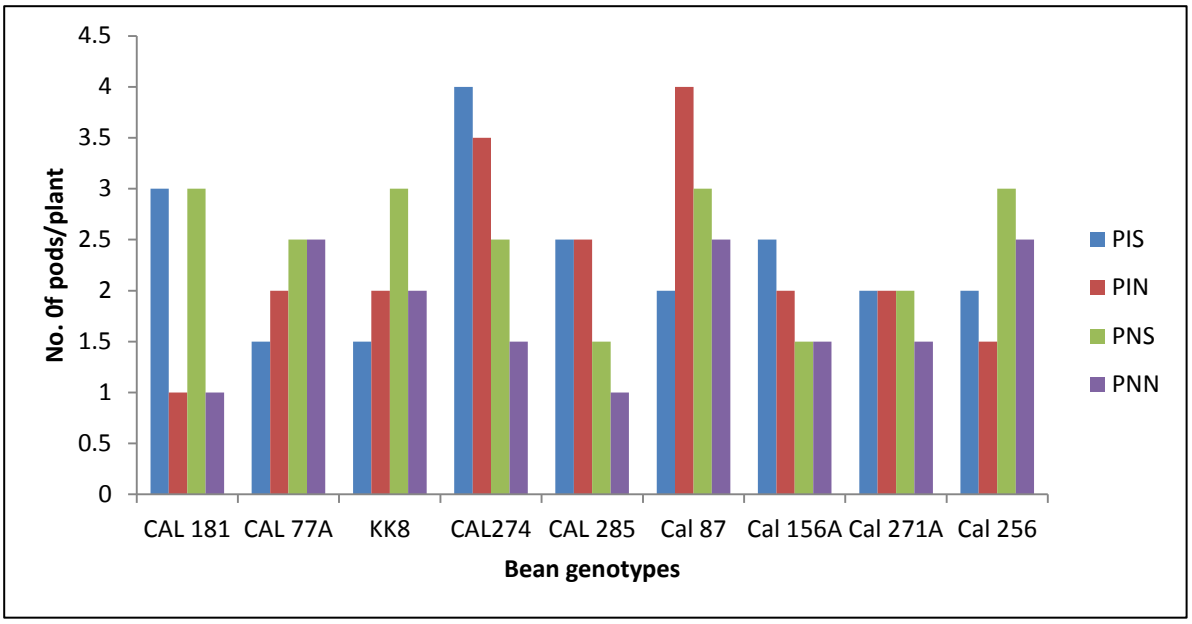

Figure 4.3: Number of pods per plant among the nine bean genotypes in the greenhouse

PIS- No. of pods per plant in inoculated plants in sterile soil, PIN- No. of pods per plant in inoculated plants in non-sterile soil, PNS- No. of pods per plant in non- inoculated plants in sterile soil, PNN- No. of pods per plant in non-inoculated plants in non-sterile soil.

4.2.2 Field experiment on screening of bean varieties to Common bacterial blight

4.2.2.1 Effect of the pathogen *Xanthomonas axonopodis pv. phaseoli* on growth parameters

The field trials carried out at KALRO-Kakamega during the long rains of 2014 revealed that the nine bean genotypes varied significantly ($P<0.05$) for all the traits studied. Field experiment (Table 4.3) revealed that the mean CBB disease severity was lower in bean plants that were not inoculated (control) compared to those that were inoculated (Table 4.3). There was a significant ($P<0.05$) variation between inoculated and non-inoculated plants in disease severity rating (CBB score) indicating that all the bean genotypes responded differently to CBB infection (Table 4.3). The bean genotypes that were inoculated with *Xap* bacteria recorded a significantly ($P<0.05$) higher CBB score

compared with the bean genotypes that were not inoculated. There was a significant interaction ($P<0.05$) in CBB severity between the inoculated and non-inoculated bean genotypes (Table 4.3). The genotype response to *Xap* infection in the field conditions varied from slightly resistant to tolerant with all the genotypes being tolerant except Cal 156A, Cal 256, Cal 271A and Cal 274 that were slightly resistant based on the CIAT score index (Table 4.3).

The field weight of various bean genotypes varied significantly ($P<0.05$) with CAL 87A recording significantly higher weight (Table 4.3). Inoculated bean genotypes recorded significantly ($P<0.05$) lower plant weight compared to non-inoculated indicating a significant difference in the treatment (Table 4.3). In addition, the interaction between the treatments and genotypes varied significantly ($P<0.05$) as indicated in Table 4.3.

The bean yields per plot in the field differed significantly among the genotypes and between the treatments with inoculated genotypes recording significantly ($P<0.05$) lower yields compared to the non-inoculated bean genotypes (Table 4.3). Genotype Cal 87A had significantly higher yield.

Table 4.3. CBB severity and yield components of bean genotypes in the field long rains 2014 KALRO-Kakamega

	CBB score#	Plant field weight (g)	Yield/ Plot (g)	Plant height (cm)	No.Seed / pod	No.Pod / plant	Pod length (cm)
Genotype**							
CAL 156A	4.50±0.72 a*	85.21±50.02c	26.32±6.66c	20.50±1.52b	3.33±0.42a	6.33±1.40a	8.83±0.40c
CAL 181	2.83±0.16bcd	169.51±27.63abc	60.91±13.39bc	29.50±2.55ab	3.67 ±0.42a	11.00±2.26a	8.83±0.70c
CAL 256	4.00 ±0.44ab	83.90±29.87c	32.47±10.04bc	24.50±1.92ab	3.83± 0.70a	6.00±1.41a	9.50±0.84bc
CAL 271A	3.66±0.61ab	158.56±25.89abc	64.40±10.50bc	31.00±3.60ab	4.50± 0.56a	8.50±0.99a	11.16±0.60a
CAL 274	3.50±0.56abc	129.86±35.14bc	48.35±17.66bc	35.83±4.14a	3.33±0.55a	9.66±3.09a	9.83±0.70bc
CAL 285	2.33±0.21cd	172.63±40.86abc	58.80±18.44bc	37.33±4.13a	3.50±0.34a	8.83±1.24a	10.33±0.33ab
CAL 77	2.16±0.16d	266.95±35.35ab	102.63±10.17ab	32.50±2.27ab	4.17±0.30a	9.33±1.70a	11.16±0.79a
CAL 87A	2.16±0.16d	316.81±65.34a	167.25±28.72a	29.83±2.12ab	4.00±0.57a	9.50±1.40a	11.16±0.70a
KK8	2.33±0.21cd	119.88±30.50bc	49.01±16.51bc	25.83±1.72ab	4.00±0.25a	9.16±1.90a	9.00±0.25bc
Treatment							
Non inoculated	2.52±0.11	205.49±23.58	80.46±11.30	32.15±1.59	4.44± 0.17	10.82 ±0.80	10.74 ±0.30
Inoculated	3.59 ±0.29	128.59±19.00	55.13±9.24	27.15±1.43	3.19 ±0.19	6.59±0.68	9.22±0.29
P values							
Genotype	<.0001	< 0.0015	<.0001	0.0038	0.5001	0.5326	0.0077
Treatment	<.0001	< 0.0053	0.0259	0.0125	<.0001	0.0006	0.0003
Genotype*Treatment	0.0026	0.09747	0.9927	0.9876	0.7246	0.9783	0.9403

*Values (Mean ± SE) followed by different letters along the column are significantly different according to Tukey's HSD at 95 % probability level.

**Cal (Calima), KK (Kakamega)

#CBB was scored on a scale of 1-9 based the CIAT scale as; no symptom=1, slight=3, moderate=5, severe=7 and complete discoloration=9 (Buruchara *et al*., 2010). Resistance (R) was assigned to plants with no or limited symptoms (score 1-3), tolerance (T) was assigned to plants with 4-6 disease score, whereas plants with a score of 7-9 were considered to be susceptible (S).

Although the plant length of inoculated and non-inoculated beans differ significantly ($P<0.05$) among the genotypes and between the treatments, there was no significant interaction between the treatments and genotypes being established (Table 4.3). Non-inoculated bean plants were taller with mean plant height of 32.148cm while those that were inoculate had mean height of 27.148cm (Table 4.3). In inoculated plants, Genotype Cal 156A was the tallest (38.3 cm) while genotype Cal 181 was the shortest (24.6 cm). In non-inoculated bean plants, Cal 181 was the shortest (33.4 cm) while genotype Cal 285 tallest (39.3 cm). Cal 285 recorded the highest mean of 37.33cm while Cal 156A with 20.5cm was the lowest.

Even though the number of seeds per pod, pod per plant and pod length varied significantly ($P<0.05$) between the inoculated and non-inoculated bean plants, there was no significant difference ($P<0.05$) that was established among the genotypes in the number of seeds per pod and pods per plant (Table 4.3). There was no significant interaction observed between the bean genotypes and the treatment in the number of seeds per pod, pod per plant and pod length (Table 4.3).

4.3 Effect of soil amendment and method of cropping on incidence and severity of common bacterial blight disease

4.3.1 Effect on disease incidence and severity

Disease incidence, distribution and severity differed significantly ($P<0.05$) among the different bean genotypes. The CBB score varied significantly ($P<0.05$) among the bean genotypes and was influenced by the soil amendments applied and the method of cropping used either monocropping or intercropping (Table 4.4).

Non-inoculated plants (control) showed significant ($P<0.05$) resistant reaction to CBB disease where disease symptoms varied from moderately resistant to complete resistant reaction (Table 4.4). Genotypes Cal 285 and Cal 87 showed moderately resistant reaction for disease severity (4.28 and 4.56 respectively). Genotypes Cal 77 and Cal 156A showed complete resistance to disease reaction with scores of 1.94 to 2.28 on the CIAT scale (Table 4.6). Inoculated bean plants showed resistant reaction to CBB disease symptoms with genotypes Cal 77 and Cal 156A showing complete resistance to disease reaction with scores of 2 while genotypes Cal 285 and Cal 87 showed moderately resistant reaction with disease severity ranging from 4 to 5 on the CIAT scale (Table 4.4).

There was a significant interaction ($P<0.05$) in CBB severity between the bean genotypes and treatment, among the genotypes and among the treatments (Table 4.4). Inoculated bean plants grown in DAP recorded significantly ($P<0.05$) lower CBB score while those grown without any soil amendment (PN) had significantly ($P<0.05$) higher CBB score. All the other treatments did not differ significantly with each other (Table 4.4).

4.3.2 Effect of soil amendment on plant growth parameters

Bean plants grown with DAP were significantly ($P<0.05$) taller than those grown on soils with chicken manure with Cal 285 recording the highest value (Table 4.4). Monocropped beans were also significant ($P<0.05$) taller than those that were intercropped while beans grown without DAP or grown with manure alone were significantly ($P<0.05$) shorter. There was significant difference ($P<0.05$) in plant height among the four bean genotypes and within the farm practice. There was also significant ($P<0.05$) interaction between the bean genotypes and the farm practice.

Plant weight was significantly lower ($P<0.05$) in *Xap* inoculated plants than in the non-inoculated plants (Table 4.4). Although there was no significant difference ($P<0.05$) in plant weight among the four bean genotypes, there was significant difference ($P<0.05$) in the farm practices. There was a significantly higher plant weight when grown with DAP (Table 4.4). Monocropped bean genotypes had a significantly ($P<0.05$) higher weight than intercropped beans while beans grown alone recorded significantly ($P<0.05$) the least weight (Table 4.4).

Table 4.4. Mean effect of farm practice on disease severity and incidences in the field conditions

	CBB Score	Plant height (cm)	field weight (g)	Yield gram/plot	No.Seed per pod	No.Pod per plant
Genotype						
CAL 156A	2.28±0.17*b	38.47±2.04b	1194.44±118ba	83.27±6.69b	3.22±0.15b	10.77±1.21c
CAL 285	4.28±0.13a	47.44±2.02a	983.33±102.66a	93.47±11.88b	4.00±0.22a	10.55±0.56c
CAL 77	1.94±0.12b	46.52±1.53ab	1283.33±103.61a	110.05± 8.01b	3.61±0.16ab	13.72±0.85b
CAL 87A	4.56±0.12a	47.22±2.90a	1305.56±103.99a	167.32±16.22a	3.67±0.14a	15.22±1.58a
Farm practice						
ID	2.91±0.41c	45.58±2.19a	1341.67±106.92ab	150.29±0.59a	3.58±0.22ab	14.33±0.96b
IM	3.00±0.37bc	42.83±1.91a	1116.67±109.29ab	125.23± 15.44a	3.83±0.32ab	10.41±0.84e
IN	3.50±0.45ab	43.54±3.79a	983.33± 135.86ab	73.52 ± 6.09b	3.33±0.22b	7.75±0.62f
PD	3.00±0.36bc	47.87±2.88a	1383.33±141.33ab	138.07± 17.90a	3.33±0.18b	12.25±0.87d
PM	3.41±0.35abc	48.54 ±3.02a	1433.33 ±137.80a	120.99± 13.63a	4.00±0.17a	17.41 ±0.91a
PN	3.75±0.30a	41.71±2.70a	891.67 ±106.92b	73.07 ±8.42b	3.66±0.14ab	13.25±2.29c
P values						
Genotype	<.0001	0.0167	0.1222	<.0001	0.0002	<.0001
Farm practice	<.0001	0.4144	0.0139	<.0001	0.0051	<.0001
Genotype* Farm practice	0.0091	0.7902	0.8337	0.1779	<.0001	<.0001

*Values (Mean ± SE) followed by different letters along the column are significantly different according to Tukey's HSD at 95 % probability level

ID- intercropped bean in DAP, IM- intercropped bean in manure, IN- intercropped bean alone, PD- monocropped bean in DAP, PM- monocropped bean in manure, PN- monocropped bean alone. CAL (Calima).

Yield in grams per plot was significantly lower ($P<0.05$) in *Xap* inoculated than in non-inoculated plots (Table 4.4). There was a significant variation of yields ($P<0.05$) among the genotypes and among the treatments. In the *Xap* inoculated plots, Genotype Cal 87 had significantly ($P<0.05$) higher yield (167.32g) followed by Cal 77 (110.05g), Cal 285 (93.47g) and Cal 156A (83.27) (Table 4.4). Beans planted with DAP had a significantly ($P<0.05$) higher yield in both intercropped and monocropped plots while the intercropped plots with manure had a significantly higher yield than in the monocropped plots. The yields were lowest in plots planted with neither DAP nor chicken manure (Table 4.4).

Genotype Cal 285 and Cal 156A had significantly ($P<0.05$) less number of pods per plant (PPP) than Cal 77 and Cal 87A (Table 4.6). Genotype Cal 87A had significantly ($P<0.05$) more pods than the other genotypes intercropped and grown with either DAP or chicken manure. Genotype Cal 156A monocropped and grown with chicken manure had significantly ($P<0.05$) more number of pods while Cal 285 had the least. There was significant difference ($P<0.05$) in the number of pods per plant between monocropped and intercropped beans. The number of pods per plant was lowest when grown alone than when grown in either DAP or manure (Table 4.4). The number of seeds per pod ranged between 3 and 4 in all the genotypes with Cal 285 recording significantly ($P<0.05$) higher average mean number of seeds per pod of 4.00 while Cal 156A had the least (3.22) (Table 4.4). Monocropped bean plants grown with chicken manure had significantly more ($P<0.05$) seeds per pod (4.00) than all the other treatments with intercropped beans grown without any soil amendment having significantly ($P<0.05$) the lowest mean for seeds per pod (3.33) (Table 4.4).

There was significant difference ($P<0.05$) observed among the genotypes and between the genotypes and farm practices in CBB score, yield per plot, seeds per pod and pods per plant.

CHAPTER FIVE

DISCUSSION

5.1 Virulence of *Xanthomonas axonopodis pv. phaseoli* on bean cultivars in Kakamega

Common bacterial blight is among the most devastating bean pathogen associated with the highest yield losses in bean growing regions in Kenya. In the present study, the results on pathogenicity test and those on morphological characterization of the bacterium on the XAP1 medium established that the bacterial pathogen which was isolated and recovered from leaves was *X. axonopodis pv. phaseoli* (*Xap*). The isolates that were recovered from leaf samples plated on the semi-selective medium XAP1 were categorized as *Xanthomonas* like, with regard to their yellow pigment and convex mucoid morphology. In addition, the colonies of *Xap* on XAP1 medium were smooth, convex, mucoid, bright yellow, round with entire margins as observed by Shenge *et al*., (2006). Moreover, biochemical test results revealed that the colonies were rod shaped and gram negative. This is in agreement with the findings by Akhavan *et al*. (2013) and that of Belete and Bastas (2017) on common beans in Iran and Ethiopia respectively revealing that the pathogen is gram negative rod-shaped single flagella bacteria with yellow convex and slimy colonies when grown on glucose-containing medium.

During the study, it was observed that *Xap* was common in the various types of leaves including those from the research farm. The average frequency was 54.167 % with variation within and between the leaf sources. The leaf samples collected from research trial sites leaf lots showed lower prevalence of 12.5 % while disease prevalence was significantly higher in leaf samples collected from farmers' field leaf lots (41.67 %)

(Table 4.1). The higher disease prevalence in the farmers' fields could be attributed to the spread of the pathogen by farmers during agronomic practices as well as using already infected seeds and contaminated soil. This concurs with the findings by Njingulula *et al*. (2014) and Belete and Bastas (2017) reporting that the CBB causing pathogen is spread among the fields by farmers during agronomical practices through the seeds preserved by the farmers from the previous harvest, contaminated soils, rain water and wind leading to a higher disease prevalence. A similar study was also reported in Uganda by Opio *et al*. (1993) on the *Xap* infection levels on farmers selected seeds and those from agricultural research stations revealing higher *Xap* infection on farmers selected seed types. This is evident as seen where the *Xap* population on seeds from the farmers recorded the highest cfu compared with those seeds sourced from the research station.

The variation in CBB severity between the research site and farmers field could have been as a result of the different seeds following different seed production and handling management including cultural practices. It is also evident that most farmers use uncertified seeds and recycle the seeds for years (Belachew, 2014; Njingulula *et al*., 2014). Coupled with poor farming practices, the result is continuous built up of CBB pathogen (Fininsa, 2003; Njingulula *et al*., 2014; Belete and Bastas, 2017). The seed-borne nature of the bacteria that causes CBB makes the disease one of the most significant bean diseases universally that is very hard to control (Belete and Bastas, 2017). The higher disease prevalence could also be attributed to the prevailing warm temperatures and humidity (Belete and Bastas, 2017) in Kakamega region influenced by L. Victoria that favour rapid disease progress as supported by the findings in the present study (Tables 4.1, 4.2 and 4.3).

5.2 Screening selected bean genotypes for resistance to common bacterial blight disease

There was significant variation in disease severity rating between *Xap* inoculated and non-inoculated plants and for all the other traits as observe by Popovic *et al*. , (2012) indicating that CBB affected all the growth and yield traits of the bean genotypes. KK8, Cal 256, Cal 87, Cal 181 and Cal 274 bean genotypes exhibited CBB disease symptoms following inoculation with *Xap*. The CBB severity and incidence varied significantly between sterilized and unsterilized soil indicating that there was CBB inoculum in the forest soil that was used in the experiment. All the nine bean genotypes grown in non-sterile soil recorded the highest CBB score. The higher CBB score could be attributed to higher *Xap* population in the soil. Based on the CIAT scale, the genotype response to *Xap* infection in the field conditions varied from slightly resistant to tolerant with all the genotypes being tolerant except Cal 156A, Cal 256, Cal 271A and Cal 274 that were slightly resistant as indicated in Table 4.3.

Disease symptoms in bean plants inoculated with *Xap* were also expressed in the form of reduction in number of leaves. This effect was quite evident in genotypes Cal 87, Cal 181 and Cal 274 (Table 4.2). The reduction in number of leaves could be due to reduced plant vigor and premature defoliation resulting from CBB necrotic lesions (Dursun *et al*., 2002; Akhavan *et al*., 2013). Genotypes Cal 87, Cal 181 and Cal 274 exhibited reduction in height when inoculated with *Xap* in the field. Therefore, the five bean genotypes (Cal 271, Cal 256, Cal 87, Cal 274, and Cal 181) may be considered susceptible to CBB disease based on reduction in number of leaves and plant height. The other four

genotypes (KK 8, Cal77, Cal 285 and Cal 156A) were resistant to *Xap* based on number of leaves and plant height (Muimui *et al*., 2011).

Five genotypes; KK8, Cal 256, Cal 87, Cal 181 and Cal 274 may be considered susceptible to the pathogen based on pod counts, pod weights and mean seed weight per plant. By contrast, genotypes Cal 77, Cal 271, Cal 285 and Cal 156A did not exhibit reduction in pod counts or pod weights when inoculated with *Xap*, and could be considered more tolerant based on the three yield-related parameters. This is in agreement with a previous study by Belachew *et al*. (2015). The highly significant differences indicate the existence of large variability among genotypes to *Xap* thus there is significant difference in the reaction of bean genotypes to CBB.

With the CIAT disease severity scale, genotypes CAL 77 and Cal 156A are the only ones among the nine genotypes that were more tolerant to CBB. Indications of their CBB resistance were still evident in most of the other plant growth and yield parameters. However, the reduction in number of leaves, pod length and days to maturity is a clue that genotype Cal 77 may be less tolerant compared to CAL 156A. The direct relationship between the leaves and the pods was because the larger the photosynthetic area the higher the yield due to increased size and number of pods (Belete and Bastas, 2017).

The low disease severity scores exhibited in cultivar CAL 77 and KK 8 may indicate inhibition of plant tissue colonization by *Xap* in the bean genotypes. Although the number of leaves in genotype Cal 156A significantly reduced when inoculated with *Xap*, it recorded shortest days to 90% maturity, maximum height, pod length and the highest

yield per plot. Still, the plants had relatively more leaves than the other eight cultivars due to disease tolerance seen through enhanced production of leaves which helped the plants to quickly replace leaves that had been damaged by *Xap*-related necrosis, while sustaining photosynthetic competence in the presence of CBB infection (Lopes, and Berger, 2001).

Throughout the study, the beans that were inoculated with *Xap* showed the CBB symptoms on the leaves and the pods. The disease severity was more in the genotype Cal 271A and Cal 256 compared to the non-inoculated bean plants. The two bean genotypes can be seen to be susceptible to CBB. This resulted in the reduction on the other plant growth parameters including the height of the plant, length of the pods, number of pods per plant and the overall yield. The degree of yield decline caused by CBB in the present study agrees with the results obtained by Wendland *et al*. (2010) and Gillard *et al*. (2009) who reported reduction on yield on susceptible bean genotypes. There is a direct relationship between the CBB severity, seed weight and yield loss. This was seen in Cal 256 that recorded the lowest yield per plant (33.12). This agrees with the findings of

The relative CBB rating was similar for both green house and field conditions, but most bean genotypes were more resistant when grown in the field condition.

5.3 Effect of soil amendment and method of cropping on incidences and severity of Common bacterial blight disease on the available bean genotypes

Bean plants grown with DAP and poultry manure were more resistant to *Xap* than those grown without. This could be attributed to plant nutrition that has a great influence on

disease development either by facilitating disease escape or by enhancing physiological resistance to the plant.

Christos (2008) also noted that there is an increased tolerance to disease through compensation. Besides, bean genotypes grown with DAP recorded better plant growth parameters that were assessed with Cal 77 and Cal 156A having taller and more number of pods and generally higher yield. The high yield could be as a result of increased photosynthetic rate that lead to increased plant growth vigor especially during flowering as observed by Befrozfar *et al*. (2013). The higher yields could also be attributed to increased flowering rate that might have resulted in more pods per plant.

It was also noted that beans planted with DAP did better than those in chicken manure. This could be due to the difference in their chemical composition (Hira *et al*., 2016) or their rate of absorption by the plants as observed by Sallam (2011). The higher performance of the bean plants in DAP indicates its ease of dissolution of the nutrients because it is in a more soluble form compared to poultry manure, which contain important and useful soil nutrients (Ayeni *et al*., 2012), but being in a crude form, that are slowly released into the soil (Nyankanga *et al*., 2012). This could be the reason for the slow growth, few numbers of pods and low yield in the control.

Monocropped beans were also better and more productive than the intercropped with Cal 156A having the highest number of pods and pod length. Intercropping practice used was planting maize and beans in the same planting hole and resulted in interspecies competition for water, light and other nutrients in the soil as observed by Fininsa (2003) and Hauggaard, *et al* ., (2007). The yield was high in Cal87 when intercropped and this

could be attributed to reduced disease incidence because the maize restricted the movement of *Xap* between bean plants by providing a physical barrier.

In this study, the range and mean values suggest the existence of sufficient variability among the bean genotypes under test for the majority of the characters. This means that genotype with short pod length produced low seed yield per plants. This result agrees with Fininsa (2003) who reported that both pod length and number of pods per plant have negative significant associated with seed yield per plants.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. The experiments confirmed the presence of *Xap* pathogen in diverse leaf sources in the area under study with the leaves obtained from research trial site having lower levels of pathogen infection compared to leaf from farmer's field.

ii. Results on CBB disease severity and incidence in the green house and in the field indicate that CAL77 and Cal 156A genotypes exhibited high level of resistance to CBB, thus a better variety to use. Seven genotypes namely Cal 285, Cal 256, CAL271A, Cal274, KK 8, CAL 181 and Cal 87 exhibited moderate level of tolerance reaction based on the CIAT disease severity scale.

iii. Common Bacterial Blight disease caused both quantitative and qualitative yield losses depending on bean genotype and its resistance.It was also noted that beans planted with DAP were taller, had more pods and higher yield than those in chicken manure and that monocropped beans were better and more productive than the intercropped beans.

6.2 Recommendations

i. Further evaluation to be done using other isolates of *Xap* that are known to commonly occur in Western Kenya.

ii. The actual factors that confer high levels of tolerance to CBB in genotype CAL 77 and Cal 156A need to be established through identification of mode and the nature of defensive mechanisms that could be protecting the genotype from CBB disease and could potentially be used for breeding more resistant genotypes. It could be incorporated in CBB-susceptible bean cultivars. Susceptible genotypes may be tried in other locations that are less prone to *Xap.*

iii. Integrating the use of resistant varieties, treatment of seeds with chemical and use of correct cultural farm practices could be one of the best substitutes in controlling common bacterial blight of common bean and avoiding yield loss.

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APPENDICES

APPENDIX I: Composition of poultry manure

PARTICULARS	Deep litter	Broiler house	Cage manure
C/N Ratio	9.5-11.5	9.4-11.2	5.8-7.6
Total N%	1.70-2.20	2.40-3.60	3.63-5.30
Total P_2O_5%	1.41-1.81	1.56-2.80	1.54-2.90
Total K_2O%	0.93-1.30	1.40-2.30	2.5-2.9
Fe(ppm)	930-380	970-1370	970-1450
Zn (ppm)	90-308	160-315	290-460
Cu (ppm)	24-42	27-47	80-172
Mn (ppm)	210-380	190-350	370-590
Mn (ppm)	0.90-1.10	0.86-1.11	0.80-1.02
Ca%	0.45-0.68	0.42-0.65	0.40-0.46

Amanullah *et al*., 2007

APPENDIX II: Diammonium Phosphate Composition

Chemical formula	$(NH4)_2HPO_4$
Composition	18% N 46% P2O5 (20% P)
Water solubility (20 ºC	588 g/L
Solution Ph	7.5 to 8

(Source; IPNI. 2014)

APPENDIX III: Scoring at growth stages

Scale	**Plant parts affected.**
1	No visible disease symptoms.
3	Approximately 2% of the leaf surface area covered with a few small lesions. Pods are generally free of lesions.
5	Approximately 5% of the leaf surface area covered by small lesions that are beginning to coalesce and sometimes encircled by yellow halos resulting in minor blight. Lesions on the pods are generally small and not coalescing.
7	Approximately 10% of the leaf surface area covered with medium and large lesions which are usually accompanied by yellow halos and necrosis. Lesions on pods are large and coalescing and often show bacterial exudates.
9	More than 25% of the leaf surface area with large coalescing and generally necrotic lesions resulting in defoliation. Lesions on pods coalesce to cover extensive areas, exhibit abundant bacterial exudation which sometimes causes pod malformation and empty pods.

Source; Buruchara et al., 2010; Manandhar et al., 2016

APPENDIX IV: Map of Kakamega County