

**REACTIONS OF 45 BEAN LINES TO BACTERIAL BLIGHT (*Xanthomonas axonopodis phaseoli*) ISOLATES FROM MAJOR BEAN PRODUCING AREA OF
TANZANIA**

BY

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ABSTRACT

Studies were conducted, to screen 45 BC₁F₅ bean lines for common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli* L) resistance using bacterial blight isolates collected from lowland and mid altitude bean growing areas in Tanzania. Infected samples with common bacterial blight (CBB) symptoms were used to obtain CBB bacterium isolation in a semi-selective bacterial blight differential (BBD) medium. Isolates in the growth medium were incubated at 25 – 30°C for 4 days for the normal CBB strain and up to 6-7 days for its variant “fuscous” strains. Local susceptible check (Canadian wonder) and resistance check (VAX 6) were used to inoculate 45 F₅ bean lines in the field and screen house experiments. Results from the field experiment, inoculated with *Xap* isolate from Morogoro district showed that there were significant differences in reactions ($P \leq 0.05$ and $P \leq 0.01$) among bean lines evaluated. Five lines; PSO3 – 557 (C) – 15, PSO4 – 120 (A) – 15, PSO4 – 120 (A) – 8, PSO3 – 557 (C) – 12 and PSO3 – 557 (C) – 11 showed to be highly resistant. From the screen house experiment, line PSO4 – 72 (B) – 4 was resistant to all isolates. Lines PSO4 – 148 (A) – 11, PSO3 – 585 (A) – 12, PSO3 – 585 (A) – 21, PSO4 – 148 (A) – 15, PSO3 – 585 (A) – 8, PSO4 – 122 (D) – 5, PSO3 – 557 (C) – 7, PSO3 – 557 (C) – 11, PSO4 – 122 (D) – 4 and PSO3 – 557 (C) – 2, were resistant to three isolates hence highly resistant. Twenty six lines were resistant to two or one isolates. Isolate obtained from Mbeya was more virulent than isolates from Ismani, Mgeta and Morogoro. From these findings it was concluded that, transferring genes for CBB resistance in adapted bean varieties was successful since resistant lines were identified among the BC₁F₅ lines. Therefore, there is a need for further studies on *Xap* reaction of bean breeding materials to different isolates from major bean producing areas in the country.

DECLARATION

I, Hezron Kumbuka Mwaitwalile Tusekelege, do here declare to Senate of the Sokoine University of Agriculture that this dissertation is my own original work and has not been submitted for a degree award in any other University.

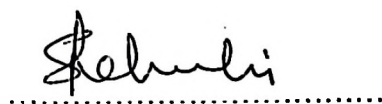


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DEDICATION

This dissertation is dedicated to my beloved parents; Silibwila Ntende Tusekelege and the late Mr. Kumbuka Tusekelege who has enriched my life beyond any wealth I will ever dream.

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LIST OF ABBREVIATION AND SYMBOLS

ARI	Agriculture Research Institute
ANOVA	Analysis of variance
BBD	Bacterial blight differential
BC ₁	Back cross one
°C	Celsius degree.
CBB	Common bacterial blight
Cfu	Colony forming unit (s)
CIAT	Centro Internacional de Agrcultura Tropical
Cm	Centimetre.
CRSP	Collaborative Research Support Program
Cv	Cultivar.
DAP	Days after planting
DF	diffusible factor
et al.,	et alei (and others)
F ₅	Filiar generation five.
g	gramme (s)
ha	hactare
hr	hour
kg	kilogramme
LSD	Least significant difference
MAS	Marker-assisted selection
ml	millilitre
mm	millimeter
NA	Nutrient agar

PCR	Polymerase Chain Reaction
Pv	pathovar
QTL	quantitative trait loci
r	Simple correlation coefficient
R5	Reproductive phase stage five (Pre flowering)
RFLP	Restriction Fragment Length Polymorphism
SADC	Southern Africa Development Cooperation
SE	Standard error.
SUA	Sokoine University of Agriculture
TARP II	Tanzania Agricultural Research Project Phase Two
Xap	Xanthomonas axonopodis pathovar phaseoli
Xapf	Xanthomonas axonopodis pathovar var. fuscuous
YDC	Yeast dextrose carbonate agar.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Common bean (*Phaseolus vulgaris* L.) is one of the most important crops worldwide in both economic and nutritional aspects. Common bean is also an important legume crop which plays a crucial role in food protein supply for direct human consumption in the world and in developing countries (Popelka *et al.*, 2004; Broughton *et al.*, 2003). Common bean (*P. vulgaris*) is a major food crop that provides an inexpensive source of protein for both rural and urban households in East Africa (Mkandawire *et al.*, 2004).

Common bean is the major staple in the Eastern and Southern Africa, and is recognized as the second source of human dietary protein and the third most important source of calories. These are indispensable nutritional supplement in diets based on cereal and /or starchy tuber crops (Pachico, 1993). Common beans is ranked first among the leguminous crops in Tanzania, due to its importance as an exceptionally high potential for alleviating hunger and malnutrition for most people in rural areas to children and the sick (Mduruma, 1996). The importance of this crop is not only due to its use as a less expensive source of dietary protein to both urban and rural communities, but also as an income earner crop (Broughton *et al.*, 2003; TARP II SUA Project, 2003).

Despite the fact that common bean is viewed as one of the most important crops in Tanzania and other developing countries in the world, most producers are small-scale resource-poor farmers whose production is largely based on unimproved local cultivars that have been selected over many years in their localities (Mduruma, 1996); Gepts and

Debouck, 1999). Furthermore, production of the crop is limited by numerous biotic and abiotic problems (Mkandawire *et al.*, 2004).

According to Venette and Lame. (1998) and; Rosana *et al.* (1999) among the major problems affecting crop yield losses of beans in Tanzania and in the world, is the common bacterial blight (CBB) disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin *et al.* (1995) *Dye (Xap)*. Common bacterial blight (CBB) disease is a problem to sustainable bean production in Tanzania and other developing countries (Kelly and Miklas, 1998; Mkandawire *et al.*, 2004 and Singh *et al.*, 1992). In Tanzania, the disease is endemic in almost all bean growing areas, however, more severe epidemics occur in hot (25 – 30°C) lowland and mid altitude areas ranging between 450 to 1500 meter above sea level (MAFS, 2002).

Common bacterial blight {(Xap) and (Xapf)} diseases of bean can cause epidemics with losses exceeding 75% of potential yield under severe conditions of infection in East Africa and in the Southern Africa Development Cooperation (SADC) countries (Opio *et al.*, 1996). Hanounik *et al.* (1993) reported that, given the complexity of the diseases and their soil-borne nature, breeding for resistance is one of the most difficult challenges for bean breeders and pathologists. Many of the bean varieties planted by bean farmers in Tanzania have susceptibility to the CBB disease (Mduruma, 1996).

Infection late in the season result into lower disease incidence and severity (Morales and Niessen, 1988). Various techniques have been used in testing the reactions to virulent isolates of *Xap* in the field screening and in the screen house inoculation, combining with

MAS (Marker Assisted Selection) for CBB resistant genotypes in common bean (Mutlu *et al.*, 2005a).

There is no satisfactory chemical control for CBB disease (Opio *et al.*, 1996; Ariyaratne *et al.*, 1998 and Aggour *et al.*, 1988b), control seems most likely achieved through the development of resistant breeding program through development of cultivars and or lines with resistance to *Xap* pathogens.

Studies on pathogenic variation in *Xanthomonas axonopodis* pv. *phaseoli*, the causal agent of common bacterial blight disease in bean have been conducted in East Africa. Finding reveal that there is considerable genetic diversity within the population of *Xanthomonas axonopodis* pv. *phaseoli* in Eastern Africa (Opio *et al.*, 1996). Mkandawire *et al.* (2004) have reported on diversity and pathogenic variation of common bacterial blight and its variant fuscans (*Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans*) in East Africa indicating the existence of genetically and geographically distinct variants of this pathogen. Miklas *et al.* (1996) and Mahuku *et al.* (2006) reported on genotypic characterization of the common bean bacterial blight pathogens, *Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans* (*Xapf*), that the two CBB pathogens are genetically distinct as observed by using rep-PCR and PCR- RFLP of the ribosomal genes.

Breeding for effective deployment of disease resistance genes improvement and productivity within the farming systems is best addressed through a combination of genetic and phytotechnical management (the use of resistance genes sources) solutions that are accessible to poor farmers (Pastor-Corrales *et al.*, 2003; Snoeck *et al.*, 2003) in

varied cropping systems and agro – geographical regions. Increased productivity may be emerging from work on edaphically resistance of aerial parts of the plants which are the major avenues of bacterial colonization. Lack of money to purchase pesticides and the dangers of pesticide toxicity also make breeding for disease resistance a desirable priority. High levels of cultivar resistance would minimize yield losses; reduce antibiotics use and production costs and hence would facilitate integrated disease and crop management cum production and distribution of pathogen – free seed.

In spite of records of the occurrence of various seed- borne bacterial diseases in Tanzania, not much is known about the reactions of bean lines (*P. vulgaris* L.) to bacterial blight isolates found in some major growing areas and their economic yield losses on crops grown in the country. Although for other diseases like Angular leaf spot and halo blight studies have been conducted, for common bacterial blight (CBB) disease reactions on breeding lines detailed studies have not yet been done in the country. This study was conducted to evaluate the reaction of BC₁F₅ breeding materials to common bacterial blight isolates. These crosses were made by SUA- CRSP Bean project (Nchimbi *et al.*, 1990) and the selected lines with resistance to CBB were recommended for further evaluation.

The main objective of this study was to identify bean lines with resistance to CBB bacterial isolates among those developed by hybridization at SUA for low and mid altitudes bean growing areas in Tanzania. The specific objective is;

- (i) To determine the reaction of bean lines to different *Xanthomonas* isolates.
- (ii) To identify lines with CBB resistant genes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Occurrence and Distribution of Common bacterial blight in common beans

Common bacterial blight (CBB) is a serious disease of common beans which has worldwide occurrence in the tropical and sub – tropical countries (Wortmann *et al.*, 1998). Common and fuscous blights of bean caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and its variant *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (*Xapf*) respectively, occur frequently in temperate and tropical climate (Gilbertson and Maxwell, 1992).

Symptoms, epidemiology and ecology of these two *Xap* pathogens are thought to be similar, fuscous strains being generally more aggressive than *Xap* strains as was reported by Vidaver. (1993). Sanitary control of this seed borne disease is complicated by epiphytic and asymptomatic growth of the bacteria on the bean canopy (Weller and Saettler, 1980a). Weller and Saettler. (1980b) reported that symptoms development on leaves require inoculum density of at least 5×10^6 cfu/20 cm² of leaf tissue after inoculation.

Expression of some traits in plant – microbe interaction is density dependent through quorum sensing (von Bodman *et al.*, 2003; Dow *et al.*, 2003). It has been shown that for *Xanthomonas campestris* pv. *campestris*, epiphytic survival is also dependent on the production of diffusible factor (DF) implicated in xanthomonadin and exopolysaccharide production in a cell – density – dependent manner (Poplawsky and Chun, 1989; Poplawsky *et al.*, 2000). Epiphytic multiplication is affected by environmental factors and / or host physiology (Weller and Saettler, 1980b). For colonization of aerial parts of plant,

bacteria need to deal with frequent and major environmental changes (Hirano and Upper, 1989). The most common sites of bacterial colonization are stomata, the base of trichomes and depressions along veins (Mansvet and Hatting, 1989; Timmer *et al.*, 1987). Elucidating these issues mentioned is of valuable importance for understanding the reaction, ecology and biology of an epiphytic plant – pathogenic bacterium such as *Xanthomonas axonopodis* pv. *phaseoli*.

Common bacterial blight is one of the five major diseases of beans which are occurring in the tropic and sub – tropical climates. Countries where CBB is epidemic are Argentina, Brazil, Colombia, Costa Rica, Dominican Republic, Guatemala, Mexico, Nicaragua, Peru, Puerto Rico, Venezuela in Latin America and Angola, Burundi, Cameroon, Gabon, Kenya, Madagascar, Malawi, Mozambique, Nigeria, Rwanda, South Africa, Tanzania, Togo, Uganda, Republic of Congo, Zimbabwe and Zambia in Africa. Other regions where CBB has occurred are Australia, some parts of Europe, India, Iran, Israel, Japan, New Zealand and United States of America (Wortmann *et al.*, 1998). The Pathogen *Xap* has a host range that includes the common bean, Cassava, Cotton, Cucurbits, Rice, Tomato, Pepper and Plantain (Bradbury, 1986).

The distribution of CBB in Tanzania shows the prevalence of CBB and is ranked low to medium in major growing areas with the exception of high ranking in some areas in Musoma region. Their occurrence and distribution of common bacterial blight in Tanzania is as shown in the map (Appendix 1). It is also generally believed that common bacterial blight is a disease with two strains, *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscous* (*Xapf*). The former occurs in low to medium altitudes and the later occurs in high altitudes bean growing areas.

2.2 Effect CBB on yield and quality of beans

Many diseases limit dry bean and snap bean yields. Common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* is one of the most important major diseases of bean leading to important crop yield losses in East Africa and central Africa including Tanzania (Singh, 1992; Rosana *et al.*, 1999). In some cases CBB cause epidemics with losses that may exceed 40% - 75% (Coyne and Hoeschle-Zeledon, 2001; Massomo *et al.*, 2005). Opio *et al.* (1992) and Mahuku *et al.* (2002) reported the losses of 26% - 62% which was recorded in Uganda and Zaire. It was estimated that each 1% increase in blight severity causes yield loss of about 10.5 kg – 78 kg/ha, depending on season and crop growth stage.

Due to lack of effectiveness of chemical application control management, breeding for cultivar resistance is the most reliable/effective control strategy for this disease (Opio *et al.*, 1996; Massomo *et al.*, 2003). However, it was observed that resistance to CBB is difficult in evaluation and selection breeding populations because of its complex inheritance and low heritability (Coyne and Schuster, 1974d).

2.3 Etiology

The bacterium *Xanthomonas axonopodis* pv. *phaseoli* is a gram-negative straight rod that is strictly aerobic and mobile by a polar flagellum (Goodwin and Sopher, (1994). There are two forms of this bacterium, the non- pigment producing form, *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and its brown- pigment producing variant, *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (*Xapf*). Both cause similar symptoms and are seed-borne, but *Xapf* has been shown to be more aggressive than *Xap* (Vidaver, 1993). *Xanthomonas axonopodis* pv *phaseoli* var *fuscans* produces a melanin- like pigment on tyrosine medium, which results from the secretion and oxidation of homoglenic acid, an

intermediate in tyrosine catabolic pathway. In the non-fuscans strain (*Xap*), a complete catabolic pathway that permits the use of tyrosine as a nutrient is present. Bacteria (*Xap*) produce a yellow water-insoluble carotenoid and mucoid growth on nutrient glucose agar. The *Xap* grows well on potato dextrose, nutrient and yeast-extract-dextrose calcium carbonate (YDC) agars (Trujillo and Saettler, 1987). It is a plant pathogen found only in association with plant materials/residues (Venette and Lame, 1998).

2.4 Hosts

The principal host is *Phaseolus vulgaris*, but other legume species are naturally infested, including: *Phaseolus lunatus*, *Vigna aconitifolia*, *Vigna radiata*, *Lablab purpureus* and *Mucuna deeringiana* are possibly natural hosts. *Phaseolus coccineus*, *P. acutifolius* and *Lupinus polyphyllus* are hosts only by artificial inoculation (Bradbury, 1986; Robert, 1994).

2.5 Symptomology

Common bacterial blight is favoured by warm temperature, high moisture and plant wounds during and after flowering as reported by Schwartz *et al.* (2004). On leaves, the disease symptoms first appear as small, angular, light green water-soaked or translucent spots/lesions. They become necrotic with light brown lesions of irregular shape and distinct bright yellow margin. The affected leaves may be killed or wither and even drop off. Similarly, water-soaked spots are formed on the pods and enlarge into reddish brown sunken irregular lesions and in severe cases entire pods shrivel and die. Infected developing seeds many fail to grow, abort or shrivel and discoloured as they mature (Venette and Lame, 1998). Stem symptoms of seedlings include water-soaked, sunken areas that enlarge and develop into reddish streaks. Affected stems commonly crack and

become girdled by water-soaked cankers or rot and the top part of the affected stem may break over during heavy rain or strong winds. In humid weather, yellow ooze exudates from the affected area, which later dries to form, crust (Mohamad, 2000).

2.6 Epidemiology

Common bacterial blight causes severe damage under warm temperatures, high rainfall and high humidity. Maximum disease development is around 28°C. Bacteria grow best in vitro at 25°C – 30°C and declines gradually as temperature is lowered. The disease is more severe with short photoperiod (12 hrs or below) than longer photoperiods (16 hrs or more) (Webster *et al.*, 1983; Sautana, 1985).

2.7 Management options

Bacterial pathogens of dry and snap beans can be managed, but not eliminated, only by carefully implementing integrated crop production strategies. These approaches reduce or delay disease severity during the critical periods of vegetative and reproductive growth stages (Schwartz *et al.*, 2004). The commonly suggested disease management options are discussed here below.

2.7.1 Cultural practices

2.7.1.1 Certified disease-free seeds

The best management option for bacterial blight of bean diseases is prevention. Only certified disease- free seeds should be used as planting materials. This is because the chance of acquiring seed-borne bacterial diseases is significantly reduced by using certified seed from disease free field, hence reduces disease spread to other fields. All seeds must be tested for internal CBB contamination because studies have shown that

symptomless bean plants can still produce contaminated seeds (Cafati and Saettler, 1980a).

2.7.1.2 Crop rotation

Crop rotation with resistant crops gives time for the CBB population in bean debris within a field to decline. In a field where a bacterial disease is a problem, avoid re - using the field again for bean production, until the crop residues are completely decomposed. This can be achieved through deep ploughing to incorporate plant debris into the soil for decomposition and crop rotation out of beans production for a period of at least two years and more. Most bean growers like cabbage growers in Tanzania practice double and/or continuous cropping without proper disposal of infected debris which is thought to be a major avenue of spreading CBB pathogens to the crop (Massomo, 2002). Prevent the spread of the bacterial diseases through equipments or spraying water by avoiding; working, spraying or doing cultural practices when the plants' leaves are wet.

2.7.2 Chemical control

Chemical control includes treating seeds with antibiotics such as streptomycin and/or copper that help to reduce contamination on the surface of the seed coat. Seed treatment will not provide 100% control, but is very effective against surface contamination although it is very expensive for poor resource farmers to be able to afford purchasing the chemicals (Schwartz *et al.*, 2004).

2.7.3 Breeding for resistance

Effective long term control of CBB is best achieved by use of resistant varieties. Considerable effort has been directed to finding new progenies or germplasm with

resistance to CBB in breeding for tolerant varieties (Rava *et al.*, 1987; Musaana, 1991). Sources of resistance to CBB have been identified and observed that the resistance to CBB is quantitatively and qualitatively controlled depending on the source of germplasm with pod and leaf resistance controlled by different genes (Park *et al.*, 1998b; Singh and Muñoz, 1999). Systemic infection of bacterial diseases may be effectively reduced or controlled by the use of resistant and or tolerant improved cultivars. However, most reports indicate that the reaction of CBB in common bean is quantitatively inherited. Molecular marker linked to genes, can assist in selection and offer opportunities to incorporate both quantitative and qualitative resistance in the common bean (Saettler, 1989; Venette and Lame, 1998; Helene and Legard, 1991).

Breeding for resistance to CBB is complicated by pathogenic variation among isolates of CBB as reported by Schuster and Coyne. (1981); Valladares *et al.* (1977) and Maraite. (1989). Moreover, breeding for resistance to CBB is complex because of pathogen variability, resistance linkage with undesirable traits and different genes conditioning resistance in leaves, pods and seeds (Arnaud- Santana *et al.*, 1994; Miklas *et al.*, 1996; Zapata, 1996b). The identification of quantitative trait loci (QTL) with moderate influence on resistance to CBB, combine with phenotypic data implying few genes are involved (Jang *et al.*, 1999; Miklas *et al.*, 1996; Nodari *et al.*, 1993). Kelly and Miklas. (1998) and Beebe. (1988), suggested that marker- assisted selection (MAS) may be useful in breeding for bean resistance improvement to CBB.

Variation in virulence of isolates of CBB within and between geographical locations from which they are collected demonstrate the importance of selecting appropriate strain for breeding for resistance Schuster and Coyne. (1971), Mkandawire *et al.* (2004) and Zapata

et al. (1985) reported that isolates of CBB var. *fuscous* are more aggressive in virulence and were associated with more stem collapse than the common blight isolates in East Africa. Host specialization of the pathogen has been reported on *Phaseolus acutifolius* var. *latifolius* by Zapata (1996a).

The knowledge of pathogenic variation for CBB is still a potential problem in the search for germplasm with resistant to this bacterium and in breeding for resistant and / or tolerant genotypes. Further, investigation is required especially for Tanzania. The information is fundamental to appropriate formulating a breeding programme for resistance to CBB research. The overall objective of this study was to determine the reaction of newly bred common bean lines incorporated with resistant genes to CBB using *Xap* isolates from major production areas of Tanzania.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location

The study was conducted at Sokoine University of Agriculture (SUA). SUA is located about 5 km south east of Morogoro – township, at latitude 6°5' south and longitude 37°39' east at 549 meters above sea level (m. a. s. l) on the foot of mount Uluguru plateau. Annual rainfall totals range between 600 to 800 mm with a bi – modal distribution. There are two cropping seasons corresponding to the rainy seasons. The first season is from October to January; and the second season is from Mid February to May. The mean monthly temperature ranges between 25 and 28 °C with daily mean minimum of 26 °C and mean maximum of 30 °C. The actual mean monthly temperature when conducting the experiment ranged between 25 and 30 °C with daily mean minimum of 25 °C and mean maximum of 34 °C.

3.2 Materials

Forty-five bean lines were used in this study with two varieties serving as checks VAX 6 line (an introduced resistant line) and Canadian Wonder (susceptible variety) respectively (Table 1). These lines were in the BC₁ F₅ generations.

Table 1: Shows the parents sources used in obtaining F₅ generations

Progeny Name	Parents
PSO4 - 120	LYAMUNGU 90 / PSO3 - 556A
PSO4 - 90	LYAMUNGU 90 / PSO3 - 552D
PSO4 - 122	LYAMUNGU 90 / PSO3 - 556D
PSO3 - 557	LYAMUNGU 90 / PSO3 - 118 - I
PSO3 - 92	NJANO / PSO3 - 523B
PSO3 - 585	KABLANKETI / PSO3 -092
PSO4 - 72	BWANA SHAMBA / PSO3 - 500 D
PSO4 - 148	KABLANKETI / PSO3 - 585 A
PSO3 - 556	LY 90 / PSO3 - 117
PSO3 - 552	LY 90 / PSO3 - 115
PSO3 - 118	LY 90 / OTO237 - 17
PSO3 - 094	KABLANKETI / C4 - 1308C
PSO3 - 095	BWANA SHAMBA / BEL NEB - RR - I
PSO3 - 106	NJANO / BEL NEB - RR - I
PSO3 - 115	LY 90 / VSCR - 9
PSO3 - 117	LY 90 / OTO23 -233 (B)

3.3 Collection of isolates

Naturally infected leaves and seeds with characteristic symptoms of CBB were collected from Morogoro, Mgeta, Njombe, Ismani and Mbeya- Uyole ARI. These locations were selected to be representative in terms of occurrence and the distribution of CBB in the Tanzania (Table 2 and Appendix 1). The sample materials of infected leaves were stored between newspapers and left to dry completely before isolation. The maximum storage period before isolation was two months. Data on rainfall, elevation and altitudes were taken at the time of collection of the samples (Table 2).

Table 2: Collection sites, genotype per sample, altitudes and annual rainfall (mm)

Collection site/Areas	Genotypes/Samples	Altitude m.a.s.l	Annual rainfall
Morogoro SUA	Kablanketi var.	494	600 - 800
Morogoro Kihonda	Kablanketi var.	494	600 - 800
Mbeya Uyole	Amendion var.	1800	above 800
Mbeya Uyole	Bilfa corioca var.	1700	above 800
Mbeya	G2333 Var.	1800	above 800
Njombe	Salundo var.	1770	above 800
Njombe	Uyole 98 var.	1770	above 800
Iringa	Uyole 98	1460	600 - 800
Ismani	Wanja var.	1400	600 - 800
Mgeta	Lyamungu 90	1746	above 800



Plate 1: Bean leaf affected by CBB disease used for isolation

3.3.1 Preparations of media

Bacterial blight differential media (BBD) was prepared following the procedures described by Mortensen (1999). Bacterial blight differential media contained in grams per litre (g/l) of distilled water, Sodium- Deoxycholate 0.25 g, Proteose peptone 15.0 g, Yeast extracts 0.5 g, Glycerol 2.5 ml and Agar 7.5 g. These ingredients were added little by little with constant stirring to wet the ingredients thoroughly to avoid formation of lumps. The mixture was heated to dissolve agar for about 3 – 10 minutes in the microwave – oven.

The media also contained solution casein (dried skimmed milk 10.0 g in 250 ml distilled water. The mixture was stirred and heated in a microwave – oven as above.

The two solutions were separately autoclaved and sterilized for 15 minutes at a temperature of 121°C and then were allowed to cool to about 45°C - 50°C. The two solutions were then mixed together and a filter antibiotic stock solution added. The stock solution included cycloheximide (actidione) 80 mg (– 0.8 ml of a 100 mg/ml solution, dissolve in 75% ethanol); cephalexin 20 mg (– 2 ml of a 10 mg/ml aqueous solution which inhibits enterobacteria); vancomycin 10 mg (– 2 ml of a 5 mg/ml aqueous solution) and trimethoprim 20 mg (– 8 ml of 2.5 mg/ml methanol) adjusted to 100 ml with distilled water. Then the mixed solutions were poured in sterilized Petri – dishes of about 20 – 25 ml of solution per Petri dish.

Nutrient agar (NA) was prepared in 1000 ml distilled water to which were added peptone 5.0 g, beef extract 3.0 g and agar 15.0 g. The mixture was stirred for about 10 minutes and heated in a microwave – oven for 5 minutes, finally the solution autoclaved for 15 minutes at 121°C. It was cooled to 40 – 45°C, and then 20 – 25 ml of solution was poured into each petri – dish.

3.3.2 Bacterial isolation

Xanthomonas axonopodis pv *phaseoli* were isolated from infected leaves; about 2 – 4 millimetres (mm) diameter pieces of infected leaves were excised or cut aseptically from individual lesions using a flame – sterilized scalpel and forceps. The pieces were surface sterilized in 0.5 % sodium hypochlorite solution for 2 – 5 minutes and rinsed thoroughly for a few times in sterile distilled water. Then the excised pieces of leaf were grounded

with sterilized glass rod, the grinded tissue segments were teased in a few drops of sterile distilled water, allowed to stand for 5 – 10 minutes in a lamina airflow chamber. A loopful of the resulting suspension was streaked onto plates of growth medium BBD (A semi - selective growth medium for CBB) and incubated at $28 (\pm 2) ^\circ\text{C}$ for 4 – 7 days. The medium were observed daily for the growth of the colonies. Plate 2 and Plate 3, shows the growth of colonies in the medium.

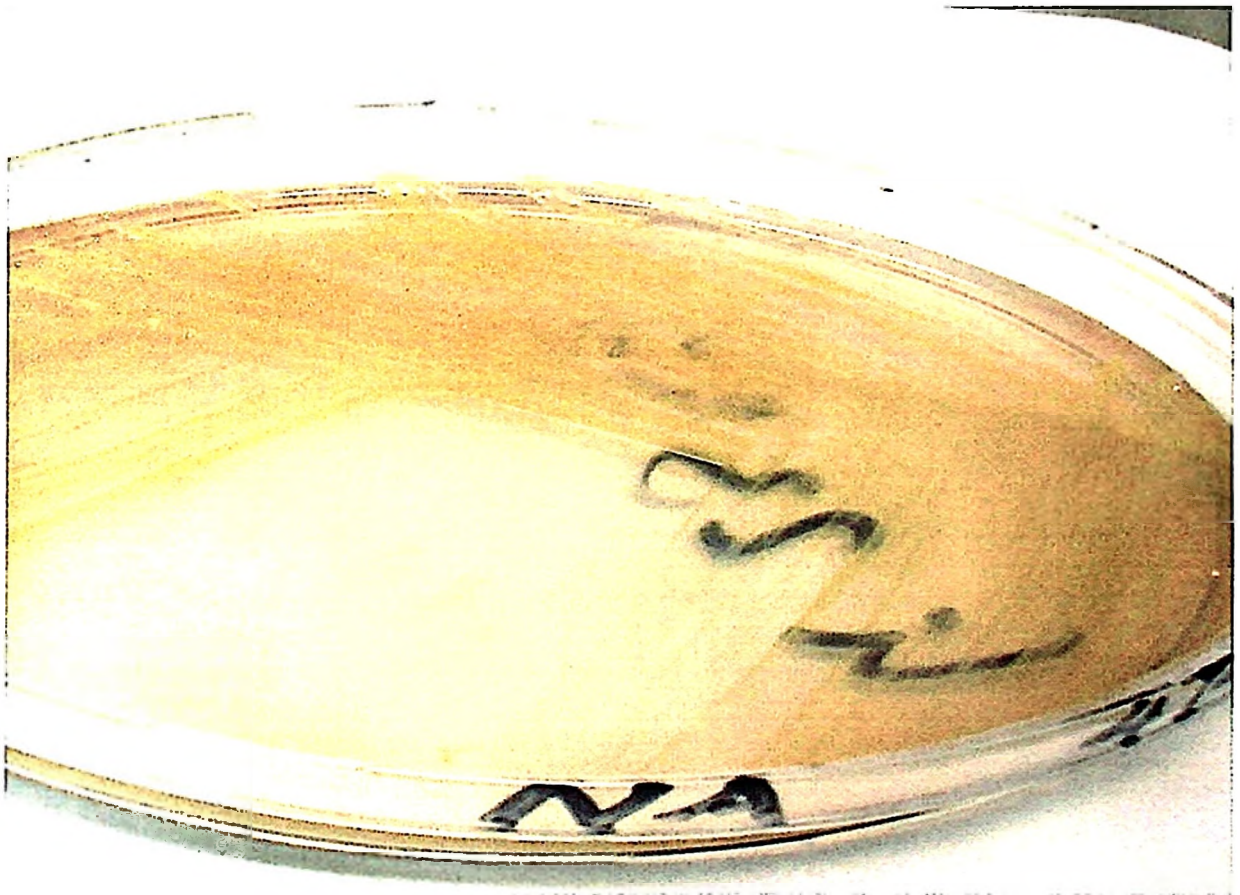


Plate 2: Growth of bacterium in nutrient agar growth media with showing brown pigmentation a typical characteristic of CBB *variant of fuscans* (Xapf)



Plate 3: Colonies growing on a nutrient agar (growth media) after 48 hrs of incubation with a typical characteristic of common blight (*Xap*).

3.3.3 Identification of bacterial isolates

Presumptive colonies of *Xanthomonas axonopodis* pv. *phaseoli* on BBD that were yellow and hydrolyzed starch were selected and considered to be CBB and those with brown pigments were considered as CBB variant - fuscous. A single colony was purified by sub culturing on NA (nutrient agar). After 48 hours of growth on NA, two to three colonies with typical characteristics of CBB were increased by streaking on NA (one colony per plate). The increased inoculum was used in pathogenicity tests to confirm the isolate as CBB.

3.3.4 Pathogenicity tests

Susceptible genotype Canadian wonder was used in all pathogenicity tests. The plants were grown in the screenhouse and were injected below the primary leaves at 6 cm above the soil surface, when the second trifoliolate leaf had fully expanded. Two millilitres of inoculum was injected using a 25 gauge needle and 2 ml syringe. Observations of

symptom on susceptible genotype were used for conformation of the reaction of CBB isolate.

3.3.5 Stem inoculations

The inoculation technique used in screenhouse was stem injection (SI) technique as recommended by Perreaux *et al.* (1986) and Saettler (1971) as explained in the section 3. 3. 4 and as shown in Plate 4.



Plate 4: A stem inoculated by hypodermic injection needle

3.4 Experimental designs and layout

A randomized complete block design (RCBD) was used, where 45 bean lines were replicated three times in a single row plot in the field experiment. In the screen house experiment the design used was split plot arranged in randomized complete block with factor A (isolates) as main plots, and factor B (bean lines) as sub plot. Spraying and hypodermic injection needle were used as inoculation techniques respectively.

3.4.1 Field experiment

The field was prepared to a fine seedbed before planting. Pre-plant fertilizers of farm yard manure (one metric tonne per hectare) and 150 kg/ha of granular 25- 5 -5 NPK was applied in the field. Forty five advanced BC₁F₃ bean lines were planted in single row plots of three metres in length, bean seeds were sown at a spacing of 50 cm x 20 cm between rows and within plants respectively. The plants were inoculated at the R 5 (pre flowering) stage of development according to Shenge. (2006), use a backpack solo sprayer, positioned at 20 – 30 cm from the foliage using a bacterial concentration of 10^9 cfu ml⁻¹ to runoff by spraying the abaxial and adaxial leaf surfaces.

For this study, the bacterial cell suspensions were prepared in sterilized distilled water from the cultures grown 48 hr. at 26 – 28°C. All concentrates were adjusted to a 10^9 cfu/ml by using a loopful wire equivalent to 10^9 cfu/ml dilution techniques. This dilution technique of the inoculum concentration were used for all inoculations in the field and screen house experiments

3.4.2 Screen house experiment

The plants were grown in the screen house at SUA in pots of 15 cm diameter in a design arranged in split plot. Two seeds were sown per pot and thinned to one plant per pot after germination. The soil used was previously heat sterilized at $80 \pm 5^\circ\text{C}$ for 4 hours, sieved and mixed with sand (in a ratio of (2: 1). A compound fertilizer containing nitrogen, phosphorus and potassium (25 -5 -5 NPK) was added at a rate of 1 g per 8 kg of soil. The plants were watered when need arose.

Temperatures were recorded twice daily at 9 a. m. and 3 p. m. The recorded temperatures ranged from 30°C to 34 °C. Each plant was injected by hypodermic injection needle with bacterial isolates inoculum at a bacterial concentration suspension of $10^7 - 10^9$ cfu/ml, 10 days after seedling emergence. The hypodermic needle was passed through the stem at the primary trifoliate leaf node. The inoculated plants were thereafter covered with a polyethylene bags for 72 – 96 hrs, then removed after that period and observation for bacterial blight symptom development was done from 7 days after inoculation, then weekly intervals. Plants showing a typical symptom of bacterial blight were scored for disease severity.

3.4.3 CBB scoring

The lines were evaluated 15 days after inoculation in the field and 7 days after inoculation in the screen house for CBB reaction using the CIAT 1 – 9 scales (1986) with modifications and percentage of leaf area affected. The reactions of CBB were rated visually as;

- 1 = no visible symptoms
- 2 = small flecks/ lesions covering 5% of leaf surface
- 3 = small lesions covering 6 – 20% of leaf surface
- 4 = lesions of various sizes covering 21– 40% of leaf surface with some chlorosis
- 5 = lesions coalescing to cover 41 – 60% surrounded by orange – yellow chlorosis
- 6 = large necrotic lesions with spreading chlorosis covering 61 – 80% of leaf surface
- 7 = more than 81 % lesions and chlorosis spreading, leaf wilting and defoliation of the affected plant may occur.

3.5 Statistical analysis

Analysis of variance (ANOVA) was performed using the Microsoft-based statistical package for crop science (MSTAT-C), treatment means separated by the new Duncan's multiple range test (NDMRT), to determine the variations among bean lines.

Statistical model for screen house experiment: $X_{ij} = \mu + \alpha_i + \beta_j + e_i + \alpha\beta_{ij} + e_{ij}$

Where

- μ = overall mean
- α_i = effect of factor (A) isolates
- β_j = effect of factor (B) bean lines
- e_i = random error of factor (A)
- $\alpha\beta_{ij}$ = interaction effect of factor (A) X factor (B)
- e_{ij} = random error of factor B and AB

Statistical model for field experiment:

$$X_{ij} = \mu + T_i + B_j + e_{ij}$$

Where

- μ = Overall mean
- T_i = treatment effect for treatments = 1, 2t
- B_j = block effect for blocks j =1, 2b
- E_{ij} = random error peculiar to each observation in the i^{th} treatments and blocks

CHAPTER FOUR

4.0 RESULTS AND DISCUSION

4.1 Common bacterial blight isolates

Among the ten samples of infected plant leaves, only six *Xap* or isolates were obtained and were found to be positive to the targeted pathogen that causes CBB disease. The typical starchy hydrolysis, yellow colour and or brown pigment diffusion in the medium culture were observed in BBD and NA media respectively (Table 3).

These results confirm the presence of *Xap* pathogen (*Xanthomonas axonopodis* pv. *phaseoli*) in the selected study areas. The positive isolation of *Xap* pathogen also revealed the presence of two types of CBB strains. The non – pigment forming type had a yellow colour (*Xap*) in the medium and the pigment forming type fuscous, the variant of *Xap* with brown diffusible pigments (*Xapf*) in the medium. The pigment forming type (*Xapf*) were relatively small of about 0.5 – 2 mm, compared to non – pigment forming type (*Xap*) 1.5 – 4 mm, when grown in the BBD and NA medium respectively (Opio *et al.*, 1996; Shenge, 2006). The incubation period for these isolated pathogens in the medium ranged from 1 – 6 days (Table 3).

Table 3 : Identification of CBB isolates from infected leaves samples

Isolate serial No.	Incubation period (Days)		Colour changes		Source of samples
	NA	BBD	NA	BBD	
1	2-5	2-3	Yellow	Yellow	SUA
2	2-5	2-3	Yellow	Yellow	Mgeta
3	2-5	2-3	Yellow	Yellow	Ismani
4	2-5	2-5	Nil.	Brown	Mbeya U.A.C
5	2-5	2-3	Yellow	Yellow	Njombe
6	2-5	2-3	Yellow	Yellow	Mbeya U.A.C
7	2-5	2-3	Negative	Negative	Njombe
8	2-5	2-3	Negative	Negative	Mbeya
9	2-5	2-3	Negative	Negative	Morogoro Kihonda
10	2-5	2-3	Negative	Negative	Iringa

4.2 Pathogenicity tests

Among the ten collected samples, four samples did not isolated successfully but the other remaining six samples were successfully isolated (Table 3). These six isolates that were confirmed by pathogenicity tests was regarded as CBB isolates and hence induces a typical CBB symptoms to a susceptible genotype Canadian wonder (local variety), when inoculated in the greenhouse after 7 -14 days. The symptoms appeared as small lesion characterized by a brown necrotic area at the point of entry and on leaves. Water soaking symptoms were also observed followed by chlorosis and necrosis. Symptoms produced by the common bacterial blight and its fuscous variant isolates were similar with the exception of fuscous variant which produced cracks on the stem in addition. Several different common bacterial blight symptoms; small black greasy lesions, burned soaked lesions with and or without yellow borders and chlorotic lesions were observed in the foliage/leaves and pods as shown in Plate 6.

From the observations of the symptoms obtained, it was concluded that the reaction symptoms observed were of CBB infection (Plates 5 and 6).



Plate 5: Stem cracking symptoms characteristic of *Xapf*



Plate 6: CBB symptoms on both leaf and pod after inoculation with *Xap* isolate in the field

4.3 Field experiment

The results obtained from the field experiment also showed that there were significant differences ($P \leq 0.05$ and $P \leq 0.01$) among the tested bean lines in disease reaction (Table 4). The disease scores were at the range of 1.3 to 6 with an overall grand means score of 3.4. Among the tested lines only eight lines (18.6%) had lower disease scores of 1.3 to 2.3. According to CIAT 1-9 scale (1986) these lines are CBB resistant. Six lines namely; PSO3 – 557 (C) – 15, PSO4 – 122 (A) – 5, PSO3 – 557 (C) – 12, PSO4 – 90 (C) – 16, PSO3 – 557 (C) – 6 and PSO3 – 557 (C) – 11 had a score ranging from 1.3 to 2, hence had a higher level of resistance compared to the other two lines. Nineteen lines (44.2%) had scores ranging from 2.7 to 3.7 which were regarded as moderately resistant. Sixteen bean lines (37.2%) had disease scores levels of 4 to 6 which were regarded as very susceptible.

The results indicate that transfer of CBB resistant genes into the different genotypes has been effective in some genotypes only but not to others. This can be attributed to the fact that breeding for resistance to CBB, according to Arnaud- Santana *et al.* (1994) and Miklas *et al.* (1996) is complex because of pathogen variability and resistance linkage with undesirable traits and there are different genes conditioning resistance in leaves, pods and seeds.

Table 4: Reaction of bean lines to Morogoro *Xap* isolate in the field

Bean lines/Varieties	CBB Mean score		Seed colour	100 seed weight	Plant habit
PSO4 - 585 (A) - 4	4.0	b-f	Kablanketi	40.0	Climber
PSO4 - 120 (A) - 10	4.0	b-f	Cream/red stripes	61.5	Determinate
PSO3 - 557 (C) - 15	1.3	h	Red/white stripes	49.0	Determinate
PSO4 - 92 (D) - 13	4.3	a-e	Yellow	33.0	climber prostrate
PSO4 - 92 (D) - 14	5.3	ab	Yellow	32.2	Climber
PSO3 - 585 (A) - 15	3.0	d-h	Kablanketi	34.5	Climber
PSO3 - 585 (A) - 17	4.0	b-f	Kablanketi	35.6	Climber
PSO4 - 148 (A) - 3	3.7	b-g	Kablanketi	45.2	Climber
PSO4 - 92 (D) - 10	4.3	a-e	Yellow	33.0	climber prostrate
PSO4 - 92 (D) - 15	3.3	c-h	Yellow	28.8	climber prostrate
PSO3 - 585 (A) - 13	3.3	c-h	Kablanketi	41.0	Climber
PSO4 - 148 (A) - 5	4.3	a-e	Kablanketi	40.6	Climber
PSO4 - 148 (A) - 17	4.0	b-f	Kablanketi	38.4	Climber
PSO4 - 90 (A) - 5	6.0	a	Yellow	37.0	Climber
PSO4 - 148 (A) - 11	4.0	a-e	Kablanketi	47.2	Climber
PSO3 - 585 (A) - 20	4.7	a-d	Kablanketi	45.8	Climber
PSO3 - 585 (A) - 10	4.7	a-d	Kablanketi	38.6	Climber
PSO3 - 585 (A) - 12	4.3	a-e	Kablanketi	47.8	Climber
USDK - CBB - 15	3.7	b-g	Red	58.0	Determinate
PSO3 - 585 (A) - 2	3.0	d-h	Kablanketi	45.0	Climber
PSO3 - 585 (A) - 21	3.0	d-h	Kablanketi	40.6	Climber
PSO4 - 148 (A) - 15	3.0	d-h	Kablanketi	35.4	Climber
PSO4 - 72 (B) - 4	4.3	a-e	Red	54.7	Climber
PSO3 - 585 (A) - 8	5.0	abc	Kablanketi	47.0	Climber
PSO4 - 120 (A) - 15	3.0	d-h	Brown	48.7	Determinate
PSO4 - 90 (A) - 6	2.7	d-h	Yellow	39.4	Climber
PSO4 - 122 (D) - 5	2.0	fgh	Brown	46.0	Determinate
PSO3 - 557 (C) - 7	3.0	d-h	Cream/red stripes	47.6	Determinate
PSO4 - 120 (A) - 8	2.3	efgh	Cream	50.0	Determinate
PSO4 - 120 (A) - 9	3.3	c-h	Cream	54.0	Determinate
PSO4 - 120 (A) - 1	2.7	d-h	Cream/red stripes	46.0	Determinate
PSO3 - 585 (A) - 11	4.7	abcd	Red/white stripes	65.0	Climber
PSO4 - 122 (D) - 19	2.7	d-h	Red/white stripes	49.4	Determinate
PSO4 - 120 (A) - 6	2.7	d-h	Cream/red stripes	47.5	Determinate
PSO4 - 120 (A) - 16	3.3	c-h	Cream	50.0	Determinate
PSO3 - 557 (C) - 12	2.0	fgh	Cream/red stripes	48.4	Determinate
PSO3 - 557 (C) - 11	1.7	gh	Red/ white stripes	42.0	Determinate
PSO4 - 122 (D) - 4	2.7	d-h	Red/ white stripes	54.5	Determinate
PSO4 - 90 (C) - 16	2.0	fgh	Yellow	38.0	Climber
PSO3 - 585 (A) - 5	3.3	c-h	Kablanketi	45.0	Climber
PSO3 - 557 (C) - 6	2.0	fgh	Red/ white stripes	52.0	Determinate
PSO3 - 557 (C) - 2	2.3	efgh	Cream/red stripes	27.0	Determinate
PSO4 - 90 (A) - 8	3.0	d-h	Red/white stripes	55.0	Determinate
Canadian Wonder	4.0	b-f	Darkish red	36.8	Determinate
Vax 6	2.3	efgh	Reddish brown	20.4	Determinate
Mean	3.4			43.7	
C V %	29.24			-	
SE±	0.57			-	

Means followed by a common letter are not significantly different Duncan's Multiple Range Test at 5% Level of significance.

These lines had 100 seed weight at a range of 20.4gm (Vax 6) to 65.0gm (PSO3 – 585 (A) – 11) Basing on 100 seeds weight results showed negative correlation effects between CBB scores and 100 seeds weight ($r = -0.032$ and Prob. = 0.835). However, the correlation was not significant to give solid conclusion on the relationship of these two traits.

4.4 Screen house experiment

Results from screen house experiment are presented in Table 5 and Appendix 2 below. The results obtained showed significant difference ($P \leq 0.05$ and $P \leq 0.01$) in *Xap* reactions among isolates strains and among bean lines. The mean disease severity scores for the four isolates evaluated were 1.8 for Morogoro isolate, 1.9 for Ismani isolate, 2.4 for Mgeta isolate and 3.8 for Mbeya isolate. Results indicate that the isolate from higher altitude that is from Mbeya (*Xapf*) and Mgeta (*Xap*) gave relatively higher disease scores than those from lower altitude that is Ismani and Morogoro. These findings mean that strains from higher altitude were more virulent in reactions to bean lines in lower altitude. These findings are similar to those reported by Beebe (1988) who showed that there was variation in virulence of isolates of CBB pathogen within and between geographical locations from which they are collected. Comparing the virulence of the isolates, the isolate collected from Mbeya were more aggressive or virulent than the other three isolates. The aggressiveness of this isolate is the characteristic virulence of CBB variant strain type fuscous (*Xapf*), which were associated more with stem collapse than the other common bacterial blight (*Xap*). Similar results were reported by other others (Valladares *et al.*, 1977 and Allen, 1983).

Basing on individual bean lines evaluated eight lines (18.6%) were found to be highly resistant to *Xap* isolate from Morogoro with disease score of 1. The lines were; PSO4 –

148 (A) – 11, PSO3 – 585 (A) – 10, PSO3 – 585 (A) – 12, PSO3 – 585 (A) – 2, PSO3 – 585 (A) – 21, PSO4 – 148 (A) – 15, PSO3 – 585 (A) – 8 and PSO3 – 557 (C) – 12. Twenty nine lines (67.4%) were resistant with a disease score ranging 1.7 to 2.0. Six lines (14%) had a score of 2.7 and 3.0 hence rated as susceptible. These findings reveal the presence of CBB resistant genes in all tested lines with the exception of six lines which showed susceptibility (Table 5).

For the isolate from Mbeya results obtained showed that fourteen lines (32.6%) were moderately resistant with disease score of 2.0 to 3.3. Twenty nine lines (67.4%) had a disease scores ranging from 3.7 to 6, were rated as susceptible lines. From these results there were significant differences ($P \leq 0.01$) in reactions between the moderately resistant lines with disease scores of 2, 2.3 and 2.7 and those lines with disease score of 3.7, 4.0, 4.7, 5.0, 5.3 and 6.0 which are regarded as susceptible.

Table 5: Disease scores of 45 bean lines to four different *Xap* isolates in the screen house.

Bean lines/variety	Isolates				Mean scores
	Isolate 1 (Morogoro)	Isolate 2 (Mbeya)	Isolate 3 (Ismani)	Isolate 4 (Mgeta)	
PSO3 – 585 (A) – 4	2.0	2.7	2.7	1.3	2.2
PSO4 – 120 (A) – 10	1.7	2.3	1.7	1.0	1.9
PSO3 – 557 (C) – 15	1.7	4.0	1.7	2.7	2.3
PSO4 – 92 (D) – 13	3.0	3.7	1.7	2.0	2.3
PSO4 – 92 (D) – 14	2.7	4.0	1.7	3.7	2.3
PSO3 – 585 (A) – 15	2.0	4.7	2.3	2.0	2.4
PSO3 – 585(A) – 17	2.0	4.7	2.0	3.0	2.3
PSO4 – 148 (A) – 3	2.0	5.0	2.7	2.0	2.3
PSO4 – 92 (D) – 10	2.7	4.7	1.0	2.7	2.3
PSO4 – 92 (D) – 15	1.7	4.7	2.7	3.0	2.6
PSO3 – 585 (A) – 13	1.7	4.7	2.0	3.0	2.6
PSO4 – 148 (A) – 5	2.0	3.7	2.0	1.7	2.1
PSO4 – 148 (A) – 17	2.0	3.7	2.7	2.0	2.7
PSO4 – 90 (A) – 5	1.7	3.0	1.0	2.0	1.7
PSO4 – 148 (A) – 11	1.0	3.0	1.7	1.7	1.5
PSO3 – 585 (A) – 20	1.7	3.0	3.0	3.0	2.3
PSO3 – 585 (A) – 10	1.0	4.0	3.0	1.7	2.2
PSO3 – 585 (A) – 12	1.0	6.0	1.0	1.7	1.9
USDK – CBB – 15	1.7	4.0	1.7	1.0	1.8
PSO3 – 585 (A) – 2	1.0	4.0	1.0	2.7	1.8
PSO3 – 585 (A) – 21	1.0	3.7	1.7	2.0	1.8
PSO4 – 148 (A) – 15	1.0	5.3	1.7	1.0	1.8
PSO4 – 72 (B) – 4	2.0	2.0	2.0	1.7	1.8
PSO3 – 585 (A) – 8	1.0	3.7	1.0	1.0	1.5
PSO4 – 120 (A) – 15	1.7	4.0	3.0	3.7	2.8
PSO4 – 90 (A) – 6	2.7	3.0	2.3	3.7	2.3
PSO4 – 122 (D) – 5	1.7	2.0	2.0	2.7	1.9
PSO3 – 557 (C) – 7	1.7	4.7	1.0	2.0	2.0
PSO4 – 120 (A) – 8	2.0	4.0	1.7	3.3	2.2
PSO4 – 120 (A) – 9	2.0	3.7	1.7	2.7	2.1
PSO4 – 120 (A) – 1	2.0	4.7	1.7	3.7	2.4
PSO3 – 585 (A) – 11	1.7	4.7	1.3	2.0	1.9
PSO4 – 122 (D) – 19	2.7	3.0	2.7	1.7	2.6
PSO4 – 120 (A) – 6	2.0	3.7	3.0	2.7	2.7
PSO4 – 120 (A) – 16	1.0	3.3	2.7	3.7	2.4
PSO3 – 557 (C) – 12	2.0	4.0	1.0	3.0	2.8
PSO3 – 557 (C) – 11	2.3	3.0	1.0	2.0	2.3
PSO4 – 122 (D) – 4	1.7	3.7	1.0	3.0	2.3
PSO4 – 90 (A) – 16	2.0	4.7	2.0	2.0	2.2
PSO3 – 585 (A) – 5	2.0	5.3	1.7	2.0	2.3
PSO3 – 557 (A) – 6	1.7	3.0	2.0	2.0	1.7
PSO3 – 557 (C) – 2	1.7	3.0	1.0	4.0	2.0
PSO4 – 90 (A) – 8	2.0	2.7	1.7	2.0	2.2
Canadian wonder	3.0	3.7	3.3	3.7	3.7
VAX 6	1.0	1.7	1.0	2.0	1.5
Mean	1.8	3.8	1.9	2.4	2.5
CV %	27.3	25.2	24.9	36.3	42.5
LSD	0.8	1.5	0.8	1.4	0.8
SE±	0.30	0.40	0.29	0.40	0.26

Means in the same column followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test at 5% Level of significance

The results obtained from Ismani isolate; shows that there were significant differences ($P \leq 0.05$) in reaction to *Xap* among lines tested. Ten lines (23.3%) with disease score of 1 were resistant and thirty three lines (76.7%) were moderately resistant respectively. However, susceptible line was the local susceptible check Canadian wonder variety. From the above results all lines except the local check were resistant and moderately resistant respectively to the Ismani isolate. The results imply that the tested lines have CBB resistant genes for this particular isolate. These resistant lines can be suitable for growing in Ismani location (Table 5).

For the Mgeta isolate, there were significant differences ($P \leq 0.05$) among the lines tested (Table 5). There were eleven lines with disease score of 1.0, 1.3 and 1.7 were found to be highly resistant, these are; PSO4 – 585 (A) - 4, PSO4 – 120 (A) – 10, PSO4 – 148 (A) – 5, PSO4 – 148 (A) – 11, PSO3 – 585 (A) – 10, PSO3 – 585 (A) – 12, USDK – CBB – 15, PSO4 – 148 (A) – 15, PSO4 – 72 (B) – 4, PSO3 – 585 (A) – 8 and PSO4 – 120 (A) – 6. Thirteen lines were resistant and thirteen lines were moderately resistant, while eight lines were susceptible to Mgeta isolate. Generally, these findings show that CBB resistant gene was effectively incorporated to bean lines for this isolate. From the findings above, all resistant lines are suitable improved bean seeds for Mgeta location (Table 5).

Mean scores for the four *Xap* isolates to the tests materials, lines PSO4 – 148 (A) – 11 and PSO3 – 585 (A) - 8 were resistant to all isolates. Lines PSO3 – 585 (A) – 21, PSO4 – 148 (A) – 15, PSO3 – 585 (A) – 2, USDK – CBB – 15, PSO4 – 90 (A) – 5, PSO4 – 72 (B) – 4 and PSO3 – 557 (C) – 6, were resistant to one or more isolates tested. However, lines; PSO4 – 148 (A) – 11, PSO3 – 585 (A) – 12 and PSO3 – 585 (A) – 21 were resistant to three isolates and lines; PSO4 – 148 (A) – 15, PSO3 – 585 (A) – 8, PSO3 – 585 (A) – 10,

PSO3 – 557 (C) – 12, PSO3 – 585 (A) – 15, PSO4 – 90 (A) – 5, PSO4 – 92 (D) – 13, PSO3 – 557 (C) – 11, PSO3 – 557 (C) – 7, PSO4 – 120 (A) – 10, PSO4 – 122 (D) – 4, and PSO3 – 557 (C) – 5 were resistant in two isolates among the four isolates evaluated. Twenty one lines were resistant to only one isolate among the four isolates tested. The results obtained from combine data of the four isolates showed that there were no significant interaction ($P \leq 0.05$) between bean lines and isolates.

Brown pigmented type (*Xapf*) was more aggressive than the yellow type (*Xap*) as also reported by Allen, (1983). There were however, no differences in the type of symptoms produced on the tested materials between the fuscous and the yellow type. This finding confirms earlier reports that the fuscous and the yellow types of (*Xap*) cannot be differentiated on the basis of symptoms they causes (Bradbury, 1986; Opio, 1993; Mkandawire *et al.*, 2004).

The type of reaction/ symptoms induced on 45 common bean lines showed differences for each of the 4 *Xap* isolates tested under similar environmental conditions (Plate 7). These results indicate that what were observed were the uniform ranking of genotypes, aggressiveness in their reaction, resistance to different *Xap* isolates and their ranked order of aggressiveness among this BC₁F₅ generation of bean lines. It is therefore noted that the relationship between the beans lines tested are controlled with quantitative gene sources as also have been reported by (Opio, 1993). From this findings observed, it is important to combines these resistant genes sources into a commercial bean cultivar in breeding program.



Plate 7: Reaction of tested bean line (PSO4 – 120 (A) – 15) (left), against resistant line (VAX 6) (centre) and susceptible variety (Canadian wonder far right).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In the view of the above results of this study it is concluded that;

- (i) Variation in reaction among these advanced bean lines to *Xap* isolates was observed. The results in screen house indicate that there were significant differences in reactions among the isolates and the bean lines evaluated. In the field conditions, there was also evidence of variation among the test breeding materials. These findings suggest it is possible to select resistant lines among the tested lines.
- (ii) The levels of resistance observed in advanced bean lines provide moderate to sufficient protection against CBB in the low and mid altitudes of bean production areas in Tanzania. Genotype with higher and or equal level of resistance to Vax 6, if well adapted in the country can be utilized by breeders in their breeding programs.
- (iii) From this studies the following lines have been identified as resistant to CBB; PSO4 – 120 (A) – 10, PSO4 – 120 (A) – 8, PSO3 – 557 (C) – 12, PSO3 – 557 (C) – 11, PSO4 – 148 (A) – 11, PSO3 – 585 (A) – 12, PSO3 – 585 (A) – 21, PSO4 – 148 (A) – 15, PSO3 – 585 (A) – 8, PSO4 – 122 (D) – 4, PSO3 – 585 (A) – 2, PSO3 – 585 (A) – 10, PSO3 – 585 (A) – 15, PSO4 – 92 (D) – 13, PSO4 – 90 (A) – 5, PSO3 – 557 (C) – 5, PSO4 – 120 (A) – 9, PSO4 – 120 (A) – 1, PSO4 – 92 (D) – 14, PSO3 – 557 (C) – 15, USDK – CBB – 15, PSO3 – 585 (A) – 4, PSO4 – 72 (B) 4, PSO4 – 122 (D) – 19, PSO3 – 585 (A) – 11, PSO4 – 90 (A) – 16, PSO4 – 90 (A) – 8, PSO3 – 557 (C) – 6, PSO4 – 148 (A) – 3, PSO4 – 148 (A) – 5, PSO4 – 148 (A) – 17,

PSO3 – 557 (C) – 7, and PSO3 – 557 (C) – 2, they were resistant across the four isolates evaluated against *Xap*.

- (iv) From this study, findings have shown that there were differences in isolates, strains from higher altitude were more virulent in reaction to bean lines in low and mid altitudes. These findings imply that this *Xap* pathogen is geographical specific.

5.2 Recommendations

According to the findings obtained in this study the following recommendation are made;

- (i) There is a need for further research on the reaction of bean breeding materials to different CBB isolates found in major growing areas in low and mid altitudes in the country. Such studies will provide useful information on the genetic variation of CBB pathogen that may contribute to successful management of the disease in Tanzania.
- (ii) There is a need for conducting yield loss studies for these resistant materials evaluated to determine the economic impact of the disease for bean production in the bean growing areas in the country.
- (iii) There is a need to conduct further evaluations of these lines identified as resistant in this study in farmers' fields to confirm their resistance.

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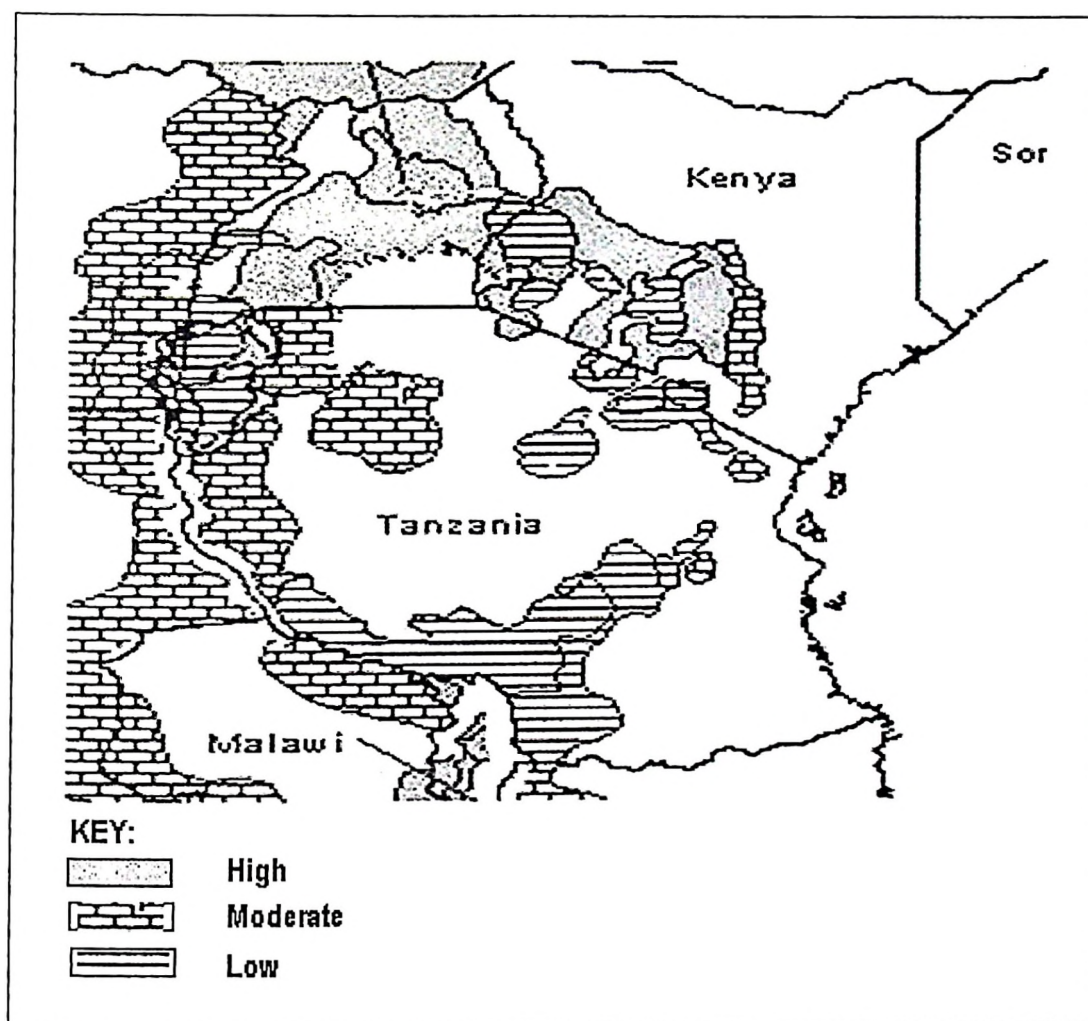
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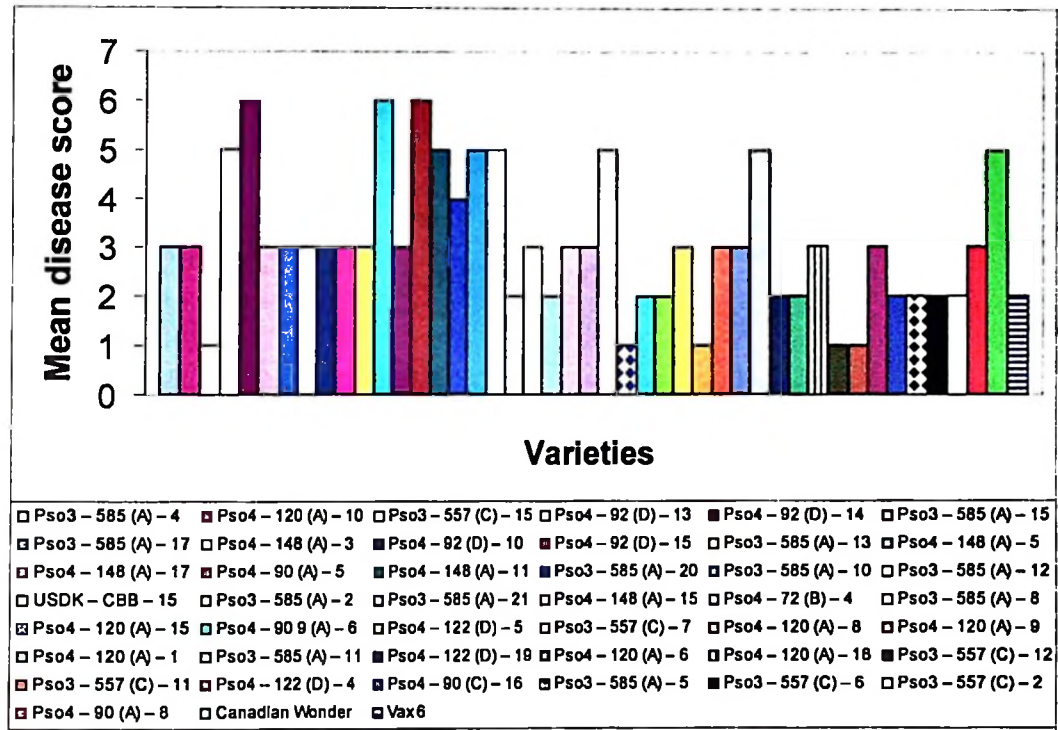
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APPENDICES

Appendix 1: A map showing distribution of CBB disease in major growing areas of Tanzania.



Appendix 2: Reaction of Morogoro isolate disease score in the field



Appendix 3: Analysis of variance for screen house experiment

K Value	Source	Degree of freedom	Sum of squares	Mean square	F value	Prob.
1	Replication	2	74.444	37.222	7.4592	0.0236
2	Factor A	3	285.487	95.162	19.0701**	0.0018
-3	Error	6	29.941	4.99		
4	Factor B	44	85.167	1.936	2.2294**	0
6	AB	132	141.93	1.075	1.2384NS	0.0636
-7	Error	352	305.615	0.868		
	Total	539	922.583			

Coefficiency of variation: 42.46 %