

**INTROGRESSION OF BEAN ANTHRACNOSE RESISTANCE GENES IN
COMMON BEAN (*PHASEOLUS VULGARIS* L.) LINES WITH ALS, CBB
AND BCMV/BCMNV DISEASES RESISTANCES**

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT ON
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ABSTRACT

The common bean (*Phaseolus vulgaris* L) is the most important food legume consumed worldwide. Sustainable production of the common bean in Tanzania is hampered by number of constrains including angular leaf spot (ALS), common bacterial blight (CBB), bean common mosaic virus/bean common mosaic necrosis virus (BCMV/BCMNV) and anthracnose (ANTH) diseases. The objective of this study was to develop common bean lines which will have resistances to ALS, CBB, BCMV/BCMNV and ANTH diseases by incorporating *Co-4*² and *Co-6* resistance gene for anthracnose in bean line that has angular leaf spot, common bacterial blight, bean common mosaic virus/bean common mosaic necrotic virus and anthracnose disease resistance. Two donor parents of common bean cultivar C4-1308B-3E-8-B and AB 136 were used as a source of anthracnose resistance and one recipient parent line Vax3 x Mex54 x Mshindi. Crosses for creating generation of segregating population and evaluation for anthracnose disease was done under screen house and field conditions. Significant genetic variation (≤ 0.05) for anthracnose disease was noted. Moreover F_1 and F_2 plants in all crosses showed significant (≤ 0.05) level of resistance to anthracnose. Thus research work was successful in incorporating anthracnose resistance genes in adapted bean lines and recommended that hybridization by pyramiding genes for disease resistances may therefore be used to improve bean genotype by incorporating the disease traits from donors to a single genotype comprising many diseases in it. It is recommended that the F_2 populations created in this study be advanced for further evaluation to ascertain anthracnose, CBB, ALS, BCMV/BCMNV disease resistance, and agronomic data.

. DECLARATION

I, Ayoub Kivuru Ndee, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that done within the period of registration has neither been submitted nor being concurrently submitted in any other institution.

.....

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.....

Date

The above declaration is confirmed

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Date

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DEDICATION

To my wife and my daughters Shamim and Asha, for their inspiration, encouragement and support throughout my studies

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LIST OF ABBREVIATION AND SYMBOLS

AFLP	Amplified Fragment Length Polymorphism
ALS	Angular Leaf Spot
ANT	Anthraxnose
BCMV	Bean Common Mosaic Virus
BCMNV	Bean Common Mosaic Necrotic Virus
Ca	Calcium
CBB	Common Bacterial Blight
CIAT	International Centre for Tropical Agriculture
<i>Co Colletotrichium</i>	
Cu	Copper
CV	Coefficient of Variance
DAP	Di Ammonium Phosphate
F ₁	Filial one generation
F ₂	Filial two generations
Fe	Ferrous
Ha	Hectare
I	Intermediate
LSD	Least Significant Difference
MAS	Markers Assisted Selection
Masl	meter above sea level
MDL	Multiple Disease Lines

Mex54	Mexico 54
PhD	Doctor of Philosophy
R	Resistant
RAPD	Randomly Amplified Polymorphic DNA
S	Susceptible
SE	Standard error
SUA	Sokoine University of Agriculture

CHAPTER ONE

1.0 INTRODUCTION, JUSTIFICATION AND OBJECTIVES

1.1 Introduction

The common bean (*Phaseolous vulgaris* L.) is the most important food legume consumed worldwide (Broughton *et al.*, 2003). It is widely cultivated in tropical and subtropical countries. Its production in sub-Saharan Africa is around 3.5 metric tons ha⁻¹ with 62% being produced in East African countries namely Burundi, DR Congo, Ethiopia, Kenya, Rwanda, Tanzania and Uganda (Broughton *et al.*, 2003). In Tanzania, common bean is cultivated for cash and home consumption (Hillocks *et al.*, 2006). Beans are major staple food in Eastern and Southern Africa, and are recognized as the second source of human dietary protein after maize and third most important source of calories after maize and cassava (Kelly, 2004).

Common bean is the first leguminous crops in Tanzania due to its importance as an exceptionally high potential for alleviating hunger in rural areas (Mduruma, 1996). It is estimated that, over 80% of rural and urban poor household in Tanzania depend on common bean as a food crop for their livelihood (Nchimbi, 1989). It is the source of proteins, vitamins, and minerals (Cu, Ca, Fe, Mg, Mn, Zn) in human diets (Tryphone and Msolla, 2010). Intake of common beans is also protective against diseases like cancer, diabetes and heart disease (Hangen and Bennink, 2003).

The importance of this crop is not only because of its use as less expensive source of dietary protein to both urban and rural community but also as an income earner crop

(Broughton *et al.*, 2003). Despite its importance, bean yields in developing countries are among the lowest in the world, with average of 0.5 tones ha⁻¹ while the potential yields being 1.5 tones ha⁻¹(Hillocks *et al.*, 2006).

These may be due to the fact that its production is carried under low input agriculture on small scale farms in developing countries. Varieties cultivated are vulnerable to attack by biotic and abiotic stresses. Biotic constraints in Africa include Angular leaf spot disease, anthracnose disease, Common bean blight disease, Common bean mosaic virus disease and bean stem maggot infestation (Wortman and Allen, 1994). Among diseases mentioned, bean anthracnose caused by the fungus *Colletotrichum lindemuthianum* (Sacc.and Magn.) is the most destructive disease of common beans. It affects all major bean plant parts like leaves, stems and pods. In Tanzania, the disease is particularly important in high altitude areas that are characterized by cool temperature and high relative humidity (Shao, 1980). It thrives best during wet conditions. The pathogen survives on infected crop debris,common bean seeds and in the soil for up to three years. In the field, the disease spreads through rain splash and wind.

Bean anthracnose is managed through use of disease free certified seed, use of resistant varieties, crop rotation, field sanitation and many other cultural and chemical methods (Tesfaye, 2003). Among the named control strategies, the most effective and appropriate method is one that integrates host plant resistance (Schwartz *et al.*, 1982; Allen *et al.*, 1998). It is therefore important to develop plant resistant materialsfor small scale resource poor farmers who are not able to incur the cost of buying chemicals for the disease control (Opio *et al.*, 2001).

1.2 Justification

Lack of improved common bean disease resistant cultivars has been identified as a major constraint in production (Pastor-Corrales and Tu, 1989). Common bean bacterial blight, Angular leaf spot, Common bean mosaic virus and Anthracnose diseases are extremely devastating causing high yield losses in common bean. Yield losses of up to 100% were reported to Anthracnose when infected seeds of susceptible varieties were grown in favorable conditions (Pastor – Corrales and Tu, 1989). Disease management using chemicals is expensive thus; many smallholder farmers of Tanzania cannot afford to buy them. Among several control strategies, integration of host plant resistance is the most effective and appropriate method which is affordable to small scale resource poor farmers (Allen *et al.*, 1998). Improvement of bean genotypes for single traits is laborious and time consuming, but using genotypes that have resistance to multiple constraints can increase the efficiency of improving bean genotype.

Foliar and soil-borne diseases in common bean are becoming major problems, especially for smallholder farmers. Therefore, resistant varieties to several disease causing pathogens are becoming a priority for the farmers. Thus, there is a need to produce varieties which are resistant to both major foliar and soil borne pathogens. In Tanzania, four genotypes were identified in 2004 by CIAT bean project to be resistant to angular leaf spot, anthracnose and ashy stem blight (*Macrophomina phaseolina*) and several lines were identified with combined resistance to rust, CBB, anthracnose and ALS (CIAT, 2004).

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to develop common bean lines which were are resistant to Angular leaf spot(ALS), Bean common mosaic virus/Bean common mosaic necrosis virus (BCMV/BCMNV, Common bacterial blight(CBB) and Anthracnose (ANTH) diseases.

1.3.2 Specific Objectives

- (i) To incorporate Co-4² and Co-6 resistant genes for anthracnose in bean lines that have ALS, BCMV and CBB disease resistance
- (ii) To evaluate segregating materials for Anthracnose disease reaction.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bean genetic improvements

Collection, characterization and understanding of genetic diversity in phaseolus bean in the late half of twenty century have been remarkable. During this period, the principle production constraints and traits different in common bean cultivars were determined. Breeding for early maturity, adaptation to higher latitude, high yielding, improved pod and seed quality, upright plant type and resistant to diseases Angular leaf spot, Bean common mosaic virus, Common bacterial blight and Anthracnose are the major achievements (Kelly, 2004).

The recent comprehensive map of disease resistance traits in common bean reveals numerous resistance gene clusters. Including co-evolution of genes for resistance to anthracnose and rust Gene cluster and defense related gene is becoming more visible in the genome (Kelly *et al.*, 2004).

2.2 Bean Diseases

2.2.1 Angular leaf spot disease (ALS)

Angular leaf spot (ALS) disease has been reported previously over 70 countries, in tropical and subtropical regions such as Brazil, Columbia, Costa Rica, Mexico and Africa (Mahuku *et al.*, 2003). It was known to exist also in other regions such as in Europe and USA (Inglis and Hegdon, 1984). The disease was considered as minor nuisance at first but is now one of the most economically important diseases of dry bean (Mahuku *et al.*, 2003). The pathogen can infect common bean (*Phaseolus*

vulgaris L) Lima bean (*P. lanatus* L) Scarlet runner bean (*P. coccineus*.L) Tepary bean (*P. auctifolius*). Previous studies conducted by CIAT (International Centre for Tropical Agriculture) demonstrated that cultivar Mexico 54 is resistant to most African ALS isolates (Pastor-Corrales *et al.*, 1998; Mahuku *et al.*, 2003).

Most pathogens exhibit high race variation, fungus that cause ALS has many strains that are genetically different. That is why a bean variety that is resistant in one region or season may not necessarily be effective in another; resistance thus breaks down (Aggarwal *et al.*, 2004). The greatest setback to development and deployment of resistant bean varieties is the high pathogenic variability occurring in *P. griseola* that renders varieties that are resistant in one location or year susceptible in another (Mahuku, *et al.*, 2003). Characterization based on differential cultivars, random amplified polymorphic DNA (RAPD) markers, microsatellites, isozymes and amplified fragment length polymorphism (AFLP) have revealed high levels of pathogenic and genetic variation in *P. griseola* (Guzman *et al.*, 1999; Mahuku *et al.*, 2003).

2.2.2 Common bacterial blight (CBB)

Common bacterial blight (CBB) is endemic to all regions of the world where dry beans (*Phaseolus vulgaris*) are cultivated, and represent a significant barrier to crop production. The disease is caused by the bacteria known as *Xanthomonas campestris* pv. *Phaseoli* and results in reduced seed yield and seed quality (Broughton *et al.*, 2003). Common bacterial blight resistance has been studied for a number of years, and has led to the development of several lines which have demonstrated resistance

to *Xanthomonas campestris* pv. *Phaseoli*. The VAX lines that were developed from XAN 159 with combined resistance from *P. vulgaris* and *P. acutifolius* possess the highest level of CBB resistance. The Centro Internacional de Agricultura Tropical (CIAT) has developed several lines which are used as good sources of resistance to Xap: they include; Vax 1, Vax 2, Vax 3, Vax 4, Vax 5 and Vax 6 (Singh et al., 1999).

2.2.3 Bean common mosaic virus (BCMV) / Bean common mosaic necrotic virus (BCMNV)

Bean common mosaic virus is a seed transmitted polyvirus that represents a worldwide constraint to common bean production (Miklas *et al.*, 2006). Systematically infected plants, especially those from infected seeds, have leaves with green mosaic patterns and distortions of tissues along leaf veins. Plants may be stunted and have only a few pods, which mature later than uninfected pods. Vascular tissue can become necrotic, producing dark streaks on petioles and stems. The dominant allele of the I gene confers resistance to all known races of BCMV (Kelly *et al.*, 2004). The development and release of dry bean cultivars with enhanced levels of disease resistance is an important goal in breeding programmes. Mshindi was derived from the cross 'Rojo' x Kablanketi made in 1992-93. Rojo is a larger-seeded variety released by SUA in 1997 with *bc-f* BCMV/BCMNV resistance, race specific angular leafspot resistance, and moderate common bacterial blight and halo blight resistance. Rojo was derived from 'SUA 90' x 86EP5034-B (Hillock *et al.*, 2006). BCMV/BCMNV symptoms were never observed on Mshindi in field trials. Mshindi was evaluated in 15 trials for reaction to ALS, 12 trials to CBB, and three trials to bean rust. Based on trials with moderate disease pressure, Mshindi was

classified as moderately susceptible to ALS, and resistant to moderately susceptible to CBB. There were two serotypes of the bean mosaic virus that are now recognized as separate viruses. Strains in Serotype A do not cause symptoms of root necrosis, known as 'black root' and are classified as BCMV. Strains in Serotype B cause black root in bean cultivars carrying the 'I' gene for resistance, and are classified as Bean common mosaic necrosis virus (BCMNV). BCMNV is predominant in eastern and southern Africa and therefore cultivars carrying the 'I' gene are prone to black root. This problem can be overcome by combining I-gene resistance with recessive resistance gene that prevents the systemic necrosis reaction (Mukoko *et al.*, 1994).

2.2.4 Anthracnose disease

2.2.4.1 The anthracnose pathogen (*Colletotrichum lindemuthianum*)

Anthracnose is perhaps the most economically important and widespread disease of the common beans (CIAT, 1997). Common bean anthracnose was first described and recorded in 1875 on plant specimens which had been obtained from Germany. *Colletotrichum lindemuthianum* (Sacc. and Magn) Scrib, causing the disease had, however, been collected by mycologists as early as 1843. The fungus is known to have races that vary from, country, region, location, and variety, to another (CIAT, 1997). Today, the disease is reportedly one of the most important and widely distributed throughout the world. It is found in Latin America, Asia, Europe, USA and Africa (Ansari, 2004). In Africa, it is particularly important in Uganda, Kenya, Tanzania, Rwanda, Burundi, Ethiopia and DR Congo.

2.2.4.2 Classification and biology of *Colletotrichum lindemuthianum*

C. lindemuthianum is an ascomycete and produces its conidia in acervuli. This fungus belongs to the genus *Colletotrichum*, order Melanconiales, family Melanconiaceae and section Hyalosporae (Alexopoulos, 1962). The fungus is found in nature in a conidial (imperfect) stage, but can overwinter as mycelia or conidia. The pathogen's perfect stage, *Glomerella cingulata* is rarely found in nature. Its conidia are oval shaped and dark brown in colour (Agrios, 1997). On the host, they form pink masses of conidia packed into the acervuli. *Colletotrichum lindemuthianum* differs from other species in this genus by its growth characteristics and a dark pigmentation on cultures (Tesfaye, 2003).

2.2.4.3 Morphology and etiology of *Colletotrichum lindemuthianum*

The conidia of *Colletotrichum lindemuthianum* are born on acervuli. The acervuli are mostly in groups, coalescing and covering lesions on infected plant parts. Setae are few, longer than the conidial mass. The conidial masses are orange to bright orange. Mycelia are scanty and white. Conidia are hyaline, oblong to dumbbell shaped, one-celled, straight ends rounded. Conidial size is about 9-15x3-4µm (Mathur and Kongsdad, 2000). Conidia are uninucleate, and usually have a clear vacuole-like body near the centre. *Colletotrichum lindemuthianum* has a unicellular conidium which, in an aqueous environment, produces a single germ tube. The conidium germinates within six to nine hours and produces one to four germ tubes (Zaumeyer and Thomas, 1957). Upon contact with a hard surface, the germ tube tip swells and differentiates into a thick-walled, heavily melanized appressorium. A penetration hypha arises from below the appressorium and penetrates cuticle and host cell wall during pathogenesis. Inside the cell lumen, a globose infection vesicle develops,

which in turn gives rise to a primary hypha (Zaumeyer and Thomas, 1957). Following infection, the symptoms begin to show after three to seven days depending on the prevailing environmental conditions (Hirst and Steadman, 1963).

2.2.4.4 Epidemiology

Colletotrichum lindemuthianum survives in bean crop residue and seed (Barrus, 1921; Tu, 1983; Pastor-Corrales and Tu, 1989). In areas where beans are continuously cropped, previous seasons inoculum can initiate epidemics of anthracnose (Dillard *et al.*, 1993). Its longevity in infected pods and seed varies considerably depending on environmental conditions; moisture and temperature being the most important factors influencing the survival of the fungus. Anthracnose conidia are spread from one plant to another mainly by splashing raindrops (Hirst and Steadman, 1963). The average distance of conidia spread is reported to range from 3 to 4.6 m per rainstorm of 10 mm or more (Tu, 1992). A 10-h wet period in a humid (>92%) environment are necessary for *C. lindemuthianum* conidia to infect, and new lesions usually appear in 3 to 7 days (Hirst and Steadman, 1963). Although plant residues contribute greatly to pathogen survival and distribution, infected seed serves an important role in the long distance distribution of the anthracnose pathogen (Tesfaye, 2003). In cases where poor farmers continuously exchange and use infected seed, the pathogen is capable of being distributed throughout all bean growing regions of the country (Opio *et al.*, 2001).

The spread of anthracnose from the initial infection point in the field depends on the speed and direction of wind. Prevailing wind associated with rain splash is an

important factor determining spread of anthracnose (Ntahimpera *et al.*, 1996). Long distance dissemination (3-5m) may develop from raindrops being blown by gusting winds (Tu, 1983). The number of foci of initial inoculum in the field also contributes, and is linearly related to the incidence of anthracnose on plant leaves (Tesfaye, 2003). Under field conditions, anthracnose incidence is highest on leaves during the rainy season and highest on pods during the dry season (Tesfaye, 2003). The disease spreads rapidly by spores carried in splashing raindrops, or through human activities or implements that come in contact with diseased plants (Tu, 1983) and in one growing season, one diseased plant can spread the disease to other plants within a 30m radius.

2.2.4.5 Major symptoms of anthracnose diseases.

Seedlings grown from anthracnose-infected seeds often have dark brown to black sunken lesions on the cotyledons and stems. Severely infected cotyledons die prematurely, and growth of the plants is retarded (Kelly and Vallejo, 2004). Diseased areas may girdle the stem and kill the seedling. Under moist conditions, small, pink masses of spores are produced in the lesions. Spores produced on cotyledon and stem lesions may spread to the leaves. On leaves, symptoms generally occur on the underside as linear, dark brick-red to black lesions on the leaf veins. As the disease progresses, discoloration appears on the upper leaf surface. Leaf symptoms often are not obvious and may be overlooked when examining bean fields (Kelly and Vallejo, 2004). The most striking symptoms develop on the pods (Kelly and Vallejo, 2004). Small, reddish brown to black blemishes and distinct circular, reddish brown lesions are typical symptoms. Mature lesions are surrounded by a circular, reddish

brown to black border with a grayish black interior. During moist periods, the interior of the lesion may exude pink masses of spores. Severely infected pods may shrivel, and the seeds they carry are usually infected. Infected seeds have brown to black blemishes and sunken lesions and are usually discoloured (Kelly and Vallejo, 2004).



Plate 1: Symptoms of anthracnose disease on leaf and pods

2.2.4.6 Control strategies of bean anthracnose

Bean anthracnose is best controlled by using disease-free seed. Seed produced under wet and humid conditions should not be planted as it is in most cases already infected. Crop rotation of at least three years also helps to eliminate or reduce the inoculums in the fields. In addition, fields should not be worked when plants are wet because fungal spores are easily spread from diseased to healthy plants under these conditions (Tu, 1992). Scouting the fields weekly for symptoms of the disease is recommended so that seed from plants that are infected with the anthracnose pathogen is not harvested as such will spread the disease in future.

Fungicides containing chlorothalonil, maneb, zineb, benomyl, captafol or folpet have also been recommended Use of resistant varieties is pivotal to any effective, economical and environmentally friendly strategy of managing the disease,

especially for small-scale farmers in sub Saharan Africa including Tanzania. The advantage of host plant resistance is that once the technology has been developed, it is packaged in seed which is easier to disseminate and deploy, and does not require any additional or specialized handling on the part of the farmers (Mahuku *et al.*, 2003). The greatest setback to development and deployment of resistant bean varieties is the high pathogenic variability occurring in *Colletotrichum lindemuthianum* that renders varieties that are resistant in one location or year susceptible in another (Mahuku, *et al.*, 2002). Integrating resistance with the above described measures gives a good control of the disease. Cultivar mixtures containing at least 60% of a resistant cultivar have been reported to offer a good control of anthracnose (Tesfaye, 2003).

2.2.4.7 Breeding for resistance and its requirements

Durable resistance is the most important way of controlling *Colletotrichum lindemuthianum* (Schwartz *et al.*, 1982). The pathogen has got variable physiological races and new ones keep emerging from time to time (Ansari *et al.*, 2004; Tesfaye, 2003; Alzate-Marin and Sartorato *et al.*, 2004; Mahuku and Riascos, 2004).

The resistance of common bean to *Colletotrichum lindemuthianum* is controlled by a number of race-specific genes. Breeding for resistance to *C.lindemuthianum* using several physiological races of anthracnose identified in Bulgaria was performed from F₁ to F₅ generation. Seven RILs of F₆ progeny, possessing more than one specific gene for resistance were selected. The line DG 2-36-58-3 was determined as the most promising by quality complex of growth habit type, vegetation period, type of seeds,

yield and presence of Co-1 and Co- 4 genes that confer resistance to 74 out of 78 worldwide recognized anthracnose races. Several common bean varieties with useful breeding traits were investigated as a source of Co-2 specific gene. Cultivar 'Drezden.'and the new Bulgarian variety 'Beslet' carrying of two genes for resistance in the Co-2 locus were determined as promising. There was strong evidence for co evolution of many bean pathogens with their host within the two centres of origin on *P. vulgaris*. Varieties that originated from Central America are resistant while those from Andean Region are susceptible to races of the anthracnose pathogen (Pastor-Corrales, 1991).

When 20,144 bean accessions were evaluated by CIAT in Colombia, 350 of them were found to be resistant to Andean and Mesoamerican isolates of the pathogen e.g. Mex 222, Ecuador 299,PI207262, G2333, G811 and G2641 (Pastor–Corrales et al., 1994). G2333 of Mexican origin has for many years continued to exhibit resistance to anthracnose and is a valuable source of resistance (Allen et al.1998). Due to the variability of the pathogen, durable resistance to anthracnose requires a combination of genes. The gene *Co-4*² confers resistance to 97% of American races of the pathogen (Balardin and Kelly, 2001). Cultivar AB 136 carrying resistance of Co-6 has been proved to be effective against *C.lindemuthianum* and it is an important source of resistance used by many breeding programmers around the world (Young and Kelly, 1996b, Young and Kelly 1997).Durable resistance is also not easily achieved since many genes are required in the same background to guard against the variable races. Knowing the biology, ecology and diversity of *Colletotrichum lindemuthianum* races from major bean growing regions (Tesfaye, 2003) is a pre-

requisite for most anthracnose resistance breeding programs (Buruchara, 1991) and this knowledge is needed to successfully develop and deploy resistance against the pathogen in particular regions (CIAT, 1996). Durable resistance can be created based on the known races by introducing the right genes to control them (Ogallo, 1991).

2.2.4.8 Pathogen variability

(a) Origins of pathogenic variability

New races of plant pathogens arise by sexual mechanisms like recombination of nuclear genes during sexual reproduction, exchange of genetic materials in somatic cells, mutation, or by extra chromosomal variation (Ogallo, 1991). Pathogens which reproduce sexually (like *Colletotrichum* species) are expected to produce variants more readily than those which are mostly asexual (Ogallo, 1991). Recombination of genes of two parental nuclei take place in a zygote during sexual reproduction, and the haploid nuclei or gametes resulting from meiosis are different, both from those of the parents and from each other. Therefore, every haploid pathogen individual is generally genetically different from any other pathogen, even within the same species. It is important to note that when parasites evolve faster than their hosts (which is the case of *C. lindemuthianum*); they have an evolutionary advantage because they can quickly track the changes of the local host population, leading to their local adaptation (Capelle and Neema, 2005). It is therefore desirable for the hosts to evolve faster than their pathogens if durable host resistance is to be achieved. Crop protection scientists and breeders should therefore devise alternative management measures as well as develop resistant bean varieties so that bean improvement matches with the prevailing rate of pathogen evolution.

(b) Variability of *Colletotrichum lindemuthianum* based on pathogenesis

C. lindemuthianum attacks susceptible varieties grown under moderate to cool temperatures and high relative humidity (Pastor-Corrales and Tu, 1989). There are many anthracnose pathogen variants or physiological races (also called pathotypes or virulence phenotypes – CIAT, 1997) and are identified by their reactions on a set of host varieties commonly referred to as host differentials. Different variants (races) differ from each other primarily on the basis of their pathogenicity (Agrios, 1997). Pathogenic variability in *C. lindemuthianum* was first reported in 1911 (Barrus, 1911) and since then, several races of this fungus have been reported in literature (CIAT, 1997; Ansari *et al.*, 2004; Tesfaye, 2003; Sartorato *et al.*, 2004, Mahuku and Riascos, 2004).

Alzate-Marin *et al.* (2004) for example identified a total of 50 *C. lindemuthianum* pathotypes in Brazil between 1994 and 2002, whereas Mahuku and Riascos (2004) identified 90 races from 200 *C. lindemuthianum* isolates collected from Andean and Mesoamerican bean varieties and regions. Virulence diversity of this pathogen has also been reported in some areas of Africa (Teskaye, 2003) and Europe where common bean has not traditionally been grown, and where climatic conditions differ from those of the two centre of origin of its host. Butare (unpublished data) identified 42 races of this pathogen out of 53 isolates from Rwanda. Sartorato *et al.*, (2004) used 24 races of *C. lindemuthianum* on 23 bean genotypes and only five bean genotypes were found resistant to all the races, the rest of the beans showed diverse

reaction to the races. Generally, one bean cultivar may be resistant to some races, but not others (CIAT, 1997).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Parental Germplasm

The germplasm with three diseases resistance to ALS, CBB and BCMV/BCMNV (VAX 3 x MEX 54 x MSHINDI), the Multiple Disease Resistance Lines (MDL) was used as female parent and genotype C4-1308B-3E-8-B (Co-4² and AB 136 (Co-6) were used as the male parents (donors). These materials were collected from Sokoine University of Agriculture (SUA)-Morogoro Bean Breeding Programme.

3.1.2 Description of parental germplasm.

The VAX lines were developed from XAN 159 with combined resistance from *P. vulgaris* and *P. acutifolius* possess the highest level of CBB resistance also PRO401-259 was derived from the cross VAX 6//mus83/Be/Neb PR1/Mus83/DOR483 (Singh *et al.*, 1999). Mexico 54 is a medium seeded cream in colour and resistance to all *P. griseola* isolate so far characterized in Africa (CIAT, 1996).

Mshindi was derived from the cross 'Rojo' x Kablanketi made in 1992-93. Rojo is a larger-seeded variety released by SUA in 1997 with *Ibc-f* BCMV/BCMNV resistance, race specific angular leafspot resistance, and moderate common bacterial blight and halo blight resistance. Rojo was derived from 'SUA 90' x 86EP5034-B (Hillock *et al.*, 2006). BCMV/BCMNV symptoms were never observed on Mshindi

in field trials. Mshindi was evaluated in 15 trials for reaction to ALS, 12 trials to CBB, and three trials to bean rust. Based on trials with moderate disease pressure, Mshindi was classified as moderately susceptible to ALS, and resistant to moderately susceptible to CBB (Hillock *et al.*, 2006).

C4-1308B-3E-8-B is the best recommended resistant variety. It has the broadest known resistance and carries three complementary genes (*Co-4*², *Co-5* and *Co-7*) that confer resistance against most known races of *Colletotrichum lindemuthianum* (Young *et al.*, 1997). Cultivar AB 136 carrying resistance of *Co-6* has been proven to be effective against *C.lindemuthianum* and it is an important source of resistance used by many breeding programmes around the world (Young and Kelly, 1996, Young and Kelly 1997).

3.2 Methods

3.2.1 Hybridization

Crosses for creating generation of segregating population required for this study were made in the screen house at Sokoine University of Agriculture (SUA)-Morogoro. All parents were planted in the screen house in 4 kg plastic pots filled with sterilized soil. Two seeds were sown per pot and then thinned to one seedling two weeks after planting. DAP fertilizer was applied at a rate of 60 kg P/ha at sowing and Urea (46% N) as a top dressing at a rate of 20 kg N per hectare. Watering was done throughout the experiment.

Crosses were made between lines which combines ALS, CBB and BCMV/BCMNV resistance and Anthracnose resistance genotypes C41308B-3E-8-B and AB136. Crossing was made by emasculation of the female flowers followed by transfer of pollen from just opened flowers to the stigma of emasculated plants as following:

(VAX 3 X MEX 54 X MSHINDI) X C4-1308B-3E-8-B

(VAX 3 X MEX 54 X MSHINDI) X AB136

Then the F₁ obtained by crossing were harvested from each cross separately and grown to advance them to F₂. The parents, F₁ and F₂ populations were planted in the field at Nyandira village, Mvomero district in Morogoro region for disease evaluation. This was done because the screening for disease was not successfully in the screen house; therefore it was appropriate to grow them in the ideal conditions for infection in Nyandira.

3.2.2 Evaluation of the segregating materials for Anthracnose disease reaction.

A field experiment was conducted at Nyandira village 1700 meters above sea level (masl). The resistant Parents, F₁, F₂ and the control cultivar(susceptible) Kigomawere used in this study.

The progenies in F₁ and F₂ generations resulting from common donor and recipient parents were assigned to plot which had 12 plants each. Each sub group had a single row and the rows were spaced at 75cm apart. Seeds were planted at spacing of 10cm for bush type (Vax 3 x Mex 54 xMshindi) recipient and C4-1308B-3E-8-B donor and their resulting progenies. While AB 136 donor a climbing bean type and their resulting progenies were planted at 15 cm. The plants were inoculated with a Nyandira natural inoculum of *Colletotrichum lindemuthianum* isolates which was

available at that place. These were planted at Nyandira because of the weather condition that is suitable for development of anthracnose disease.

3.2.3 Data collection

Disease assessment was initiated 14 days after inoculation and was based on a 1-9 severity scale according to Van Schoonhoven and Pastor-Corrales (1987) (Table 1). Twelve plants were sampled per plot for each genotype and assessed for anthracnose severity by scoring three trifoliolate leaves sampled of each plant. Disease scores were recorded every week for four weeks. Mean disease scores were calculated for each plant and used to determine the level of reaction to the pathogen.

Table 1: Evaluation scales for field screening for anthracnose reaction

Rating	Category	Description
1	Resistance	Leaf with no visible symptoms
2	Resistance	Few isolated small lesions on mid veins in the lower leaf surface
3	Resistance	A higher frequency of small lesions on mid veins in the lower leaf surface
4	Intermediate resistance	Lesions in the mid-vein and occasionally in secondary veins
5	Intermediate	Many small lesions on mid and secondary veins
6	Intermediate	Many small lesions in the lower and upper leaf surface
7	Susceptible	Larger lesion scattered over the leaf blade
8	Susceptible	Many large lesions accompanied by tissue breakdown And chlorotic leaf
9	Susceptible	Severely diseased or dead leaf

Sources: Van Schoonhoven and Pastor-Corrales, 1987

3.2.4 Data Analysis

Assessment was based on a 1-9 severity scale depicting the number of the leaf covered with anthracnose lesions as described by Schoohoven and Pastor-Corrales (1987). Simple statistics of mean, range, variance and standard deviation was used in analysing variables for disease scoring. Bean genotypes with scores of 1-3 were regarded as resistant; where those with scores of 4-6 were intermediate and 7-9 were regarded as susceptible. The frequency distribution of parental plants and its progenies based on disease reaction were plotted to determine the distribution pattern.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Hybridization

Generally, it was observed that crosses made between (Vax3 x Mex54 x Mshindi) x AB 136 gave the highest number of F₁ had 137seeds compared to those crossed by C4-1308B-3E-8-B had 116 seeds Table 2, and this could be attributed to the facts that the growth habit of the donor parents are different. The donor AB136 had a climbing habit while C4-1308B-3E-8-B was a bush type like a recipient parent. For every parent many successful crosses were made.

Table 2: Number of crosses, pods and F₁ seeds harvested.

Parents	No of crosses	No of pods	F ₁ seeds harvested
(Vax3 x Mex54 x Mshindi) x C4-1308B-3E-8-B	70	47	116
(Vax3 x Mex54 xMshindi) x AB136	64	40	137
Total	134	87	253

4.2 Evaluation of segregating materials for anthracnose disease reaction.

Generally, there was sufficient disease development during the season. Symptoms of anthracnose were first observed in the second week after plants emergency (14 days after inoculation).These concur with observation reported by Pastor-Corrales *et al.*,(1998) and Mahuku *et al.*, (2003), that anthracnose developed on inoculated plants within two to three weeks after inoculation.

The effect of anthracnose on common bean depends on susceptibility of bean genotype, age of bean growth at which the disease was severe and initial inoculums

concentration factors. These may contribute to the result observed in the study. Table 3 results shows that, bean populations (Vax 3 x Mex 54 x Mshindi) x AB 136 and (Vax 3 x Mex 54 x Mshindi) x C4-1308B-3E-8-B (F₁ and F₂) expressed resistance.

There were significance ($P < 0.05$) difference among the lines and populations for anthracnose disease. Means disease score and severity of F₁, F₂ and the donors showed high level of resistant to the disease compared to the recipient parent. The recipient parent line Vax3 x Mex54 x Mshindi (P1) with a mean disease score of 4.3 showed an intermediate resistance to the disease, indicating that disease reaction was not severe.

Table 3. Mean score of disease incidences and severity

Entry no	Entry name	Mean disease
1	Vax3 xMex 54 xMshindi (P1)	4.3
2	AB136 (P2)	2.0
3	(Vax3 x Mex54 x Mshindi) x AB136 (F ₁)	2.4
4	(Vax3 xMex 54 x Mshindi) x AB 136 (F ₂)	1.9
5	C4-1308B-3E-8-B (P3)	2.2
6	(Vax3 x Mex54 x Mshindi) x C4-1308B-3E-8-B (F ₁)	2,9
7	(Vax3 x Mex54 x Mshindi)x C4-1308B-3E-8-B (F ₂)	1.9
8	Kigoma (control)	3.6
	F-test	<.001
	CV%	30.8
	LSD	0.6185
	SE+.	0.3113

Comparisons of parents, F₁ and F₂ for disease reaction

Significant ($P \leq 0.05$) variation on disease reaction was observed among lines for all crosses made to Vax3 x Mex54 x Mshindi namely, (Vax3 x Mex54 x Mshindi) x AB136 and (Vax3 x Mex 54 x Mshindi) x C4-1308B-3E-8-B (Table 4).

The F_1 and F_2 of (Vax 3 x Mex 54 x Mshindi) x AB 136 sub group had almost similar reaction to the donor parent AB 136. The F_1 , F_2 lines and a donor parent had a mean disease score of 2.4, 1.9 and 2.0 respectively while susceptible line Vax3 x Mex54 x Mshindi showed an intermediate reaction with a mean disease score of 4.3.

Crosses made by C4-1308B-3E-8-B showed that F_1 and F_2 were both resistance (Table 4). They had a mean disease score of 2.9 and 2.2 respectively, and the donor parent C4-1308B-3E-8-B had 2.2. The overall mean disease score of this subgroup was 2.4. The F_1 , F_2 and the donor C4-1308B-3E-8-B with a disease mean score of 2.9, 1.9 and 2.2 respectively were resistance to anthracnose disease. The study indicates that the donors of common bean lines were successful in transferring genes of resistance.

The susceptible parent (recipient) Vax3 x Mex54 x Mshindi showed an intermediate reaction to the anthracnose disease with a mean score of 4.3, but the susceptible Kigoma used as control showed moderate resistance had disease mean score of 3.6. The minimum mean disease score was 1.9 obtained from F_2 of all crosses while the highest mean disease score for disease reaction observed was 4.3 from recipient parent Vax 3 x Mex 54 x Mshindi, such score are low as the disease reaction was resistance to intermediate resistant. The observation implies that F_2 of both crosses (segregation population) have been improved for anthracnose resistance to a certain level.

Despite the low severity of the disease observed in all F₁ and F₂ of the crosses made. It was noted that the mean disease to susceptible cultivar Kigoma used as control plant was 3.6 which is the moderate resistance. Aggarwal *et al.*, (2004) reported that most pathogens exhibit high variation so that a resistance gene that is effective in one region or season may not necessary effective in another, resistance thus breakdown. Host response was judged susceptible when during any evaluation, most seedlings exhibit fully sporulating lesions, and producing disease score of 7 and above. All other responses were judged on resistant score of 1 to 3 or intermediate score of 4 to 6 (Plate 2, 3 and 4).

Table 4. Disease assessment for crosses made to (Vax3 Mex x 54 x Mshindi) with ANTH Resistant parents.

Parent/crosses	N of plant	Disease			
		Mean	Range	Stdev	Variance
P1 VAX 3 x MEX 54 x MSHINDI	12	4.3	3-5	0.6	0.4
P2 AB 136	12	2.0	1-3	0.8	0.5
F1 (VAX 3 x MEX 54 x MSINDI) x AB 136	12	2.4	1-4	0.8	0.4
F2 (VAX 3 x MEX 54 x MSHINDI) x AB 136	12	1.9	1-3	0.8	0.4
P2 C4-1308B-3E-8-B P3	12	2.2	1-3	0.8	0.4
F1 (VAX 3 x MEX 54 x MSHINDI) x C4-1308B-3E-8-B	12	2.9	2-4	0.8	0.6
F2 (VAX 3 x MEX 54 x MSHINDI) x C4-1308B-3E-8-B	12	1.9	1-3	0.7	0.2
Kigoma	12	3.6	2-5	0.9	0.9

Performance of parents

The donor parents AB 136 and C4-13008B-3E-8-B still displayed good resistance to anthracnose and this indicate a possibility of obtaining genotypes with genes for resistance from the donor parents they had disease mean score of 2.0 and 2.2 for AB 136 and C4-1308B-3E-8-B respectively, (Table 3). This study concur with result reported by Young and Kelly (1997) observed that the resistance of cultivar AB 136

(Co-6) and G2333 (Co-4²) not broken. The dominant resistance genes present in the AB 136 variety was first described by Schwartz et al., (1982) and Young and Kelly (1996a) reported the same observation.

Pastor-Corrales et al., (1994) showed that only the G 2333 (Co-4²) line was resistance to 380 isolates of *Colletrotrichum lindemuthianum*. Thus, lines of G2333 (Co-4²) and AB 136 (Co-6) which has wide adaptation can positively contribute to breeding programs in Tanzania, especially for areas where *Colletrotrichum lindemuthianum* are prone. The recipient parent showed an intermediate disease resistance.

The MDL(Vax 3 x Mex 54 x Mshindi) used as recipient parent used in this study was resistant to ALS, CBB and BCMV/BCMNV, this is confirmed by visually observation made during study no any symptoms of the mention disease were seen, and this is related with other studies reported by Pastor- Mahuku *at el.* (2003), Singh *at el.* (1999) and Hillock *at el.* (2006) respectively.



Plate 2: Arrows show symptoms of anthracnose disease on the upper surface of leaf on F1 line of (Vax 3 x Mex x 54 x Mshindi) x AB 136.

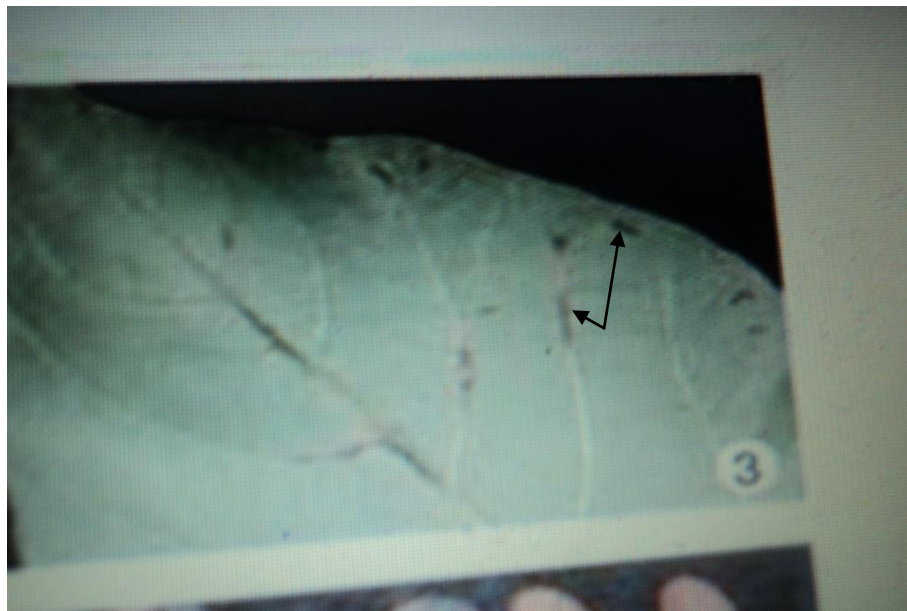


Plate 3: Arrows show symptoms of anthracnose disease under the surface of leaf on F2 line of (Vax 3 x Mex x 54 x Mshindi) x C4-1308B-3E-8-B



Plate 4: Arrows show symptoms of anthracnose disease on the trifoliolate leaves on (Vax 3 x Mex x 54 x Mshindi)

Comparison of mid parents, F₁ and F₂ for disease resistance

Results for mean disease scores of F₁, F₂ crosses and their mid parents are presented in Table 5. There was a reduction on disease reaction both based on the mid parent and the recipient parent. Although the level of disease of the recipient (susceptible) parent was not high (Score of 4.3 is moderate) this suggests that there was an improvement of resistance to anthracnose to the susceptible parent.

Table 5: Summary of mean disease score for F₁, F₂ and mid parent

Crosses	Mid parent	F₁ mean	F₂ mean
(Vax3 Mex54x Mshindi) x AB136	2.2	2.4	1.9
(Vax3x Mex54x Mshindi) x C4-1308B-3E-8-B	2.6	2.9	1.9

Frequency distribution of Disease reaction on a 1-9 scale

Results of the frequency distribution observed on leaf lesion reaction is shown in Figure 1 to 8. The frequency distribution observed on leaf lesion of the Vax 3 x Mex 54 x Mshindi, AB 136, C4-1308B-3E-8-B, F₁ and F₂ population illustrated several aspects of anthracnose resistance. Majority plants of the Vax3 x Mex54 x Mshindi (P1) population's exhibit an intermediate disease score at a range of 3 to 5 (Fig 1). All plants in AB 136 (P2) and C4-1308B-3E-8-B (P3) which were the donor parents exhibit a resistance reaction [(1-3) Fig 2 and 5]. Majority of the plants in F₁ and F₂ populations exhibit a resistance reaction with the scores of 1-3 (Fig 3, 4, 6 and 7). Since the F₁ is inclined to the resistance side this suggests the gene for resistance is dominant. These were in agreement with results reported by Schwartz *et al.*, (1982) that the variety AB 136 has the dominant resistant gene to anthracnose

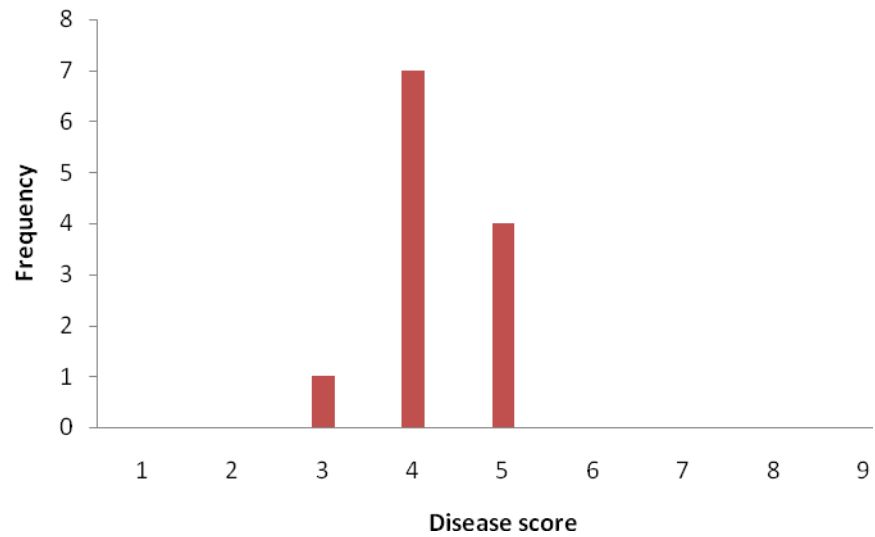


Figure 1: Distribution for anthracnose diseases of Parent 1 (Vax 3 x Mex x 54 x Mshindi)

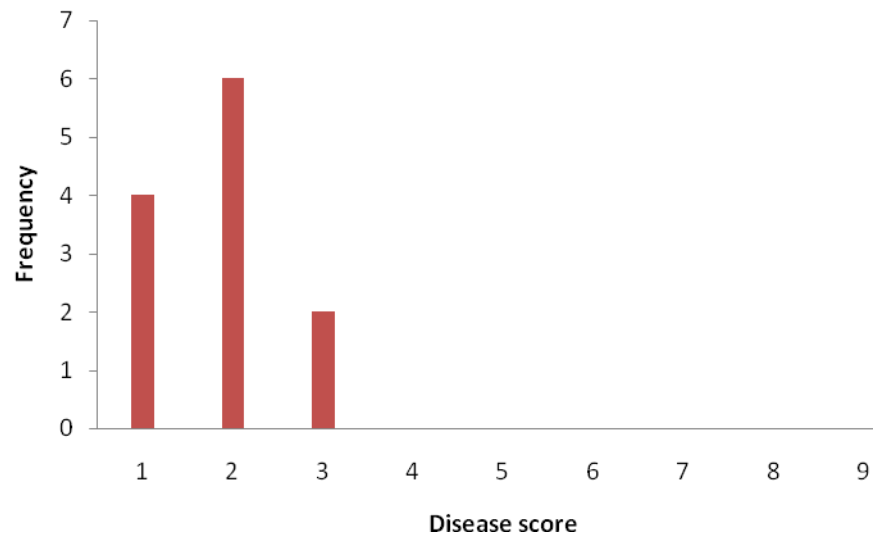


Figure 2: Distribution for anthracnose diseases of Parent 2 (AB 136)

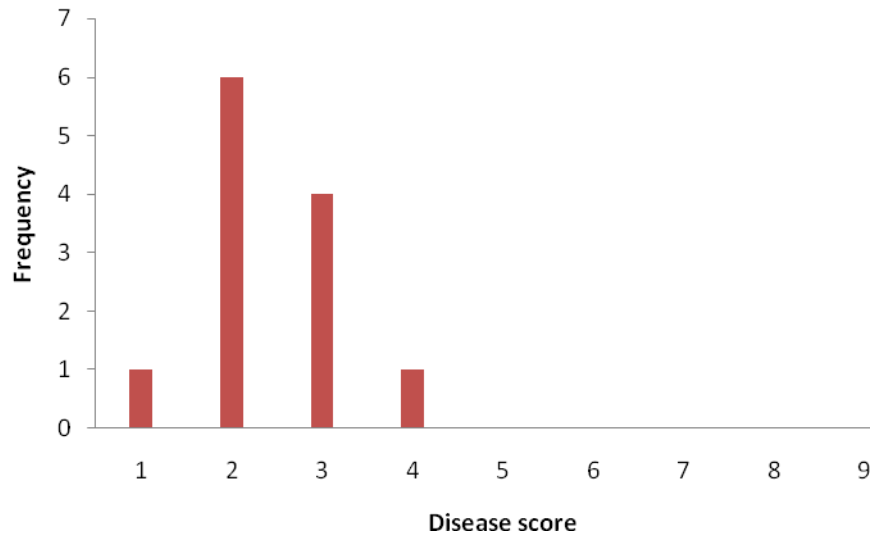


Figure 3: Distribution for Anthracnose diseases of F1 (Vax 3 x Mex x 54 x Mshindi) x AB 136

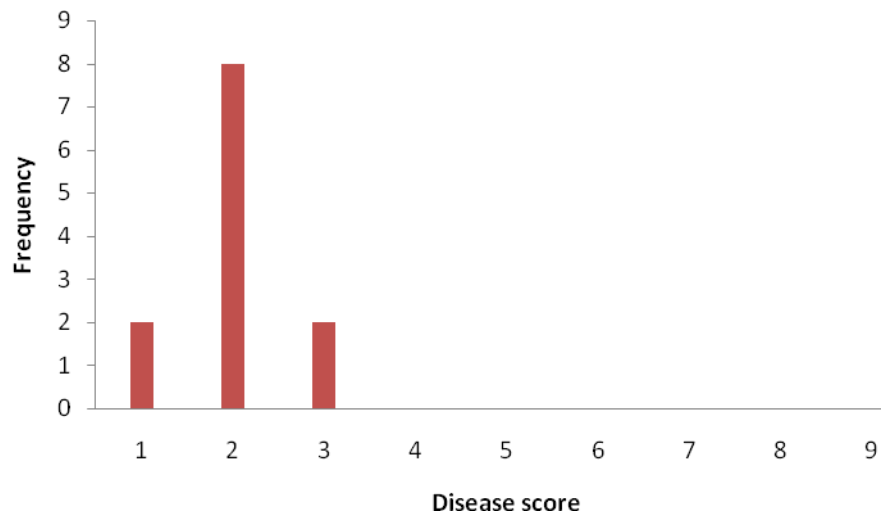


Figure 4: Distribution for anthracnose disease of F₂ (Vax 3 x Mex x 54 x Mshindi) x AB 136

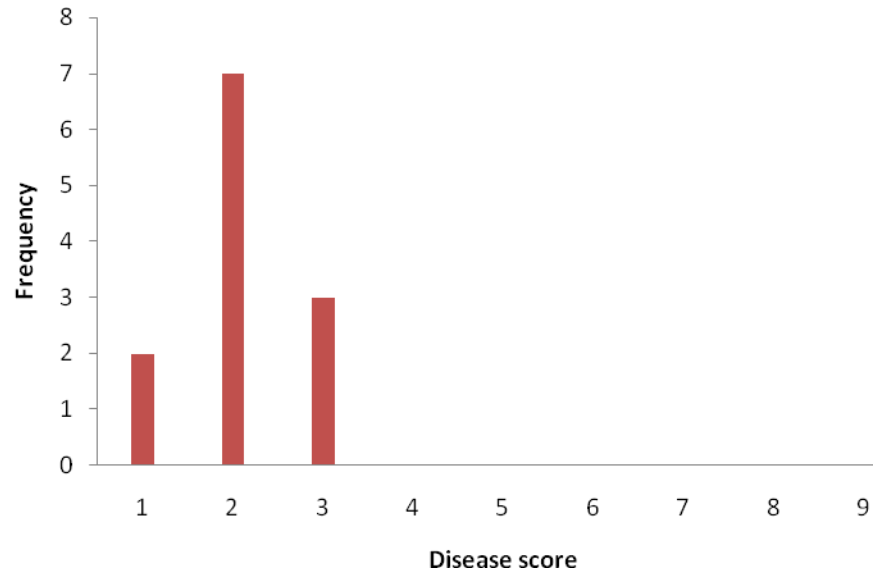


Figure 5: Distribution for anthracnose disease of Parent 3 (C4-1308B-3E-8-B)

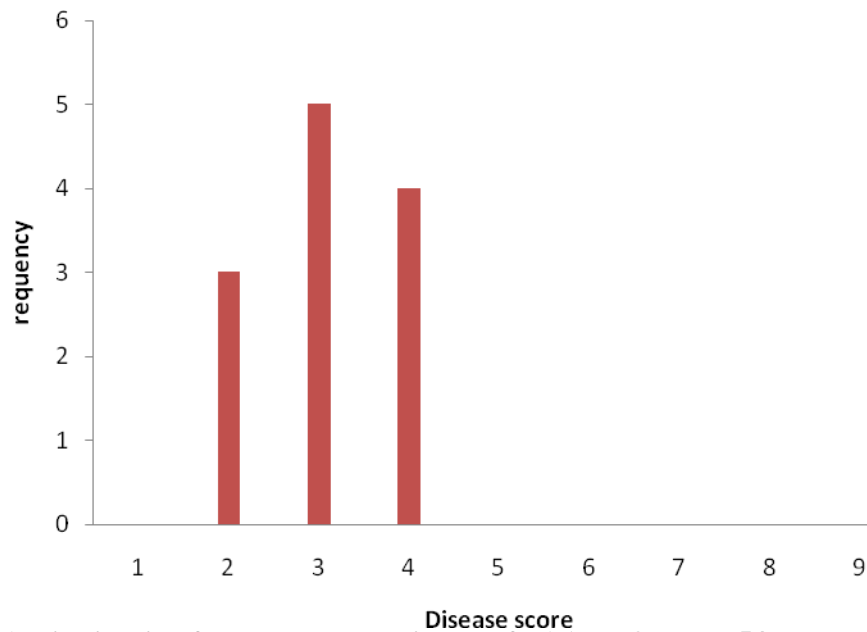


Figure 6: Distribution for anthracnose disease of F1 (Vax 3 x Mex 54 x Mshindi) x C4-1308B-3E-8-B

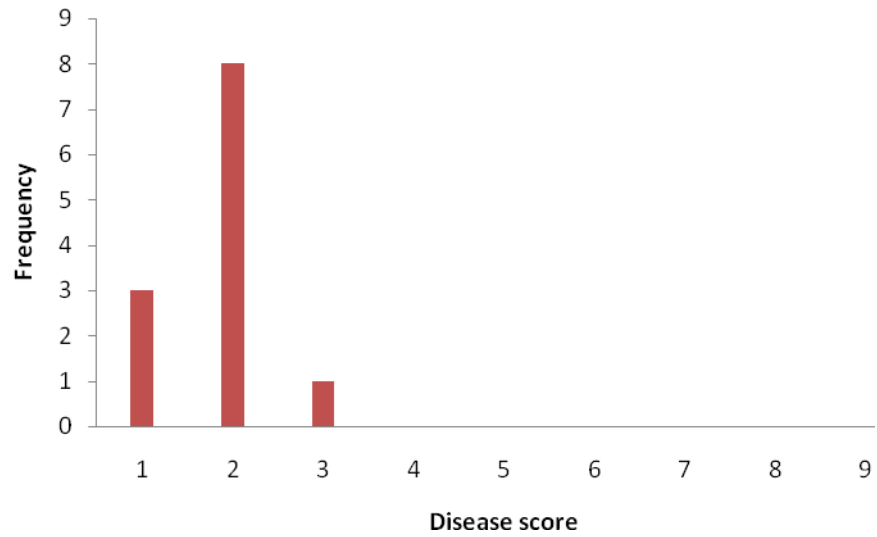


Figure 7: Distribution for Anthracnose diseases of F2 (Vax 3 x Mex x 54 x Mshindi) x C4-1308B-3E-8-B

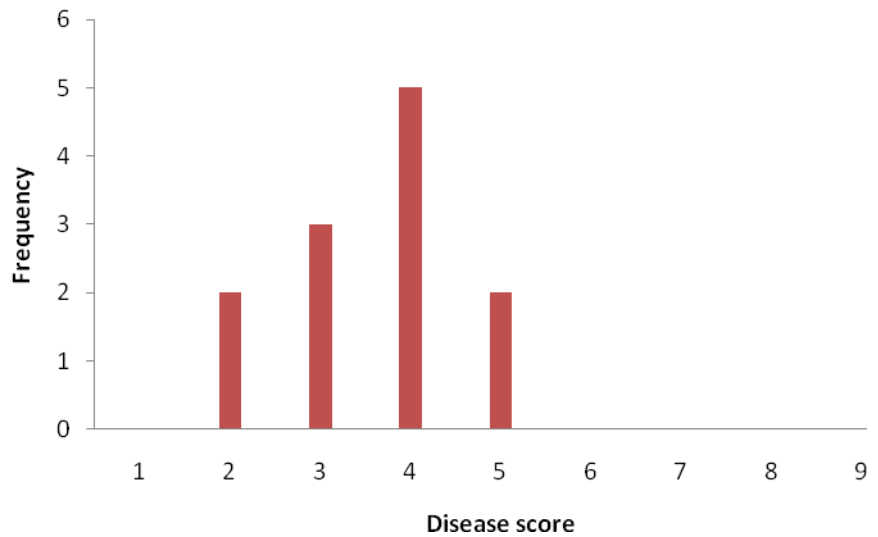


Figure 8: Distribution for Anthracnose diseases of Kigoma (control variety)

The phenotypic segregation of the F₂ progenies for reaction to anthracnose showed that plants from both (Vax 3 x Mex 54 x Mshindi) x AB 136 and (Vax 3 x Mex 54 x Mshindi) x C4-1308B-3E-8-B population was moderately variable (Table 6). This result shows that the parent that was used was not a very susceptible one since there were no plants that were in the susceptible side

Heritability, which is the proportion of the total variation in progeny that has genetic basis, was not calculated because the recipient parent was not very susceptible to the diseases

Table 6 Segregation for resistance to anthracnose (*Colletrotrichium lindemuthianum* in parental, F₁, F₂ and Kigoma

Parents/Populations	observed number of plants		
	R	I	S
Vax3 x Mex54 x Mshindi P1	1	11	0
AB 136	12	0	0
(Vax3 x Mex54 x Mshindi) x AB 136 F1	11	1	0
(Vax3 x Mex54 x Mshindi) x AB 136 F2	12	0	0
C4-1308B-3E-8-B	12	0	0
(Vax3 x Mex54 x Mshindi) x C4-1308B-3E-8-B F1	8	4	0
(Vax3 x Mex54 x Mshindi) x C4-1308B-3E-8-B F1	12	0	0
Kigoma (control)	0	7	5

R=Resistance (1-3), I=Intermediate (4-6), S=Susceptible (7-9) on CIAT scale of 1-9, (1987).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

- (i). The donor parents used in crosses C4-1308B-3E-8-B and AB 136 display good resistance to anthracnose. They can then be use as indicators of the new pathogen races in nature as (*Colltrotrichum lindemuthianum*) is highly variable.
- (ii). The Multiple Disease Line (Vax 3 x Mex 54 x Mshindi) used was observed resistance to ALS, CBB and BCMV/BCMNV, and was moderately susceptible to the pathogen. It was however improved from moderately susceptible to resistance.
- (iii). The result indicates that wider incorporation of genes for resistant for various disease within a single cultivar in particular combination of genes could be successful in developing common bean varieties with multiple disease resistance.

5.2 Recommendations

(i) Based on the results obtained the breeder may therefore consider using hybridization programme in improving genotype by introgression the desired genes from the donors.

(ii) It is recommended that the segregating population should be screened using MAS to confirm the presence of genes for resistance.

(iii) For confirming the anthracnose disease reaction on these lines, a trials should be conducted in both screen house and field at various location where the resistance genotypes will be planted in larger plots so as to get the natural inoculums of anthracnose pathogens.

(iv) F₂ population of those two crosses should be advanced to ascertain anthracnose disease and agronomic data

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