# IDENTIFICATION OF *Colletotrichum lindemuthianum* AND INTROGRESSION OF ITS RESISTANCE GENE(S) TO COMMON BEAN (*Phaseolus vulgaris* L.) ADAPTED IN TANZANIA

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## ABSTRACT

Common bean anthracnose disease caused by the fungus *Colletotrichum lindemuthianum* causes significant yield losses. It is most destructive in areas with cool temperatures and high humidity (90 - 100 %). The aim of the study was to introgress resistance gene into adapted but susceptible local cultivars Masai Red and Soya Njano using conventional breeding methods. Five races of *C. lindemuthianum* were isolated and named, from thirty two common bean diseased plants samples collected from Northern Tanzania and Nyadira, in Morogoro region. The races names varied with locations in which they were collected. The sources of resistant genes were bean cultivars G2333 and AB136. Early populations developed were evaluated under field conditions in high altitude and humid environment at Bashnet in Manyara region, in the Northern highlands of Tanzania. Both F2 and F3 populations of Soya Njano x G2333 segregated for *C. lindemuthianum* resistance at 9 : 7 ratio. Such segregation implied that two dominant epistatic genes conferred from G2333, the resistance being in mode of epistatic gene interaction. The crosses between Masai Red x G2333, both F2 and F3 populations segregation ratio was 10 : 6 which implied two dominant resistant genes were transferred to developed populations. The F2 and F3 progenies obtained from crossing Soya Njano and AB136 showed a segregation ratio of 3:1. The F2 progenies from crosses between Masai Red with AB136, segregated at a ratio of 3: 1 and also F3 progenies was 3:1. The 3:1 ratio confirmed single dominant gene inheritance conferred to developed progenies. The heritability ($h^2$) from populations of Soya Njano x G2333 and Masai Red x G2333 was between 0.41 and 0.45; while Soya Njano, Masai red and A136 was between 0.2 and 0.53, which implied moderate heritability. F2 and F3 populations developed need further testing using MAS to confirm presence of resistant genes. Multi location testing should be done for verification of resistance levels of developed bean populations in later generations.

## DECLARATION

I, Gonzaz Kyaruzi Kazimoto, do hereby declare to Senate of Sokoine University of Agriculture that this dissertation is my own original work done and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

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## COPYRIGHT

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**LIST OF ABBREVIATIONS AND ACRONYMS**

| | |
|---|---|
| Al | Aluminium |
| BCMV | Bean common Mosaic Virus |
| CATs | Conidial Anastomosis tubes |
| CIAT | Centro Internacional de Agricultural Tropical" International Centre for Tropical Agriculture |
| CBB | Common bacterial blight |
| cm | Centimetre |
| ECABREN | Eastern and Central Africa Bean Research Network |
| F1 | Filiar generation one |
| F2 | Filiar generation two |
| F3 | Filiar generation three |
| FAO STAT | Food and Agriculture Organisation Statistics |
| GIS | Geographical Information Systems |
| GPS | Global Positioning system |
| $h^2$ | Heritability in narrow sense |
| MAFS | Ministry of Agriculture and Food Security |
| MAS | Marker assisted selection |
| MDRK | Michigan Dark Red Kidney |
| Mn | Manganese |
| N | Nitrogen |
| μ = | Mi·cron also mi·kron (m kr n) n. pl. mi·crons or mi·cra (-kr) also mi·krons or mi·kra (-kr) A unit of length equal to one millionth (0-6) of a meter |
| PABRA | Pan African Bean Alliance Research |
| P | Phosphorus |

| | |
|---|---|
| RH | Relative Humidity |
| SARI | Selian Agricultural Research Institute |
| SIMLESA | Sustainable Intensification of Maize-Legume Cropping Systems for food security in Eastern and Southern Africa |
| SUA | Sokoine University of Agriculture |

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background

Common bean (*Phaseolus vulgaris* L.) is an annual crop. It belongs to the Fabaceae family. The genus *Phaseolus* comprises of 55 species. It is an important grain legume grown within the boundary between two climatic zones, the tropics and subtropics, with its primary centre of diversity in Mexico, Southern Peru, Bolivia and Argentina (Debouck, 1994). It was introduced in East Africa and Brazil by Portuguese (Jones and Mejia, 1999).

The domesticated bean species individually constitute a primary gene pool with its wild ancestral form. Wild beans distribution northwards and southwards led to formation of two geographically distinct gene pools in Meso America and the Andean (Broughton *et al.,* 2003). Domestication of common bean gave rise to several domesticated races of beans and each of the two gene pools became origin of races. Meso American gene pool races were Durango, Guatemala and Jalisco; while Andean gene pool were in New Granada, Peru and Chile (Sing *et al.,* 1991; Chacon *et al*., 2005; Hillocks *et al*., 2006).

A wide diversity of common bean cultivars is also available in developing countries for production and crop improvement for adaptation to biotic and abiotic stress; where the crop expresses wide variability in terms of maturity ranging from 60 - 150 days (Blair, 2007). According to CIAT (2013) common bean is the most vital grain legume in human diets. It is a major source of protein, carbohydrates and valuable micronutrients for more than 300 million people in the tropics. In sub-Saharan Africa, over 200 million people grow beans as a primary staple food and the most crucial source of calories after maize

(Beebe *et al.,* 2012). It enhances health promoting aspects of the diet thus vital in mitigating health risks for diseases such as obesity, cancer, diabetes and heart disease (Raatz, 2013).

The world largest producers of common bean are India, Brazil, Myanmar and Mexico (FAOSTAT, 2014). In Africa large producers are East African countries where Tanzania is the leading producer contributing 4.9 % of the production (FAOSTAT, 2013). However, production of common bean in various parts of the world is faced with a number of major biotic and abiotic constraints. Biotic stresses include those which are caused by fungi, bacteria, viruses and insect pests. The abiotic bean production constraints include macro nutrients such as nitrogen [N] and phosphorus [P], micronutrients deficiency; such as excessive rain/flooding, drought, heat and cold stress factors, each of which causes yield loss significantly (Wortmann *et al.,* 1998; Beebe *et al*., 2012).

In Africa, Latin America and the Caribbean, abiotic constraints for bean production are drought, heat, N deficiency, P deficiency, acid soil, soil toxicity caused by Mn. and Al. while biotic factors include viral diseases (BCMV), fungal diseases such as angular leaf spot, anthracnose, Pythium root rots, Fusarium root rot, rust and bacterial diseases such as common bacterial blight (Singh, 1999; Rao, 2001; Miklas *et al*., 2006; Beaver and Osorno, 2009; Beebe *et al*., 2012). All agricultural zones in Tanzania are constrained by incidences of diseases and insect pests both in the field and in storage.

In the northern zone, the biotic factors include diseases and insects pests. Major diseases are angular leaf spot (*Pseudoscespora griseola*) and anthracnose (*Colletotrichum lindemuthiamum*) and Common bacterial blight (*Xanthomonas phaseoli)*. Insect pests

include bean stem maggot (*Ophiomyia* spp), bean aphids (*Aphis fabae*), bean leaf beetle (*Ootheca benningseni* (*Acanthosceli*), bean bruchids (*Acanthoscelides obtectus*) (Nyambo, 2009).

### 1.2 Justification

Under favourable climatic conditions, anthracnose is a devastating seed borne disease of common bean. It causes significant yield loss in susceptible bean cultivars, throughout the world, resulting in 80 – 100 % yield losses (Shao and Teri 1985; Pastor-Corrales and Tu, 1989; Sharma *et al*., 1994; Fernandez *et al*., 2000; Sharma *et al.,* 2007). Infections can be quite destructive when climatic conditions are favourable to the pathogen. Economic yield losses can be as high as 100 % (Rava *et al*., 1993; Chamma Davide *et al*., 2009).

The yields are about three times as high in developed countries such as U.S.A and Canada compared to the developing countries (Porch *et al*., 2013). According to FAOSTA (2015) estimates for 2013, the world bean production was 1235 kg/ha while the yield for Africa was 799 kg/ha and it was 885 kg/ha for Tanzania, which does not suffice the demand. The yield potential is 1500 up 3000 kg/ha under reliable rainfall (Hillocks *et al*., 2006).

In Eastern Africa, anthracnose ranks the second after angular leaf spot (ALS), among bean diseases (ECABREN, 2003). In Tanzania, yield losses due to bean anthracnose range from 60 to 80 % (Dron and Bailey, 1999). The “Masai Red” and “Soya Njano” are among the leading and locally adapted bean cultivars grown by small scale farmers in the northern Tanzania (Katungi, 2009). Despite their good levels of adaptability, these two varieties are fairly susceptible to anthracnose disease. High levels of infection brings about food insecurity and low income among small scale resource poor farmers. Efforts to manage the disease through deployment of tolerant elite common bean varieties encounter

challenges caused by the frequent appearance of new physiological races of *Colletotrichum lindemuthianum* (Kelly *et al*., 1994; Pastor-Corrales *et al*., 2009). This phenomenon encourages the need to broaden the genetic base of common beans as a crop; to expand the scope of parental bean cultivars with desirable traits as potential sources of resistance genes in breeding programmes that fits to the need to overcome the frequent evolution of new races of the pathogen.

Several anthracnose management strategies including planting mixtures of bean cultivar (Tesfaye, 2003; Mwesigwa, 2009) have been advocated to alleviate deleterious effects of anthracnose disease on bean productivity. However, the success remains low due to unaffordable cost of practices and labour constraints among small holder producers. Genetic resistance is the most cost effective means to control the disease (Miklas *et al.,* 2006; Tryphone *et al.,* 2013). Development of well adapted resistant bean cultivars is considered as an effective alternative management option for control of anthracnose (Kelly *et al*., 2009). This study aimed at introgressing genes conferring resistance against anthracnose into popular local common bean cultivars ‘Masai red’ and ‘Soya Njano, ’to improve their productivity.

## 1.3 Overall Objective

To contribute in reducing common bean losses caused by anthracnose disease through incorporation of resistant genes into local cultivars Masai Red and Soya Njano, preferred by consumers in Northern Tanzania.

### 1.3.1 Specific objectives

i. To identify and characterize *Colletotrichum lindemuthianum* isolates from bean growing areas in Northern Tanzania and Morogoro region.

ii. To introgress genes for anthracnose resistance into two preferred local bean cultivars Masai Red and Soya Njano and determine the presence of resistance in segregating bean lines.

iii. To determine the inheritance pattern of anthracnose resistance.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Bean Anthracnose Disease

Bean anthracnose is a fungal disease, which means Anthrax –'coal' and nosos –'disease'. The name was coined in French from Greek according to Ragui and Shimray (2014). It is caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams.-Scrib. It is one of the most widespread and economically important fungal disease of common bean (*Phaseolus vulgaris* L.) (Pastor-Corrales and Tu, 1989; Gonçalves-Vidigal, 2001). The disease was first reported on bean (*P. vulgaris*) in France in 1843. The fundamental agent of bean anthracnose was identified as a fungus which is present in imperfect forms denominated by *Colletotrichum lindemuthianum* and *Glomerella cyngulata f. sp phaseoli*, respectively (Rodriguez and Yoder, 1987; Pastor-Colares, 1989; Martínez-Pacheco *et al*., 2009).

The symptoms of the disease were first detected in 1875, in Germany (Martinez-Pacheco *et al.,* 2009). The fungus is seed borne and has high pathogenic variability (Campa *et al*., 2011). Under favourable conditions to the pathogen, the disease causes complete yield loss on compatible genotypes, (Pastor-Corrales and Tu, 1989; Gonçalves-Vidigal, 2001; Fernandez *et al*., 2000; Sileshi, *et al.,* 2014).

In Ethiopia, bean anthracnose was reported to cause an estimated yield loss of 63 % (Beshir, 1997; Amin *et al.,* 2014). Similar losses in the range of 40 - 80 % were reported in Tanzania (Dron, 1999). According to Dillard (1988) yield is reduced due to poor seed germination, poor seedling vigour. Losses are also in terms of poor marketability of impaired seed quality of grain attributed to infection lesions on seeds, low grade grain due

to poor grain filling, seed spots and discolouration. The pathogen exists in different physiological races that varies from, country, region, location and one variety to another. More than 100 pathotypes of this fungus have been described (Silva *et al.,* 2007; Souza *et al*., 2010).

### 2.1.1 Geographical Distribution

Bean anthracnose is distributed worldwide. *Colletotrichum lindemuthianum* is particularly important in sub-tropical and temperate bean production areas of the world. (Gonçalves-Vidigal, 2007). It causes significant yield losses in temperate and sub - tropical zones than in the tropics. It has been reported in all continents including North, Central and South America, Europe, Africa, Australia and Asia (Pastor-Corrales and Tu, 1989; CIAT, 2008; Mohammed, 2013). According to PABRA (2009) anthracnose ranks second as the most important common bean disease in Eastern and Central Africa (ECA); Southern Africa (SA) and Sub-Saharan Africa. Angular leaf spot ranks first while common bacterial blight (CBB) comes third. It is of economic importance in Kenya, Uganda, Tanzania, Ethiopia. Rwanda, Burundi Kivu Province of the Democratic Republic of Congo, Malawi and Zambia (CIAT, 1981; Schwatz and Corrales, 1989; Pastor-Corrales *et al*., 1994). In Tanzania, anthracnose incidences are common in the northern Zone and Southern Highlands (Nyambo, 2009).

### 2.1.2 Classification of *Colletotrichum lindemuthianum*

The classification was made according to Lexopoulos and Mim (1979). It was proposed that *C. lindemuthianum* belongs to: Family, Melanconiaceae; Order, Melanconiales; Sub class, Coelomycetidae; Class, Deuteromycetes; Sub Division, Deuteromycotina; Division Amastigomycota; Kindom Myceteae; Super Kindom, Eucariota (Martinez-Pacheco *et al.* 2009).

### 2.1.3 Physiology and etiology of *Colletotrichum lindemuthianum*

Conidia are reproduced in Acervuli which are physiologically round or elongated. Irregular cells of an acervulus develop as setae which are brown, septate and slightly swollen at the base and taper gently to the rounded paler apex. Acervuli are few in number and longer than the conidial mass. Setae are 4 - 9 µm wide and usually less than 100 pm long. Conidia are unicellular, hyaline, cylindrical with both ends having narrow and truncate base (Mathur and Kongsdal, 2003).

### 2.1.4 Life cycle of *Colletotrichum lindemuthianum* pathogens

#### 2.1.4.1 Pre entry into the host phase

During saprophytic phase of the fungus, the spore germination process begins with the spore adhesion to the plant surface under favourable humidity conditions; specifically, correct aqueous content in the spore mucilage. At this level, the spores of the fungus are rounded off by water absorption and active growth. Later, germinating tubes are formed and the hypha elongates to dominate in the substrate.

The aerial mycelia appear; then the fungal reproductive structures are formed where the spores are stored (Martínez-Pacheco *et al.,* 2009). Spore germination starts by the development of appressorium on a plant surface which is followed by the turgor pressure which is driven to penetrate on the cuticle (Deising *et al.,* 2000; Cannon *et al.,* 2012).

#### 2.1.4.2 Infection

The infection begins with the adhesion of conidia to the plant surface; the conidia then germinate to form darkly pigmented, domeshaped appressoria that pierce the plant cuticle and cell wall directly and mediate entry into host epidermal cells (Takahara *et al.,* 2000). At initial stages of interaction between fungus and host plants, fungal pathogens produce

surface proteins that is important for adhesion and invasion (Herbert *et al*., 2004; Mohammed, 2005).

After successful penetration, bulbous primary hyphae grow biotrophically inside living host epidermal cells and invaginate the host plasma membrane. This haustorium-like structure is entirely restricted to the first infected epidermal cell, and seems to obtain nutrients and water from the plant apoplast. Subsequently, sufficient nutrients are probably released during the destructive necrotrophic stage of host infection through the activity of secreted cell wall degrading enzymes. At this stage, the pathogen also undergoes a morphological switch, producing narrow secondary hyphae, which rapidly colonise the entire host tissues (Takahara *et al*., 2012). This fungus exhibits two phases namely, biotrophic and a necrotrophic during its life cycle, hence classified as hemibiotrophic due to succession of these two phases (Perfect *et al*., 1999; Nogueira *et al*., 2013).

The first phase lasts 3 to 4 days, a stage where the fungus establishes biotrophic interaction inside the infected epidermal cells. This phase is referred to as the biotrophic phase, during which the fungus differentiates infection vesicles and primary hyphae (Dufresne *et al*., 2000). The fungus deploys various strategies to overcome defense responses, such as masking of invading hyphae or active suppression of defense, are essential for a biotrophic parasitic mode of life. During initial invasion and biotrophic development, the pathogen masks its surface by converting the hyphal surface-exposed chitin by deacetylation. This mechanism helps avoiding degradation of chitin by plant chitinases and recognition of chitin (Munch *et al*., 2008).

#### 2.1.4.3 Necrotrophic growth phase

The necrotrophic phase, which is accomplished between 6 to 8 days after inoculation; it corresponds to the appearance of anthracnose symptoms (Dufresne *et al*., 2000). However, symptom appearances are influenced by favourable temperatures, genotype of the bean cultivar and age of the bean tissues (Dillard, 1988). In this phase, the fungus develops secondary hyphae that grow both between and within host cells hence acting as a typical necrotrophic pathogen (Dufresne *et al*., 2000).

### 2.1.5 Imperfect phase

Reproduction occurs asexually which is also termed as imperfect form of *Colletotrichum lindemuthianum.* An asexual form involves spores which are produced inside acervulus and immersed in water soluble pre-formed mucilage (O'Connel, 1996; Martínez-Pacheco *et al.,* 2009). During development, spores of the fungus show two phases of life behaviour, that is biotrophic and saprophytic hence classified as hemi bitroph. The saprophytic fungus growth occurs in any carbon sources which can be converted into molecules of energy by extra cellulolytic enzymes (Martínez-Pacheco *et al.,* 2009). At this level, the spores of the fungus round off by water absorption and active growth. Later, a phase of germinule takes place where the germinating tube is formed and the hyphae elongates to occupy the substrate. The aerial mycelia appear; then the fungal reproductive structures are formed where the spores are stored (Martínez-Pacheco *et al.,* 2009).

### 2.1.6 Perfect stage

Sexual reproduction allows for the generation of new combinations of alleles in each recombination cycle, creating a high level of genetic variability in a population of a pathogen. This results into periodic occurrence of sexual reproduction that can clarify

much of the variability existing in *C. lindemuthianum* populations (Souza *et al.,* 2010). Liu *et al*. (2012) reported on unavailability of any data that show *C. lindemuthianum* a sexual stage forms. However, noted on assumption that sexual stages linked to *C. lindemuthianum* in the past belonged to other species.

## 2.2 Epidemiology

Anthracnose disease pathogens are introduced into fields through infested seed (CIAT, 1989). Farmers continuous exchange and use of infected seed, encourages pathogen distribution throughout all bean growing regions of the country according to (Opio *et al*., 2001). The pathogen is able to overwinter on infested residue for up to two years and can survive in infected seed for up to five years (Rio and Bradley, 2001). When infected seed germinates, lesion development occurs on cotyledons, spores from these lesions are spread by rain splashes, irrigation water, insects, animal and farm implements **(**Hagedorn, 1989). Spores primarily are splash-dispersed and most will spread only a short distance (150 cm or less) in gentle rain storms which affect adjacent fields (CIAT, 1989). The favourable environment support epidemics of pathogens of bean anthracnose disease However, spores can spread much further when wind-driven rain occurs. Temperatures of 13 – 26ºC with an optimum temperature of 17 ºC favour production of spores and initial infection (CIAT, 1989). Relative humidity above 92 % and free moisture encourage occurrence of an infection (Schwartz *et al*., 2005; Silva *et al*., 2013).

## 2.3 Symptomatology

Anthracnose disease symptoms in compatible varieties can be visible on any plant part. Primary symptoms can occur on cotyledonary leaves, occurrence of small lesions with dark brown to black lesions (Alzate-Marin, 1997). The stems and leaves exhibit lesions which are sunken, elongated and circular. Pods have sunken and circular lesions (Markell

*et al.*, 2000). The pathogen can infect all aerial parts of the bean plant and produces round black sunken lesions containing flesh coloured spores on leaves, stem, pods and seeds. When the pathogen establishes on a compatible cultivar, the first symptoms are yellowish spots that later develop into ulcerous necrotic wounds that affect all plant structures such as leaves, stems, flowers and fruits. Later on, general infection occurs in the plant; by the growth of the mycelia. The fungal fruiting structures can be detected over the surface of the plant which ultimately become fatal to the plant (Martínez-Pacheco *et al.,* 2009). Infection causes premature defoliation, premature fall of flowers and pods, seed deterioration in extreme cases, plant death. Infected seeds are the major means of dispersal of the pathogen (Schwartz *et al*., 2005; Campa *et al*., 2014).

## 2.4 Control Measures

### 2.4.1 Cultural control

#### 2.4.1.1 Sanitation and solarization

The disease can be managed by using pathogen free bean certified seed, resistant varieties, crop rotation for at least 2 to 3 years as well as field sanitation (Tesfaye, 2003; Mwesiga, 2006). An evaluation of the effect of integrated management of bean anthracnose through soil solarization and fungicide applications on disease development and seed health of common bean, was conducted using variety Mexican-142. The results showed that, mixture of integration of soil solarization, seed treatment and foliar spray were effective in minimizing bean anthracnose epidemics and seed infection (Mohammed *et al.,* 2013).

#### 2.4.1.2 Crop Rotation

Where bean anthracnose is already established in the field, it is recommended to adopt a 2 - 3 year rotation (CABI, 2014). Corn and Solanaceous crops like tomatoes, potatoes and

eggplant are suitable non-host rotation crops. The bean crop debris which is infected must be buried to keep them away from spreading the disease in the following season.

#### 2.4.1.3 Mixture of bean varieties

Deployment of mixtures with 50 % resistant bean cultivar exhibited significant reduction of anthracnose development in the field. Twenty five percent resistant cultivar conferred satisfactory protection level, while ten percent gave variable results (Ntahimpera *et al*., 1996).

#### 2.4.1.4 Clean seed

Use of clean seeds prevents the spreading of the anthracnose pathogen, but seed movement across locations, regions, countries by farmers and plant breeders can bring in new anthracnose races (Hegay *et al*., 2013).

### 2.4.2 Biological control

#### 2.4.2.1 Use of bioagents

Seed treatment using bio agents such as *Pseudomonas fluorescence*, *Trichoderma harzianum* and *Trichoderma viride* exhibited potential suppression of seed borne fungal pathogens. They are safe means for controlling seed born diseases such as bean anthracnose (Amin *et al*., 2014).

#### 2.4.2.2 Use of resistant varieties

**Genes for bean Anthracnose pathogens resistance**

Breeding for resistant cultivars that are resistant to races of pathogens, confers a long lasting solution to the economic losses of beans caused by anthracnose (Mahuku *et al*., 2002; Vidigal Goncalves *et al*., 2011). It is biologically safe and cost effective

(Kour *et al*., 2012). Resistant varieties are essentially useful in the crossing to incorporate alleles with resistance to pathogens in elite lines and commercial varieties (Rocha *et al*., 2012). Some of these genetic materials would be valuable in bean breeding program as new sources of resistance to anthracnose.

The differential cultivars possess genes for resistance but a clear knowledge is needed on their nature and inheritance of their resistance sources, since this information enables early planning of transferring of disease resistance into different commercial bean cultivars (Vidigal, 2007). The great majority of anthracnose resistance genes in dry bean (*Co-2, Co-3, Co-3², Co-4, Co-4², Co-4³, Co-5, Co-6, Co-7, Co-8, Co-9, Co-10, Co-11* and *Co-u*) are from beans belonging to the Mesoamerican gene pool (Kelly and Vallejo, 2004; Concalves -Vidigal *et al*., 2011). According to Kelly and Vallejo (2009) bean landrace G2333 carries three genes pyramid for anthracnose resistance: *Co-4², Co-5* and *Co-7* and the *Co-4²* gene is well characterized. Kelly and Vallejo (2004) reported that multiple alleles exist at the *Co-1, Co-3* and *Co-4* loci.

#### 2.4.2.3 Breeding for bean anthracnose disease resistance

**Inheritance**

Knowledge on the nature of inheritance of these resistant sources is vital in transferring resistance to different susceptible but otherwise preferred cultivars according to (Vidigal Filho *et al*., 2007). The results from previous studies showed that inheritance of genes for resistance is conferred by single dominant genes (Young and Kelly, 1996). Various studies (Kelly and Vallejo, 2004; Concalves-Vidigal *et al*., 2011) showed that resistance to Common bean anthracnose disease was conferred by a single gene to several physiological races. Currently, resistance to *C. lindemuthianum* is conditioned by anthracnose resistance loci identified by the *Co* symbol. Two independent dominant

genes (Campa *et al*., 2009); complementary epistatic genes (Alzate-Marin *et al*., 1997; Muhalet *et al*., 1981; Peloso *et al*., 1989), or multiple genes (Vallejo and Kelly, 2009) controlled resistance to bean anthracnose (Alzate-Marin *et al.,* 1997).

The recessive resistance gene is *co-8* in small red-seeded climbing bean variety AB 136, (Alzate-Marin *et al*., 1997). Hence all nine genes are dominant genes and multiple alleles exist at the *Co-1*, *Co-3* and *Co-4* loci, with the exception of the recessive *co-8* gene (Kelly and Vallejo, 2004; Miklas *et al.,* 2006). The nine resistance genes *Co-2* to *Co-10* are Middle American in origin and *Co-1* is the only locus from the Andean gene pool (Miklas *et al.,* 2006). However, single gene resistance alone is insufficient to offer effective and durable resistance against anthracnose disease in common bean varieties. Physiological races of *C. lindemuthianum* that cause bean anthracnose vary greatly genetically (Kiryowa *et al*., 2010**)**. Durable resistance is important and is attained through careful selection of genes providing resistance to various pathogen races producing anthracnose disease (Pastor –Corrales *et al.,* 1994).

Genetic conclusion about resistance of cultivars: Michelite, Michigan Dark Red Kidney, Perry Marrow, Cornell, 49-242, PI 207262, AB136, G 2333 and their 21 diallel hybrids were obtained in relation to the reaction to race 69 by using Hayman's method where 15-day-old bean plants of differentials were inoculated using race 69. Twenty one hybrids developed from a diallel crosses among Michelite and Perry Marrow lines were susceptible to the race 69 as well as Dark Red Kidney, Cornell 49-242, PI 207262, while lines AB 136 and G2333 were resistant to race 69. These findings indicated the predominance of the dominant genetic effects, since the estimates of the mean degree of dominance was (1.2). The variations among heritability magnitudes in narrow sense was 0.4418 according to ( Poletine *at al.,* 2006).

**Breeding efforts**

Efforts have been made to overcome the problem of bean anthracnose. Plant breeders opted for germplasm collections either for improvement of some of their traits hence news cultivars; or as sources of specific genes (Acquaah, 2007). The strategy involve short term, long term approaches. The short term programme involves exchange of accession or bean germplasm collections from other countries or; international organizations such as CIAT. After evaluation, promising accessions are released for commercial production by farmers and other end users. Pyramiding is one of the long term programmes breeding strategies. The pyramiding resistant genes capable of conferring complementary spectra of resistance has been suggested as an effective strategy against pathogen variability challenges. Pyramiding specific genes for resistance ensures durable resistance and is the main strategy in the breeding program (Genchev *et al*., 2010). An example is G2333 which is a Mexican common bean landrace which carries a three gene pyramid for anthracnose resistance: *Co-4²*, *Co-5* and *Co-7* (Vallejo and Kelly, 2009). In each one of these crosses, the resistance genes were located in independent loci and any one of them could give full resistance, even in the presence of the recessive allele of the other two; hence behaving as triplicate dominance resistance factors (Poletine *et al.,* 2000).

The cultivar AB 136 was reported to posses two independent genes that give resistance to race 73. The dominant gene is named *Co-6* while the recessive gene is *co-8*. Genotypes with *Co-6* and *co-8* conditions resistance, while susceptibility is present in genotypes with *co-6* and *Co-8*. Studies in Brazil indicated that bean cultivar AB136 has dominant genes for resistance to 25 races of *C. lindemuthianum* (Alzate-marine *et al.,* 1997). As a result of these findings, this cultivar is included as one of the donor parents in molecular marker-assisted backcross breeding programme; to develop common bean cultivars that

are resistant to anthracnose and adapted to Central Brazil (Alzate-marine *et al.,* 1997). In southern highlands of Tanzania cultivar AB136 showed complete resistance and was considered for official release for commercial production by the farmers (Dron and Bailey, 1999).

## 2.5 Chemical Control

Use of fungicide with combinations of Mancozeb seed treatment and Carbendazim spray at 10 day intervals reduce anthracnose disease severity and increased seed yield Amin *et al.* (2014). According to Beshir (1997) and Mohammed (2014), seed treatment with fungicides resulted in good anthracnose disease control. Foliar fungicide application gives positive effective when applied during flower setting, late flowering and at pod filling stages (Holmes, 2007).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study Location

This work was conducted in Long village, within Bashnet ward about 62 km west of Babati town. The field experiment was conducted in a farmer's field located at S 04°13.815; E35° 27.090, at an elevation of 2 187 metres above sea level.

The village experiences a bimodal rainfall pattern from mid November to mid January and mid March to the end of June. Major crops in this ward were common bean, maize, round potatoes and garden peas were intercropping maize with common beans and round potatoes is common practice. Mid November to mid January is the predominant season for growing common beans in Babati rural areas.

### 3.2 Bean Plant Materials

Two local adapted cultivars 'Masai red' and 'Soya Njano' were used as a female parent. They were collected from farmers at Upper Kitete village in Karatu district in Arusha region. Both varieties succumb to bean anthracnose infection. The G2333 and AB136 are resistant to bean anthracnose disease; and were used as donor parents. Masai red that belongs to Meso America gene pool, is an intermediate (climber) type IVa, weak stemmed climber. It has small oval, deep red seeds. Soya Njano is grouped into two categories which are indeterminate (climber) with weak stems and the other group is type I determinate which has strong stems. The cultivar has deep yellow round shape grain while the other is pale yellow round bigger grain compared to the former yellow, belong to climber group. Both the determinate and indeterminate are early maturing, with low flatulance.

The determinate Soya Njano was used in this study. Soya Njano is highly marketable due to its good grain quality. It exhibited good agronomic traits. G2333 and AB 136 cultivars were used as male parents.The seed types of these parental genotypes are shown in Plate 1 Both donor parent bean cultivars were obtained from Sokoine University of Agriculture (SUA) bean breeding programme. Some of their characteristics are given in Table 1. The segregating bean seeds in F1, F2, F3 generations obtained by crossing Masai Red x G2333 and selfed are shown in Plate 2 represented of other closes such as Soya Njano x G2333, Soya Njano x AB136, Masai Red x AB136 which also had segregating bean seeds.

**Table 1: Bean cultivars used for current study and some of their key phenotypic characteristics**

| Genotype | Growth Habit | Source of seed | Seed size | Seed color | Flower Color | Reaction to anthracnose |
|---|---|---|---|---|---|---|
| Soya Njano 1 | Determinate Erect, bush | Upper Kitete | Medium | Yellow | Pale pink | Susceptible |
| Soya-Njano 2 | Indeterminate | Upper Kitete and Slahamo | Small-round | Pale-Yellow | Pale pink | susceptible |
| Masai red | Indeterminate | Upper Kitete | Small | Deep red | White | Susceptible |
| G2333 | Indeterminate | SUA-Morogoro | Small | Maroon | White | Resistant |
| AB136 | Indeterminate | SUA – Morogoro | Small | Red | White | Resistant |

Source: Pastor – Corrales *et al*. (1994)


**The recipient parent cultivars Masai Red and Soya Njano**

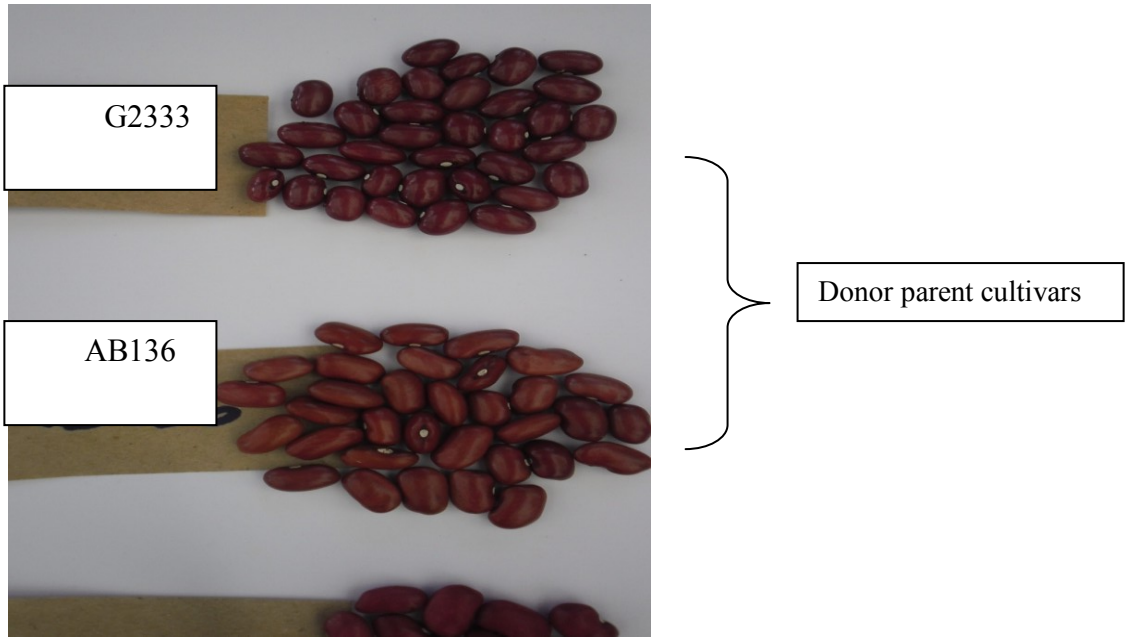



**Plate 1: Bean cultivars used as donor parents G2333 and AB36 in hybridization**

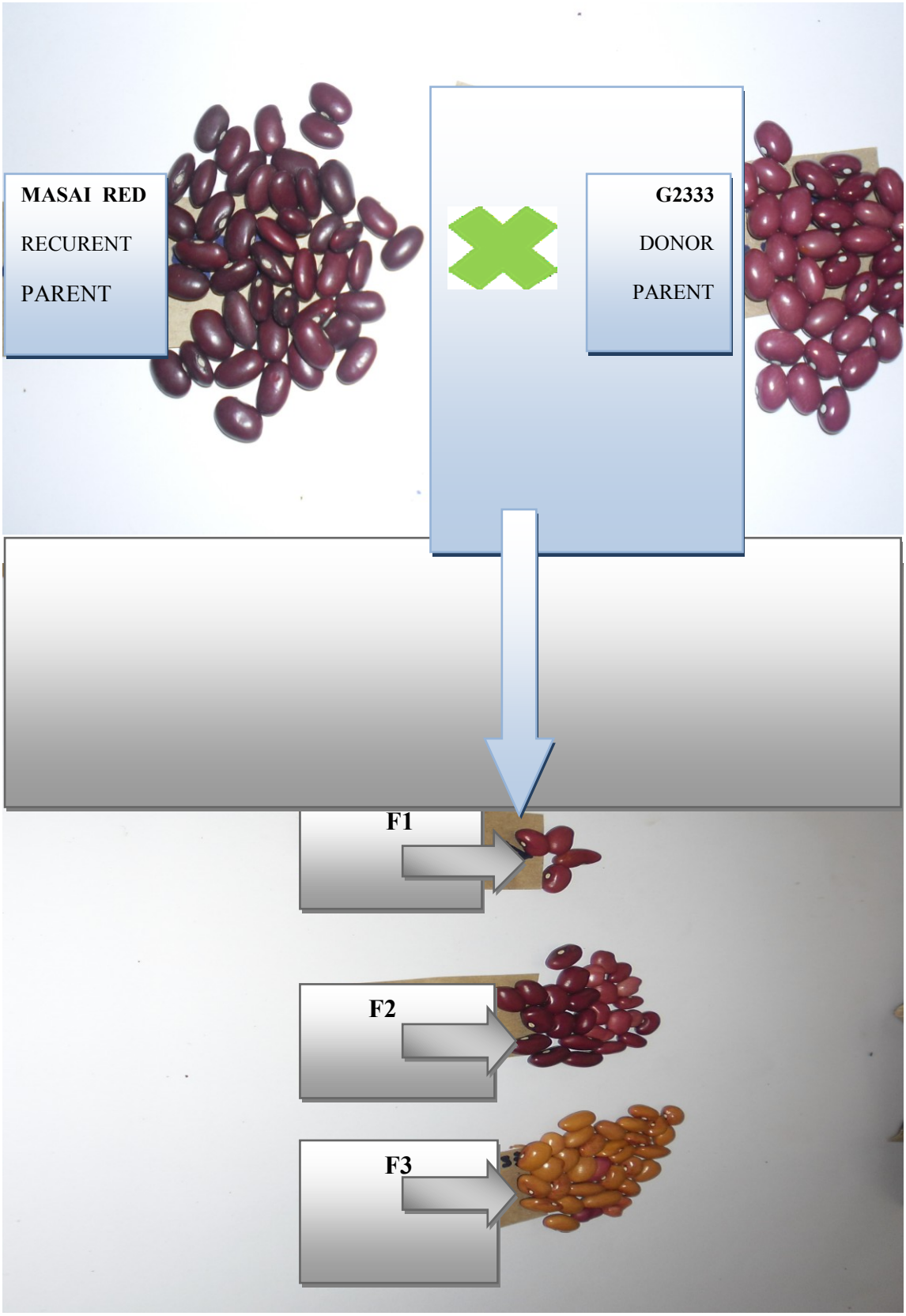



**Plate 2: Crosses between Masai Red and G2333, $F_1$, $F_2$ and $F_3$ population developed from them**

### 3.3 Generation of Breeding Lines

#### 3.3.1 Hybridization

Purification of "Masai Red" and "Soya Njano" was done by planting 5 seeds of each variety in plastic pots in the screen house. Two seeds harvested from a single plant were used in making crosses. Two seeds were sown per pot filled with sterilized forest soil and thinning was done two weeks after planting. Six pots were used per variety, for crossing establishment, recipient as well as donor parents were planted in a staggered mode at an interval of seven to fourteen days. Donor lines were planted first at an interval of seven up to fourteen days before planting recipient parents. Diamonium Phosphate (DAP) at a rate of 60 Kg/ha was used during sowing. Urea was top dressed at a rate of 20 kg N/ha.

Watering was done throughout the time of an experiment. Crosses were made between adapted local cultivars (Masai Red and Soya Njano) susceptible female parents with donor lines G2333 containing complementary genes *Co-4², Co-5, and Co-7* and AB 136, carrying complementary gene *Co-6, co-8*. Crosses were as follows:- Masai red x G2333, Masai red x AB136, Soya Njano x G2333, Soya Njano x AB136 to get F1 populations.

The procedure of crossing involved emasculation of the female flowers and transfer of pollen from just flowers to the stigma of emasculated bean plants. Both rubbing and hooking methods were used. Pollination was performed by rubbing the pollinated stigma of the male flower to the female flower. Hooking technique was done by removing the pollinated stigma of donor parent by means of forceps and hooking it against recipient parent flower (CIAT, 1989). Prior to new emasculation, forceps were sterilized by dipping in alcohol to avoid contamination with pollen or other pathogenic organisms from one flower to another. The F1 progenies obtained by crossing were harvested from each cross separately then grown to advance them to F2 then to F3 populations.

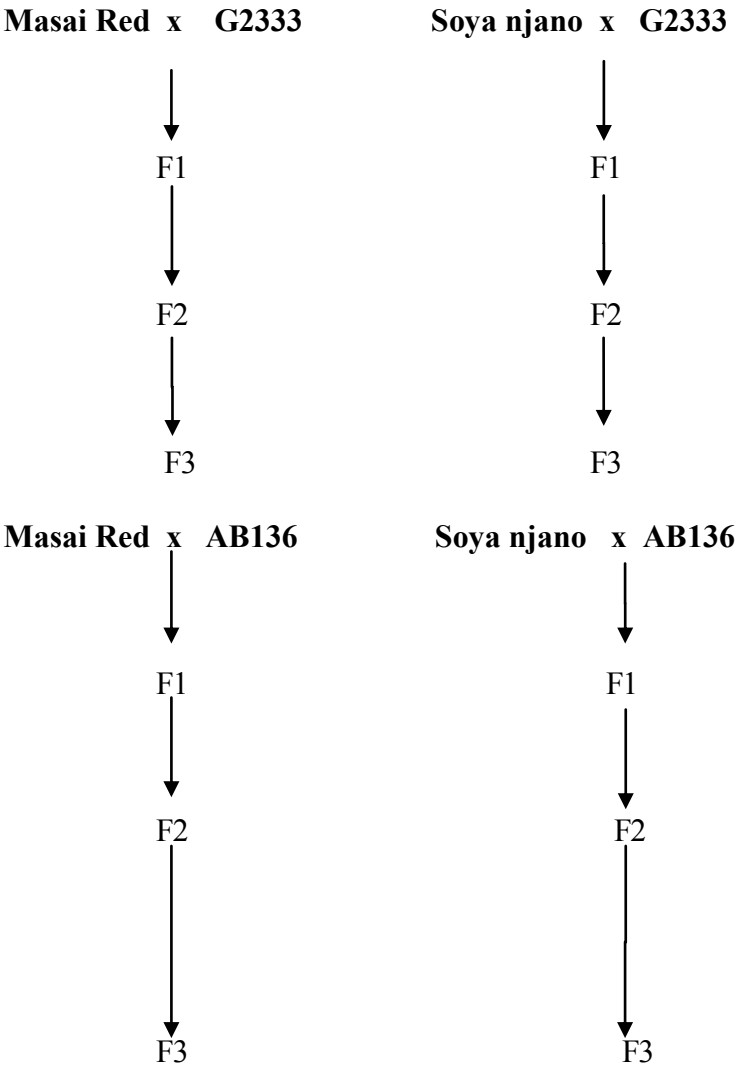



**Figure 1: Crossing design for transferring anthracnose resistance genes to *Colletotrichum lindemuthianum* to susceptible bean parent cultivars**

### 3.4 Screening for Disease Resistance

#### 3.4.1 Collection of *Colletotrichum lindemuthianum* isolates

Stem, leaf and pod samples bearing fresh symptoms of anthracnose infection were collected from farmers' fields in areas with natural infections, across different agroecologies in Arumeru, Karatu and Babati rural districts in northern Tanzania and Mvomero district in Morogoro region. In Karatu district, samples were collected from Rhotia, Slahhamo and Upper Kitete villages. In Arusha samples were collected from

(SARI), Similar samples were collected from, Long, Endaw and Ayatango villages in Babati rural district and Nyandira village in Mvomero district.

The collected samples were placed between folds of magazines and paper bags, and stored under normal room temperature at 24 °C. Paper bags with diseased samples were kept open, unpiled and separated to control decaying. Each sample was labeled carefully to depict names of varieties from which samples were picked, site, villages, district and name of the region. GPS "Garmen" type was used to mark coordinates of each site altitude, latitude and longitude.

### 3.4.2 Medium and inoculum preparation

The fungal isolates were grown in the petri plates at 24 °C, on V8 medium composed of V8 juice (200mls), $CaCO_3$ (3.0 g), Bato Agar (15g), streptomycin (10mg) and distilled autoclaved $H_2O$ (1000ml). Single spore isolates were established employing a standard procedure with modification according to Munda *et al.* (2009). The pathogens were isolated from well developed and fresh lesions of bean pods and stems. Small pieces of infected tissue were cut at intermediate part between diseased and health tissue, at least five pieces for a single culture. The pieces were surface sterilized in alcohol 7 % for less than 1 min. Using a sterile forceps, the pieces were then dipped into 2 % NaOCl (Sodium hypochlorite) for 3 minutes. The pieces were finally transferred aseptically into sterile distilled water and serially washed 3 times blotted dry on a sterile paper towel. Lastly the pieces were transferred into prepared media within petri dish and arranged, carefully leaving isolation space between each other. The petri dishes with V8 medium containing the pieces were sealed and incubated at 24 $^{o}$C temperature (Pastor-Corrales *et al*., 1994).

### 3.4.3 Inoculation

#### 3.4.3.1 Seed inoculation procedure

Six single spore isolates of the coded isolates 2CRHOT, 3ASLAHMO, 6ASAR14, 1C-BASH-L, 2D-1 collected from Long village in Bashnet, were used. Each isolate was stored in V8 media, at 4 °C. Eight petri plates with V8 medium without antibiotic were planted in with single spore of each isolate for multiplication. Inoculum containing spore suspensions of 1.2 x $10^6$ spores/ml for inoculating test plants was prepared from ten days old spore cultures according to the procedure described by Mahuku *et al*. (2002).

#### 3.4.3.2 Seed inoculation

Four seeds of each differential cultivars were germinated by being placed in humid plates more than 92 % relative humidity, at 25 °C; for 5 to 7 days. Germinated seeds were dip inoculated in a calibrated spore suspension of 1.2 x $10^6$ spores/ml for 5 min in 200 mls Bigirimana and Höfte (2001). The inoculated seeds were placed in humid plates incubated in the dark room and after 2 days seedlings were at emergence stage, were transferred to trays, covered by thin layer of sterilized soil and incubated at 19 – 22 °C. The relative humidity was above 92 %. The growth chamber contained 6 trays each planted with four germinated seeds of 12 differential cultivars.

#### 3.4.3.3 Seedling inoculation

**Planting parent cultivars and new populations**

The experiment consisted of parent cultivars, derived F1, F2 and F3 populations and bean differential cultivars planted in non replicated and un-randomized plots. The plot size was 2 rows, each 2.75 m length. The inter row spacing was 50 cm and intra hill spacing was 20 cm. Plots were planted with donor, recipient cultivars, F1, F2 and F3 populations. Each row was a plot with 12 plants for parent, spreader cultivars and F1 populations. The

F2 and F3 populations were planted in two rows per plot. The tested plant populations were irrigated every evening on rain free days to provide high relative humidity conditions for 7 days after inoculation.

**Seedling inoculation**

Inoculum was prepared from spore suspension derived from 2D-1 Long Ayt. isolate cultures; raised on V8 medium and kept in the darkness for ten days at 24 $^{\circ}$C. The ten days old spolulated cultures were flooded with 10 mls of sterile distilled water and scraped from the plates using new tooth brush. The spore suspensions were filtered through four layered gauze cloth. A haemocytometer was used to calibrate spore concentration to 1.2 x $10^6$ spores/ml (Mahuku *et al*., 2001). Seedling inoculation was made by spraying with the acqueous conidial suspension on 14 day old seedlings in the field. During inoculation and incubation period, temperatures and relative humidity ranged between 20 to 21 °C and 96 to 100 %, respectively.

### 3.4.4 Disease score and data analysis

#### 3.4.4.1 Disease score

The reaction of plants to *C. lindemuthianum* was evaluated 7 to 10 days post inoculation. The disease score was done using a scale of 1 - 9 where (Schoonhoven and Pastor-Corrales, 1987). Seedlings with no visible symptoms (severity value 1) or showing limited necrotic lesions (severity values 2 to 3) were considered resistant. Seedlings with large sporulating lesions (severity values 4 to 8) or dead (severity value 9) were considered susceptible. A set of 12 common bean anthracnose standard differential cultivars were used to confirm pathogenic identity of the *C. lindemuthianum* isolates.

### 3.4.4.2 Data analysis

Genstat statistical package was used to compute means, variance, standard deviation**,** standard error and regression coefficient, of variation between variables. The disease mean scores of parents, F1, F2 and F3 populations were generated and used in estimation of narrow sense heritability of parents using regression analysis. Chi-square was used to compare the segregation of F2, and F3, populations to Mendelian ratios. Genetic gain for disease resistance was computed using a procedure proposed by Zobel and Talbert (1991), cited by Abengmeneng, *et al*. (2010).

The selection differential (S) was estimated as shown below.

(1) $S = Xs - X\mu$

(2) Mid- parent = $\frac{P1 + P2}{2}$

(3) Deviation = grand mean score – Midparent disease score

(4) % age deviation = $\frac{\text{Deviation x 100 \% Percentage deviation}}{\text{Mean}}$

(5) % age gained = % deviation x $h^2$

(6) Genetic gain G = % age gain x $h^2$ (percentage gain x heritability)

Where, $h^2$ = Narrow sense heritability.

where, S = Selection differential (difference between mean of selected individual and the population mean Jansson, (2005); Abengmeneng, *et al*. (2010), Xμ = Mean of population, Xs = Mean phenotypic value after selection (sample mean),

Genetic gain (ΔG) was estimated as:

G = Percentage gain; Where, $h^2$ = Narrow sense heritability; Deviation x 100%

Percentage gain = percentage deviation x heritability;

### 3.5 Naming of *Colletotrichum lindemuthianum* races

Four seeds of each differential cultivars were germinated, dipped into inocula suspension of six different isolates. Six sets of differential cultivars were used. The germinated inoculated seeds were placed in a plastic tray then covered with a thin layer of the sterilized soil. Trays were placed in a growth chamber and watered daily for 14 days. Disease scoring was perfomed 10 days after sowing (Birigimana and Höfte, 2001). The disease score data was used to determine susceptible and resistant cultivars. The races derived from different *C. lindemuthianum* isolates were distinguished by using a set of differential cultivars. This set consisted of 12 cultivars, each with a designated binary number as follows: Michelite, 1; Michigan Dark Red Kidney, 2; Perry Marrow, 4; Cornell 49-242, 8; Widusa, 16; Kaboon, 32; Mexico 222, 64 ; PI 207262, 128; To, 256; Tu, 512; AB136, 1024; and G2333, 2048. The sum of the numbers assigned to each infected cultivar of the differential set determined race designation (Pastor Corales *et al.,* 1994).

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Pathogen in Isolates

Thirty two isolates of *C. lindemuthianum* were isolated from different common bean samples of infected bean cultivars from 26 sites within an elevation ranging between 1390 - 2197 metres above sea level; in five districts, namely Babati, Karatu, Mbulu, Arumeru and Mvomero (Table 2).

**Table 2: Collected isolates of common bean anthracnose disease from Babati, Karatu, Mbulu, Arumeru and Mvomero districts**

| Name of site | Sample code name | Variety | Growth habit | Gene pool | Altitude in m.absl |
|---|---|---|---|---|---|
| Bashnet-Manyara | 1ABASH | JESCA (Punda) | Bush type | Andean | 2189 |
| Long /Endaw | 1BASH | Farmer's variety | Bush type | Andean | 2193 |
| Long | 1CBASH | Experimental variety | Climber | Mesoamerican | 2195 |
| Long | 2A | Masai red | Climber | Mesoamerican | 2197 |
| Endaw | 2B | Masai red | Climber | Mesoamerican | 2186 |
| Endaw | 2C | Lyamungo 90 | Bush type | Andean | 2187 |
| Endaw | 2D | Farmer's var. | Bush | Andean | 2183 |
| Endaw | 3A | Farmer's var. | Bush type | Andean | 2146 |
| Bony | 3B | Farmer's var. | Bush type | Andean | 2156 |
| Bony | 3D | Farmer's var. | Bush type | Andean | 2166 |
| Bony | 3E | Farmer's var. (black grains) | Semi climber | Mesoamerica | 2170 |
| Masquaroda-Mbulu | 1AMASQ | Lyamungo 90 | Bush | Andean | 1895 |
| Mbulu | 1BMBLU | Lyamungo 90 | Bush | Andean | 1942 |
| Simba | 1A1KMS | Farmer's variety | Semiclimber | Mesoamerica | 1498 |
| Kambiya Simba | 1BKMS | Farmer's variety | Climber | Mesoamerica | 1523 |

| | | | | | |
|---|---|---|---|---|---|
| Kainam | 1A-2KAI | Farmer's variety | Bush | Andean | 1544 |
| Kambi ya samba | 1CKMS | Soya njano | Bush type | Andean | 1533 |
| Kambi ya Simba | 1DKMBS14 | Farmer's variety | Semiclimber | Mesoamerica | 1534 |
| Kambi ya Simba | 1EKMBS | Farmer's variety | Bush | Mesoamerican | 1537 |
| Rhotia | 1ARhotia | Soya njano | Bush | Andean | 1613 |
| Rhotia | 2BRHOT | Soya njano | Bush | Andean | 1624 |
| Rhotia | 2CRHOT-KR | Soya njano | Bush | Andean | 1621 |
| Slahhamo | 3ASLAMO | Soya njano | Bush | Andean | 1532 |
| Slahhamo | 3BSLAMO | "Bwana shamba" Canadian wonder | Bush | Andean | 1548 |
| Upper Kitete | 4AUPKIT | Soya njano | Bush | Andean | 1534 |
| Upper Kitete | 4BUPKIT | Soya njano | Bush | Andean | 1717 |
| Upper Kitete | 4CUPKIT | Soya njano | Bush | Andean | 1720 |
| Upper Kitete | 4DUPKIT | Soya njano | Bush | Andean | 1720 |
| KITETE | 5AKIT | Soya njano | Bush | Andean | 1749 |
| SARI | 6ASAR | Lyamungo 90 | Bush | Andean | 1411 |
| SARI | 6BSAR | Lyamungo 90 | Bush | Andean | 1399 |
| Mgeta | ANyd- | Lyamungo 90 | Bush | Andean | 1645 |
| Mgeta | ANyd- | Farmer,s variety | Bush | Andean | 1645 |

Key: M.a.s.l=metres Above Sea level

In Babati Rural, Karatu and Arumeru districts, the samples were drawn from bush types (Lyamungo 90, JESCA), semi climber (Canadian wonder) and climbing bean types (Masai red). The common bean cultivars collected such as Lyamungo 90, Soya Njano, JESCA, Canadian wonder and farmer were all infected with *C. lindemuthianum.* The common bean diseased plant samples collected were as indicated in Fig.2. The bean samples infected cultivars in reference to growth habit of the collected cultivars were as follows bush types (78 %), climbers (12.5 %) and Semi climbers at (9.4 %).

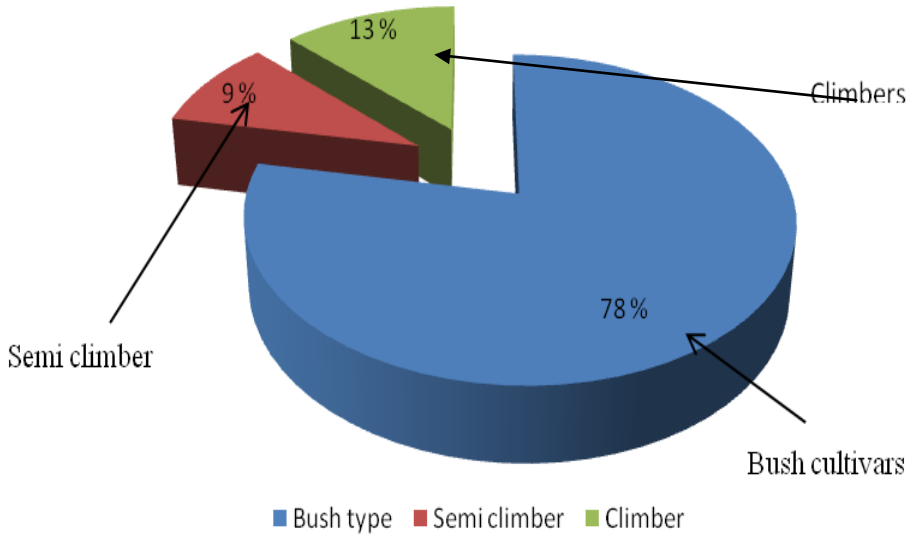



**Figure 2: Common bean cultivars diseased samples percentage infected by *Colletotrichum lindemuthianum*, their growth habit and distribution as collected from Babati, Karatu, Mbulu, Arumeru and Mvomero in Morogoro**

## 4.2 Pathogenicity Test

### 4.2.1 Pathogenicity test and race naming of *Colletotrichum lindemuthianum* under growth chamber condition

A total of nine pure single spores were obtained from 32 diseased samples collected. Six out of nine isolated single spores of *C. lindemuthianum* were named using twelve standard differential cultivars.

### 4.2.2 Pathogenicity and race classification of *Colletotrichum lindemuthianum* isolates

Under growth chamber conditions, six *C. lindemuthianum* isolates showed pathogenicity on 12 differential bean cultivars as indicated in Table 3. Six of the 12 bean differential cultivars showed susceptibility to at least one of the collected *C. lindemuthianum* isolates.

The reactions of a set of common bean differential cultivars to 6 isolates of *C. lindemuthianum* allowed the identification of the races 21, 37, 55, 161 and 533 from Karatu, SARI (Arumeru) and Babati districts. The bean cultivars Michelite was susceptible to all *C. lindemuthianum* isolates which were collected from Arumeru, Karatu, Mbulu and Babati rural.

Other findings reported Michelite as susceptible check when conducted resistance test of a number of accessions (Mahuku *et al*., 2002). A differential cultivar TU was susceptible to one isolate from Karatu. In general isolates collected in the area of the study infected both Andean and MesoAmerica cultivars, MDRK, Perry marrow, Widusa and Kaboon. on the MesoAmerican cultivar Michelite, Mexico222, PI 207262, TO and TU. Non of the isolates infected cultivars Cornel 49242, Mexique 222, AB136 and G2333. According to Mahuku *et al*. (2002) susceptibility to *C. lindemuthianum* of G2333 and AB136 was not frequently reported. They were resistant under screen house and field condition when pathogenicity test was conducted. The isolate found in Northern Tanzania was different from those reported by (Ansari *et al*., 2000) and those reported by Drone and Bailey (1999), in the aspect of bean differential cultivars it infected.

**Table 3: Pathogenicity test of *Colletotrichum lindemuthianum* under growth chamber conditions**

| | Isolate reaction on common bean differentials | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L | |
| Gene pool | M | A | A | M | M | A | M | M | M | M | M | M | Race |
| Isolate Codes | | | | | | | | | | | | | Designation |
| 2CRHOT | S | R | S | R | S | R | R | R | R | R | R | R | 21 |
| 3ASLAHMO | S | R | S | R | S | R | R | R | R | S | R | R | 533 |
| 6ASAR14 | S | R | S | R | R | S | R | R | R | R | R | R | 37 |
| 1C-BASH-L | S | R | R | R | R | S | R | R | R | R | R | R | 161 |
| 2D-1 Long Ayt | S | R | R | R | R | S | R | S | R | R | R | R | 161 |
| 1b-Bashnet-Bony | S | S | S | R | S | S | R | R | R | R | R | R | 55 |

**Key:** Differential cultivars of common bean and their binary values (in parentheses): A, Michelite (1); B, Michigan dark red, kidney (2); C, Perry marrow (4); D, Cornell 49242, (8); E, Widusa (16); F, Kaboon,(32); G, Mexique 222 (64); H, PI 207262 (128); I, TO (256); J, TU (512); K, AB136 (1024); and L, G2333 (2048). M=Mesoamerica, A=Andean gene pools, R=resistant, S=Susceptible. 2CRHOT: RHOT- Karatu Isolate; 3ASLAHMO: Slahmo-Karatu Isolate; 6ASAR14 SARI-Arusha Isolate; 1C-BASH-L;2D-1 Long Ayt. and 1b Bashnet-Bony were all from Bashnet

### 4.2.3 Pathogenicity testing of *Colletotrichum lindemuthianum* under field conditions at Bashnet Manyara

The results in Table 4 showed the compatibility of 2D-1 Long Ayt isolate of bean *C. lindemuthianum* to a set of 12 bean differential cultivars which allowed naming race 161. Isolate 2D-1 Long Ayt (161) was compatible to bean cultivars Michelite, Kaboon and PI 207262. Other nine differential cultivars were not infected. However, this race exhibited compatibility to both indeterminate and determinate bean cultivars. The differential cultivars which were not infected by race 161 were MDRK, Perry Marrow, Widusa, G2333, AB136, Cornell 49242, Mexico 222, TO, TU. However, an isolate designated 161 was not among the listed isolates collected from Africa and other parts of the world as reported by (Ansari, *et al*., 2004). It was also not in the list of the previous isolates collected in the southern highlands of Tanzania (Dron and Bailey, 1999). These results

implied that isolates collected at Bashnet consisted *C. lindemuthianum* named race 161 which was not previously reported.

**Table 4: Pathogenicity of *Colletotrichum lindemuthianum* under field conditions at Bashnet Manyara**

| Differential cultivars | Genes conferring Resistance | Place of cultivar | Binary number | Gene pool | disease score * | Isolate Reaction* |
|---|---|---|---|---|---|---|
| Michelite (A) | ----- | 0 | 1 | MA | 5 | S |
| MDRK (B) | Co – 1 | 1 | 2 | A | 1 | R |
| Perry Marrow ( C ) | Co – $1^3$ | 2 | 4 | A | 1 | R |
| Cornel 49242 ( D ) | Co – 2 | 3 | 8 | MA | 1 | R |
| Widusa ( E ) | Co – 9 | 4 | 16 | MA | 3 | R |
| Kaboon (F) | Co – $1^2$ | 5 | 32 | A | 5.5 | S |
| Mexico 222 (G) | Co – 3 | 6 | 64 | MA | 3 | R |
| PI 207262 (H) | Co – $4^3$, Co – 9, | 7 | 128 | MA | 4 | S |
| TO (I) | Co – 4 | 8 | 256 | MA | 3 | R |
| TU (J) | Co – 5 | 9 | 512 | MA | 1 | R |
| AB 136 (K) | Co – 6, Co-8 | 10 | 1024 | MA | 1 | R |
| G 2333 (L) | Co – $4^2$, Co-5, Co-7 | 11 | 2048 | MA | 1 | R |
| Race designation | | | 161 | | | |

Key:
Binary number of a specific race was computed by the summation of susceptible cultivars binary number. M A; Middle American gene pool; A: Andean gene pool of *Phaseolus vulgaris.* Binary number; $2^n$, where n is equivalent to the place of the cultivar within the series (0 - 11). Growth habit: I = Determinate; II = Indeterminate bush III = Indeterminate bush with weak main stems and prostrate branches; IV = Indeterminate climbing habit.* Disease score and *bean differential cultivars reaction on isolates

### 4.3 Introgression of Anthracnose Resistance in Preferred Varieties

Four genotypes were grown and crossed under screen house conditions during the 2014 - 2015 growing seasons at SUA. Two genotypes were resistant donor and another two were recipient cultivars. The general observation in in Table 5 showed the crosses performances between Soya Njano x G2333 which gave 37 seeds and Masai Red x G2333 had 15 seeds had lowest number of seeds. That could have been contributed by inadaptability of G2333 to the environment during crossing work. The number of seeds obtained for F1 ranged from 15 – 37. The F2 were from 45 to 138. Masai Red x AB136 gave 138 seeds which had heighest number of seeds. Both recurrent and donor parents

were climbers, compared to Soya Njano x G2333 had 45 seeds which was the lowest. F3 were from 34 to 61 seeds as indicated (Table 5). These results comply with the reported results on common bean plants by (Porch and Jahn, 2001). The Soya Njano, Masai x AB136, crosses in F2 and F3 had produced many seeds due to AB136 good environmental adaptability and had successful crosses.

**Table 5: Number of seeds of $F_1$, $F_2$, $F_3$ obtained from crossing the donor for anthracnose disease resistance and the adapted parents of common bean local cultivars**

| Parent material | No. of crosses | $F_1$ seeds | $F_2$ seeds | $F_3$ seeds |
|---|---|---|---|---|
| Soya Njano x G2333 | 58 | 37 | 45 | 40 |
| Soya Njano x AB136 | 42 | 25 | 89 | 61 |
| Masai red x G2333 | 74 | 15 | 57 | 42 |
| Masai red x AB 136 | 42 | 22 | 138 | 34 |

### 4.3.1 Inheritance pattern of anthracnose resistance in early populations of crosses of Soya Njano, Masai Red and G2333, AB136 segregation ratios

**Crosses made between Soya Njano, Masai Red and G2333**

The parental cultivars Soya Njano, Masai Red, G2333 and AB136, developed populations (F1, F2 and F3) were tested for bean anthracnose disease resistance under field conditions. Results showed that Soya Njano plants were all susceptible as indicated in plate 1d while, all plants in donor parent cultivar G2333 population were resistant. Plants in the F1 generation were all resistant to bean anthracnose disease. The F2 generation segregation ratio of Soya Njano x G2333 was 9R: 7S Resistant: Susceptible ($\chi^2 = 0.01$, P = 0.872) and F3 generation plants of had segregation ratio of 9R : 7S ($\chi^2 = 0.001$, P = 0.979) (Table 6). The segregation data obtained from F2 and F3 populations indicated that G2333 carried two dominant resistance genes. The results of bean cultivar G2333

conferring two was reported by Young and Kelly (1996). The similar results were reported by (Campa *et al*., 2011). However, segregation ratio of 9: 7 implied that two pairs of genes with duplicate recessive epistasis were expressed, by the heterozygous dominant individuals which are phenotypically distinguishable from other possible genotypes obtained from the populations being studied (Burner, 1980). González *et al*. (2015) reported that either additive main effects or epistatic effects or both functioning concurrently are responsible in controlling anthracnose disease resistance in bean.

The F1 plant population were all resistant to *C. lindemuthianum* that indicated that a dominant gene was responsible for resistance. The F2 plant population segregation was fitted to 10R: 6S ratio (5R) Resistant and (3S) susceptible ($\chi^2 = 0.714$; $P > 0.05$) and F3 population segregation ratio was 10R : 6S ratio ($\chi^2 = 0.002$; $P > 0.05$) in (Table 6). These results revealed that two dominant gene was conferring resistance to the developed populations of Masai Red and G2333. Similar inheritance was reported by Pastor-Corrales *et al.* (1994) on presence of two dominant independent genes in G2333 was controlling resistance to *C. lindemuthianum*. However, the segregation of 9: 7, 10: 6 (5: 3) ratios expressed two epistatic genes relationships that could correspond to the ratios found in this study as reported by (Diering and Tomas, 2001).In other findings showed that G2333 was a three genes pyramided cultivar, with genes situated at different loci conferring resistance independently (Vallejo and Kelly, 2009). According to Mahuku *et al*. (2002) genetic resistance to some pathotypes of *C. lindemuthianum* is conferred by various single, duplicate or complementary dominant genes. However, bean cultivar G2333 was reported to be capable of controlling more than 380 races in different areas it was used (Pastor - corrales *et al*., 1994).

**Table 6: Segregation ratios for resistance and susceptible progenies in parental cultivars and their developed populations to *Colletotrichum lindemuthianum* under field conditions**

| | | Number of plants | | Segregation | | |
|---|---|---|---|---|---|---|
| Perdegree | Generation | Resistant | Susceptible. | Ratio (R:S) | $\chi^2$ | Probability |
| Soya Njano | P1 | 0 | 12 | ……. | ……. | ……. |
| G2333 | P3 | 12 | 0 | ……. | ……. | ……. |
| Soya Njano x G2333 | F1 | 12 | 0 | ……. | ……. | ……. |
| Soya Njano x G2333 | F2 | 12 | 10 | 9:7 | 0.016 | 0.872 |
| Soya Njano x G2333 | F3 | 13 | 10 | 9:7 | 0.001 | 0.979 |
| Masai Red | P2 | 0 | 12 | ……. | ……. | ……. |
| G2333 | P3 | 12 | 0 | ……. | ……. | ……. |
| Masai Red x G2333 | F1 | 12 | 0 | ……. | ……. | ……. |
| Masai Red x G2333 | F2 | 15 | 6 | 10 : 6 | 0.714 | 0.296 |
| Masai Red x G2333 | F3 | 13 | 8 | 10 : 6 | 0.002 | 0.955 |

Key: $\chi^2$ = Chi test, P1= Soya Njano, P2 = Masai red, P3 = G2333, P3 = G2333; F1- F3 = Soya NJano x G2333 and Masai Red x ABG2333

**Crosses made between Soya Njano, Masai Red and AB136**

The donor parent AB136, Soya Njano, Masai Red recipient bean cultivars and F1, F2 and F3 populations derived from crosses made between Soya Njano, Masai Red and AB136 were inoculated with race 161. Soya Njano and Masai Red plants were all susceptible to *C. lindemuthianum* as indicated in Plate 3(a – b) also the spreader row of Lyamungo 90 was susceptible as showed in Plate 3(c – d). The donor parent AB136 plants were all resistant. The F1 plants were all resistant to common bean anthracnose disease, this indicated that resistance in AB136 was controlled by dominant gene. The F2 plants segregation fitted to 3: 1 ratio ($X^2 = 3.56$, $P = 0.04$) (Table 7). The F3 population showed significant difference from F2 population at 0.05 probability. It exhibited that AB136 conferred single dorminant gene. These results were similar to those reported by (Gonçalves-Vidigal *et al.,* 2001). The F3 population segregation ratio for resistance of *C. lindemuthianum* was also 3: 1 ($\chi^2 = 0.44$; $P < 0.50$). In the crosses between Masai Red and AB136, the F2 population segregation was 3R:1S ratio ($\chi^2 = 4.19$; $P < 0.05$) and F3 population segregated at a ratio of 3R: 1S ($\chi^2 = 0.55$; $P < 0.50$) (Table 8). The results

were in conformity with those of (Alzate-Marin *et al*., 1997) on the resistance of *C. lindemuthianum* races 89 and 64. All F1 populations developed from the crosses exhibited resistance, implied that resistance was due to dominant genes transferred into the derived generation. (Alzate- Marin *et al*., 1997) reported on the presence of two independent resistant genes of AB136 to pathogen race 73 of *C. lindemuthianum*, where (C*o-6*) gene was dominant gene and a recessive gene assigned with the genetic symbol *co-8.*

**Table 7: Segregation ratios for resistance and susceptible in parental cultivars and their derived populations to *Colletotrichum lindemuthianum* under field conditions**

| | | Number of plants | | Segregation | | |
|---|---|---|---|---|---|---|
| Pedigree | Generation | Resistant | Susc. | Ratio | $\chi^2$ | Probability |
| Soya Njano | P1 | 0 | 12 | ……. | …… | ……. |
| AB136 | P4 | 12 | 0 | ……. | ……. | ……. |
| Soya NJano x AB136 | F1 | 12 | 0 | ……. | ……. | ……. |
| Soya Njano x AB136 | F2 | 14 | 10 | 3:1 | 3.56 | 0.04 |
| Soya Njano x AB136 | F3 | 13 | 6 | 3:1 | 0.44 | 0.50 |
| Masai red | P2 | 0 | 11 | ……. | ……. | ……. |
| AB136 | P4 | 12 | 0 | ….. | …... | ……. |
| Masai Red x AB136 | F1 | 12 | 0 | …….. | …… | ….. |
| Masai Red x AB136 | F2 | 13 | 10 | 3:1 | 4.19 | 0.05 |
| Masai Red x AB136 | F3 | 15 | 7 | 3:1 | 0.55 | 0.50 |

Key: $\chi^2$ = Chi test, P1= Soya Njano, P2 = Masai red, P3 = G2333, P4 = AB136, F1 - F3 = Soya NJano x AB136 and Masai Red x AB136

(a) Masai Red- pod symptoms

(b) Soya Njano – pod symptom

(c) Lyamungo 90 ( diseases spreader variety) (d) Lyamungo at vegatative stage

**Plate 3: Pictures (a - d) show diseased bean plants with common bean antracnose symptoms on different parts of the plants**

### 4.3.2 Heritability estimation

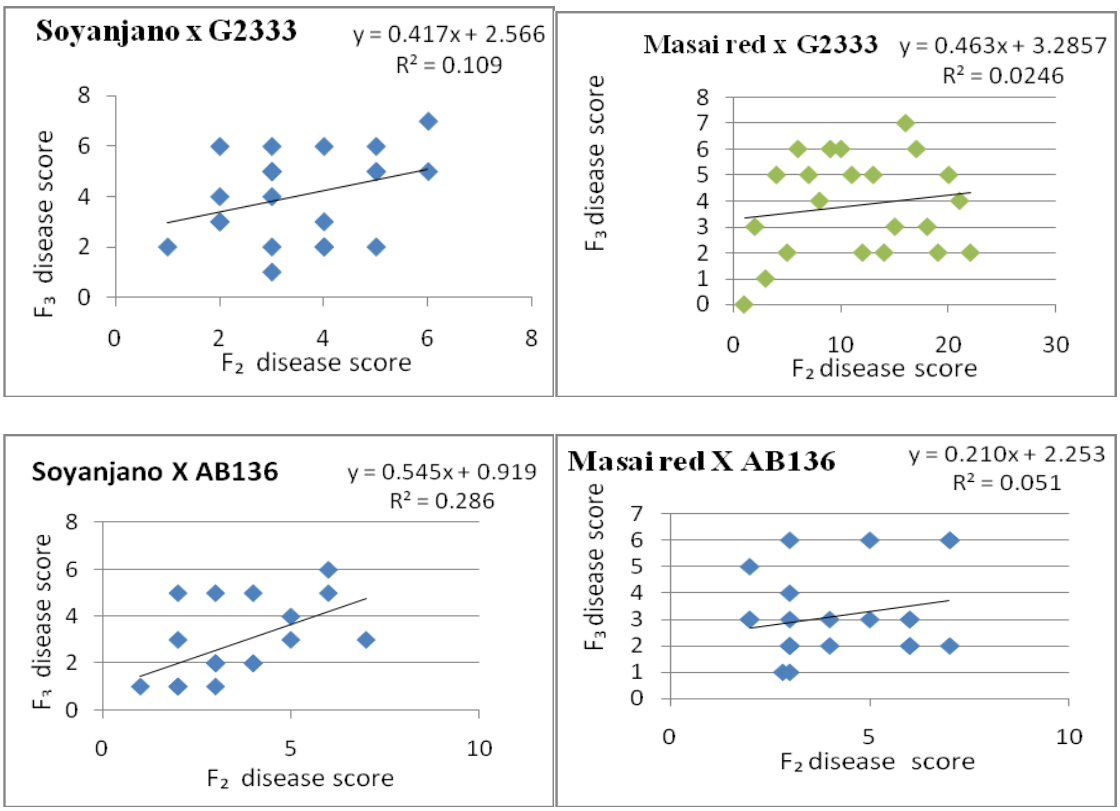
The estimated narrow sense heritability ranged between 0.42 and 0.46 in populations derived from crosses between Soya Njano, Masai Red with G2333 and Masai Red with AB136 was 0.22, (Table 8 and Fig. 3). These results implied the presence of moderate heritability in developed population. The similar results were reported by (Poletine *et al.,* 2006), that medium magnitude narrow sense heritability value, even at that moderate

magnitude, indicated the possibility of success on obtaining resistant genotypes in derived populations. The population derived from Soya Njano with AB136 had the heritability of 0.53 (53 %) that showed moderate heritability, $R^2$ = 0.29 coefficient of determination. Populations of Masai Red x AB136, Soya Njano x G2333 and Masai Red x G2333 was 5 % - 15 %. The moderate narrow sense heritability in developed F2 from Soya Njano x AB136, implied that the mean performance of the developed populations has regressed back at 53 % towards the mean of the previous resistant generation according to (Stanfield, 1991). When heritability for a trait is high, selection using phenotypic traits is effective (Falconer, 1989; Kearsey and Pooni 1996; Campa *et al.,* 2014).

**Table 8: Heritability in narrow sense estimation for *Colletotrichum lindemuthianum* in derived F2 and F3 populations**

| | **Mean disease Score** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Generations** (F2& F3) | **F2** | **F3** | **SDEVF2** | **SDEF3** | **P-value** | **b ($h^2$)** | **A** | **$R^2$** |
| P1 X P3 | 3.55 | 4.05 | 1.37 | 1.73 | 0.13 | 0.42 | 2.57 | 0.12 |
| P2 X P3 | 2.67 | 3.62 | 1.65 | 1.96 | 0.07 | 0.46 | 2.38 | 0.15 |
| P1 X P4 | 3.53 | 2.84 | 1.6 | 1.63 | 0.02 | 0.53 | 0.92 | 0.29 |
| P2 x P4 | 4.17 | 3.22 | 1.93 | 1.61 | 0.73 | 0.22 | 2.18 | 0.05 |

KEY: P1= Soya njano, P2 = Masai red, P3 = G2333, P4 = AB136, $R^2$ Regression determination, F2, F3 = Filial generation 2 and 3, SDEV: standard deviation of F2 and F3, b = Coefficient of X slope; stands for ($h^2$) heritability in narrow sense. P = P value; A = y - intercept



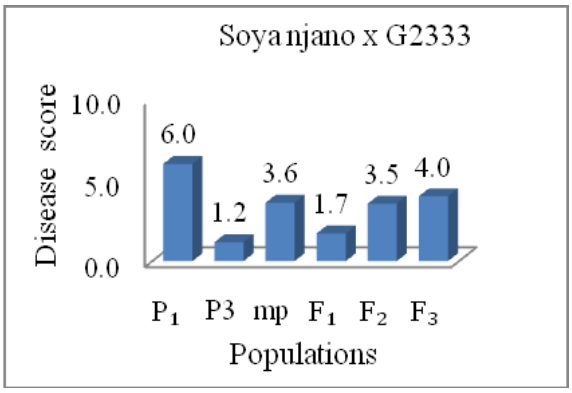

P1 = Soya njano,, P2 = Masai red, P3 = G2333, P4 AB136,
Regression graphs on inheritance of F2 and F3 populations

**Figure 3: Heritability determination of *resistance of C. lindemuthianum* using F3 populations to F2**

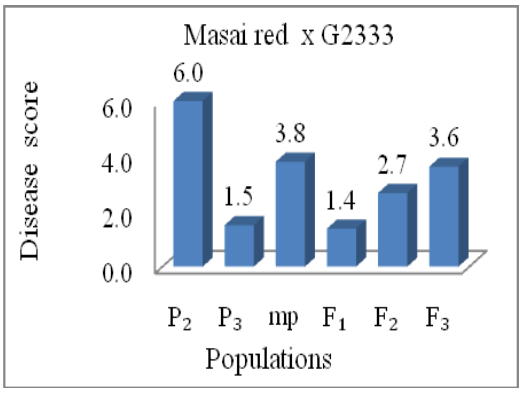
### 4.3.3 Comparisons of parents, F1, F2 and F3 for Common bean anthracnose disease Resistance

The mean disease scores of crosses of F1, F2 and F3 generated populations from Soya Njano, Masai Red with G2333 and mid parent are presented in Figure 4(a - b). The results show that there was a reduction of disease reaction in the $F_1$ populations basing on recipients and mid parent resistance performance. The F2 populations of Masai red x G2333 mean score was 2.7 more resistant than mid parent mean score 3.8. The F3 populations. The susceptible bean cultivars mean disease scores were between 6.0 - 6.5, that implied high susceptibility to common bean anthracnose. Abengmeneng *et al*. (2015) reported that genotypes with mean disease resistance above of the mean performance of the population are recommended for selection and use as seed. According to the results in

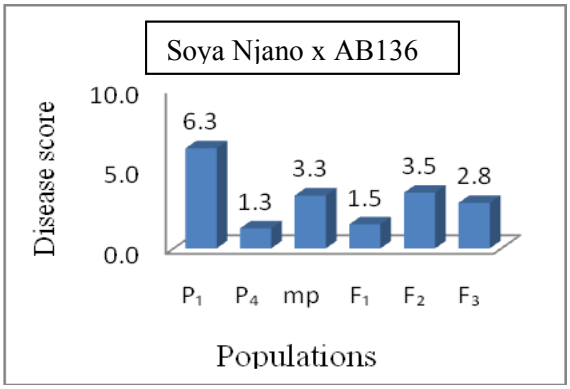
Figure 4(c - d), Soya Njano x, AB136 (F3) and Masai Red x AB136 (F3) exhibited improvement of resistance in developed populations to *C. lindemuthianum* and Soya njano xG2333, the Masai Red x G2333 (F2 and F3) showed equal performance as midparent. Namkoong *et al.* (2000) and Abengmeneng *et al*. (2015) reported that, only genotypes whose phenotypes were approximate to the population mean were good for selection as resistant plants and fit in the Northern zone environment.



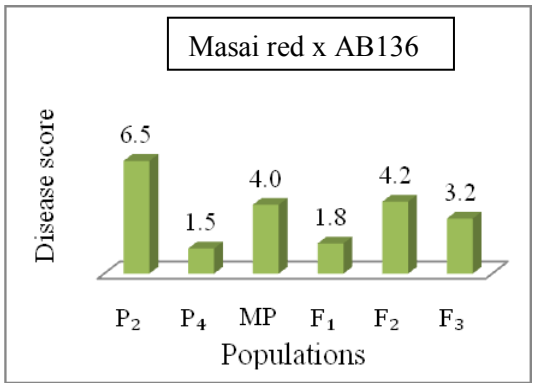

4a




4b




4c




4d

**Figure 4: Comparison of mid parents F1, F2 and F2 generations for resistance of Common bean Anthracnose disease**

Key: P1 = Soya Njano, P2 = Masai red, P3 = G2333, P4 = AB136

### 4.3.4 Estimation of genetic gain

The results indicated in Table 9 showed that mean anthracnose disease score were 6.0 for Masai Red and Soya Njano was between 6.0 to 6.5. The donor bean cultivar G2333 mean disease scores was between 1.5 and AB136 was 1.5. The maximum genetic gain through selection depends on the phenotypic variations present in the base population and maintained in the following cycles through selection (Janick, 2010). However, the genetic gain in F2 and F3 populations of Soya Njano and G2333 genetic gain is between 0.2 and 2.0. The F2 populations derived from Soya Njano, Masai Red and AB136 genetic gain were absolute and ranged between (- 0.1 to 0.8). Negative succession per season represented the stabilization of the genetic gain in disease resistance as disprayed by the figures close to zero (Chiorato *et al*., 2010). These results showed the presence of different levels of resistance to common bean anthracnose disease, hence, high potential genotypes resistant to *C. lindemuthianum* for selection.

Genetic gain results in some of the populations indicated in Table 9 showed that there was increased genetic gain through moderate narrow sense heritability. The results concur with the findings reported by Ramalho *et al*. (1993) and Souza *et al*. (2014) that genetic gain depends on the availability of moderate to high heritabilities and a useful amount of genetic variation. Population breeding methods such as lines development by standard backcrossing, pedigree or bulk and recurrent selection were suggested most perfect suited to long term genetic gains and these methods requires sufficient time (Cowling, 1996).

**Table 9: Genetic gain for *Colletotrichum lindemuthianum* resistance estimation in F2 and F3 derived populations**

| Parent material | | Mpt | Mean disease score (1-9) | deviation/ F-mid Parent | Grand mean disease scores | Percentage deviation | Herita bility in $h^2$ | % age gained | Genetic Gain (scores) |
|---|---|---|---|---|---|---|---|---|---|
| Soya Njano | | | 6.0 | | | | | | |
| Masai red | | | 6.0 | | | | | | |
| G2333 | | | 1.5 | | 1.5 | | | | |
| Soya njano x G2333 | F2 | 3.6 | 3.5 | -0.1 | 3.5 | -2.9 | 0.42 | 1.2 | 0.5 |
| Masai red x G2333 | F2 | 3.8 | 2.7 | -0.3 | 3.5 | -8.6 | 0.46 | 3.9 | 1.8 |
| Soya njano x G2333 | F3 | 3.6 | 4.0 | -0.1 | 3.5 | -2.9 | 0.26 | 0.7 | 0.2 |
| Masai red x G2333 | F3 | 3.8 | 3.6 | -0.3 | 3.5 | -8.6 | 0.48 | 4.1 | 2.0 |
| Grand mean | | | 3.5 | | | | | | |
| | | | | | | | | | |
| Soya Njano | | | 6.3 | | | | | | |
| Masai red | | | 6.5 | | | | | | |
| AB136 | | | 1.5 | | 1.5 | | | | |
| Soya njano xAB136 | F2 | 3.3 | 3.5 | 0.1 | 3.4 | 2.9 | 0.53 | 1.6 | 0.8 |
| Masai red x AB136 | F2 | 4 | 4.2 | -0.6 | 3.4 | -17.6 | 0.22 | -3.9 | -0.9 |
| Soya njano xAB136 | F3 | 3.3 | 2.8 | 0.1 | 3.4 | 2.9 | 0.52 | 1.5 | 0.8 |
| Masai red x AB136 | F3 | 4 | 3.2 | -0.6 | 3.4 | -17.6 | 0.24 | -4.2 | -1.0 |
| Grand mean | | | 3.4 | | | | | | |

Key: Mpt = midparent, F = Filiar generation

# CHAPTER FIVE

## 5.0 CONCLUSION AND RECOMMENDATIONS

### 5.1 Conclusions

i. Race 161 was determined from bean diseased plants samples collected at Bashnet in the northern Tanzania when pathogenicity testing conducted under field condition at Bashnet.

ii. Donor parent of bean cultivars G2333 and AB136 exhibited resistance to *C. lindemuthianum* pathogens when tested under field conditions, hence are potential donor parents. Other potential donor bean cultivars, were MDRK, Perry Marrow, Widusa, Cornell 49 242, Mexico 222, To, Tu, exhibited resistance under field testing at Bashnet.

iii. The genes for resistance again st common bean anthracnose disease was successfully introgressed into adapted bean cultivars Soya Njano and Masai Red using conventional breeding. The results showed that there were two genes introgresed from resistant parents G2333 and one dominant gene from AB136.

iv. Heritability in narrow sense of common bean anthracnose disease resistance was moderate in the developed populations in this study. The results showed that selection of resistant genotypes from the derived populations was possible.

## 5.2 Recommendations

i. Bean differential cultivars which showed resistance to most of the isolates should be used as donor parents to improve the resistance of local and commercial susceptible cultivars in breeding programmes. The suggested bean cultivars G2333, AB136, MDRK, Perry Marrow, Widusa, Cornell 49 242, Mexico 222, TO and TU, exhibited resistance to *C. lindemuthianum*, when tested under field conditions at Bashnet.

ii. Breeding efforts should focus on improvement for disease resistance of the available susceptible preferred bush local and semiclimber commercial susceptible bean cultivars.

iii. More research is required to advance resistant bean materials which showed resistance to *C. lindemuthianum.* The developed resistant populations should be backcrossed to the recipient parent cultivars, to retain good characteristics of the traits of the original Masai Red and Soya Njano preferred by farmers and other consumers.

iv. Some of the resistant-developed populations should be introgressed with other genotypes having resistance to other diseases in order to make them have multiple diseases resistance.

**REFERENCES**

Abengmeneng, C. S., Ofori, D. A., Jamnadass, R., Akromah R. and Kumapley, P. (2015). Estimation of heritability and genetic gain in height growth in *Ceiba pentandra. African Journal of Biotechnology* 14(22): 1880 – 1885.

Acquaah, G. (2007). *Principles of Plant Genetics and Breeding*. Blackwell Publishing Oxford, 129 pp.

Alzate-Marin, A. L., Baía, G. S., de Paula Júnior, T. J., de Carvalho, G. A., de Barros, E. G. and Moreira, M. A. (1997). Inheritance of anthracnose resistance in common bean differential cultivar, AB 136. *Plant Disease* 81: 996 – 998.

Amin, M., Teshele, J., Tesfay, A. (2014). Evaluation of Bioagents Seed Treatment Against *Colletotrichum Lindemuthianum*, in Haricot Bean Anthracnose under Field Condition. *Research in Plant Sciences* 2(1): 22 – 26.

Amin, M., Fitsum, S., Selvaraj, T., Mulugeta, N. (2014). Field Management of Anthracnose (*Colletotrichum lindemuthianum*) in Common Bean through Fungicides and Bioagents. *Advanced Crop Science and Technology* 2:124.

Ansari, K. I., Palacios, N., Araya, C., Langin, E. T. D. and Doohan, F. M. (2004). Pathogenic and genetic variability among *Colletotrichum lindemuthianum* isolates of different geographic origins. *Plant Pathology* 53(5): 635 – 642.

Beebe, S., Rao, I., Mukankusi, C. and Buruchara, R. (2012). *Improving Resources Use Efficiency and Reducing Risk of Common Bean Production in Africa, Latin America, and Caribbean, International Center for Tropical Agriculture* (CIAT). Cali, 18pp.

Bigirimana, J. and Höfte, M. (2001). Bean Anthracnose, Inoculation Methods and Influence of Plant stage on Resistance of *phaseolus vulgaris* Cultivars. *Phytopathology* 149: 403 – 408.

Blair, M. W., Fregene, M. A., Beebe, S. E and Ceballos, H. (2007). Marker-assisted selection in common bean and cassava, *in; Marker Assisted Selection: Current Status and Future perspective in Crops, livestock, forestry and fish*. "Edited by", ( *Guimaraes; E. P*., *Ruane, J*., *Scherf, B. D*., *Sonnino A*.; *Dargie, J. D*.) Food and Agriculture Organization of the United Nations, Rome, 82 pp.

Broughton, W. J., Hern´andez, G., Blair, M., Beebe, S., Gepts, P. and Vanderleyden, J. (2003). Beans (*Phaseolus* spp.) model food legumes. *Plant and Soil* 252: 55 – 128.

Burns, W.G. (1980). *The Science of Genetics*, Macmillan Publishing Company, NewYork. 608pp.

Buruchara, R., Chirwa, R., Sperling, L., Mukankusi, C., Rubyogo, J. C., Muthoni, R. and Abang, M. M. (2011). Development and delivery of bean varieties in Africa: The Pan- Africa Bean Research Alliance (PABRA) Model. *African Crop Science Journal* 19 (4): 227 – 245.

CABI Plant knowledge Bank (2014). Bean anthracnose *Colletotrichum lindemutheanum.* CSIRO Publishing, Collingwood, Victoria, 2pp.

Campa, A., Giraldez, R. and Ferreira, J. J. (2011). Genetic analysis of the resistance to eight anthracnose races in the common bean differential cultivar Kaboon. *Phytopathology* 101: 757 – 764.

Campa, A., Rodríguez-Suárez, C., Giraldez, R. and José, J. F. (2014). Genetic analysis of the response to eleven *Colletotrichum lindemuthianum* races in a RIL, population of common bean (*Phaseolus vulgaris* L.). *Plant Biology* 14:115 – 119.

Cannon, P. F., Damm, U., Johnston, P. R. and Weir, B. S. (2012). Colletotrichum- current status and future directions. *Study of Mycology* 73 (1):181 – 213.

Chamma Davide, L. M. and Aparecida de Souza, E. (2009). Pathogenic variability within race 65 of *Colletotrichum lindemuthianum* and its implications for common bean breeding. *Crop Breeding and Applied Biotechnology* 9: 23 – 30.

Chiorato, A. F., Carbonell, S. A. M., Vencovsky, R., Silva Fonseca Júnior, H. and Pinheiro, J. B. (2010). Genetic gain in the breeding program of common beansat IAC from 1989 to 2007, *Crop Breeding and Applied. Biotechnology.*10: 4 – 7.

CIAT, (1989). Bean production problems in the tropics. 2nd ed. Schwartz, H. F. and Pastor-Corrales, M. A. (eds.). Cali, Colombia. CIAT. 726pp.

CIAT. (2013). The impacts of CIAT's collaborative research. Cali, Colombia: International Center for Tropical Agriculture. (CIAT), 8pp.

Costa, M. M. C. and Carvalho, C. R. (2011). Vegetative compatibility and Hetero karyon formation between different isolates of *Colletotrichum lindemuthianum* by using the *nit* mutant system. *(Brazilian Journal of Microbiology* 42(1): 1517 – 8382.

Cowling, W. A. (1996). Plant breeding for stable agriculture. *Journal of the Royal Society of Western Australia* 79: 00 – 00.

Debouck, D. G. (1994). Beans (*Phaseolus* spp.) International Board for Plant Genetic Resource. Rome [http://www.hort.] site visited on 29/8/2013.

Diering, D. A. and Tomas, P. M. (2001). Inheritance of Male Sterility in Lesquerella Fendleri. *Journal of American Society of Horticultural Sciences* 126(6): 738 743.

Dillard, H. R. (1988). Cooperative Extension New York State Cornell University, Department of Plant Pathology New York State Agricultural Experiment Station, Geneva Cornell University, Fact Sheet 729 pp.

Dillard, H. R. and Cobb, A. C. (1993). Survival of *Colletotrichum lindemuthianum* in bean debris in \New York State. *Plant Disease.* 77: 1233 – 1238.

Divon, H. H. and Fluhr, R. (2007). Nutrition acquisition strategies during fungal infection of plants. *Journals of Microbiology* 266: 65 – 74.

Drijfhout, E. and Davis, H. J. C. (2012). Selection of a new set of homogeneously reacting bean (Phaseolus vulgaris) differential to differentiate races of common bean. *Plant Pathology* 38: 391 – 396.

Dron, M. and Bailey, J. A. (1999). *Improved control of bean anthracnose disease in Latin America and Africa through increased understanding of pathogen diversity.* published by CTA. 177pp.

Dufresne, M., Perfect, S., Peliera, A., Bailey, J. A. and Langina, T. (2000). A GAL 4-like Protein Is Involved in the Switch between Biotrophic and Necrotrophic Phases of the Infection Process of *Colletotruchum lindemuthianum* on Common Bean, *The Plant Cell* 12(9): 1579 – 1589.

Eastern and Central Africa Bean Research Network final report, (ECABREN) (2003). On *Priority-Setting Workshop*, Nairobi, Kenya. 56pp.

Food and Agricultural Organization of the United Nations (FAOSTAT). (May 2014). Dry Bean Statistical Database. [http://faostat3.fao.org] site visited on 17/6/2016.

Food and Agricultural Organization of the United Nations (2015). *State of diversity of major and minor crops*. The Second Report on the state of the world's 322pp.

Genchev, D., Christova, P., Kiryakov, I., Beleva, M. and Batchvarova, R. (2010). Breeding of Common Bean for Resistance to the Physiological Races of Anthracnose Identified in Bulgaria, *Biotechnology and Biotechnological Equipment* 24 (2): 1814 – 1823.

Gepts, P., Daniel, G. and Debouck, F. (1991). Races of common bean (Phaseolus vulgaris, Fabaceae) 45: 379 – 396.

Gonçalves-Vidigal, M. C., Poletine, J. P., Vidigal- Filho, M. C., Scapim, P. S., Silvério, C. A. L. and Thomazella, C. (2000). Inheritance of Resistance to Races 69 and 453 of C*olletotrichum lindemuthianum* in the Common Bean. *Brazilian Archives of Biology and Technology* 43 (5): 479 – 485.

Gonçalves-Vidigal, M. C., Sakiyama, N. S., Vidigal Filho, P. S., Amaral Júnior, A. T., Poletine, J. P. and Oliveira, V. R. (2001). Resistance of Common Bean Cultivar AB 136 to Races 31 and 69 of *Colletotrichum lindemuthianum*: the *Co-6* Locus. *Crop Breeding and Applied Biotechnology* 1 (2): 99 – 104.

Gonçalves-Vidigal, M. C., Rosa da Silva, C., Vidigal Filho, P. S., Gonela, A. and Kvitschal, M. V. (2007). Allelic relationships of anthracnose (*Colletotrichum lindemuthianum*) resistance in the common bean (*Phaseolus vulgaris* L.) cultivar Michelite and the proposal of a new anthracnose resistance gene, *Co*-11. *Genetics and Molecular Biology*, *Brazilian Society of Genetics* 30 (3): 589 – 593.

Goncalves-vidigal, M., Meirelles, C., Poletine, A. C., Lorenna de sousa, J. P., Anelise, S., Cruz, R., Maria, P., Nunes, U., Giselly, F., Lacanallo, W., Pedro, S. and Vidigal, T. (2011). Genetic analysis of anthracnose resistance in Pitanga_ dry bean cultivar. *Plant Breeding* 131: 423 – 429.

González, A. M., Yuste-Lisbona, F. J., Rodiño, A. P., De Ron, A. M., Capel, C., García - Alcázar, M., Lozano, R. and Santalla, M. (2015). Uncovering the genetic Arc hitecture of *Colletotrichum lindemuthianum* resistance through QTL mapping and epistatic interaction analysis in common bean. *Plant Science* 6: 141.

Goodwin, M. (2003). Crop Profile for Dry Beans, Regions – Alberta, Manitoba, southern Ontario. *Small areas of Quebec and Saskatchewan,* 41pp.

Hagedorn, D. J. and Inglis, D. A. (1986). *Handbook of Bean Disease, Cooperative Extension Publications*, University of Wisconsin-Extension 25pp.

Hegay, S., Geleta, M., Bryngelsson, T., Asanaliev, A., Garkava-Gustavsson, L., Hovmalm, H. P. and Ortiz, R. (2013). Introducing host-plant resistance to anthracnose in Kyrgyz common bean through inoculation-based and marker-aided selection. *Plant Breeding* 133: 86 – 91.

Hillocks, R. J., Madata, C. S., Chirwa, R., Minja, E. M. and Msolla, S. N. (2006). *Phaseolus* bean improvement in Tanzania 1959-2005. *Euphytica* 150: 225 – 231.

Janick, J. (2010). Plant Breeding Reviews; A John Willey and Sons, Inc., Hoboken, New York, 175pp.

Jones, A. L. and Mejia, D. (1999). *Phaseolus vulgaris: beans; production; processing; harvesting; postharvest losses; pest control.* Food and Agriculture Organization of the United Nations, Rome. 25pp.

Katungi, E., Farrow, A., Chianu, J., Sperling, L. and Beebe, S. (2009). *Common bean in Eastern and Southern Africa: a situation and outlook analysis report.* ICRISAT. 56pp.

Kelly, J. D. and Vallejo, V. A. (2004). A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. *Horticultural Science* 39: 1196 – 1207.

Kelly, J. D. and Vallejo, V. A. (2009). New insights into the anthracnose resistance of common bean landrace G2333. *Horticulture Journal* 2: 29 – 33.

Kiryowa, M., Nkalubo, S., Mukankusi, C., Talwana, H. L. and Tukamuhabwa, P. L. (2010). Assessing the efficacy of pyramided genes in conferring dual and durable resistance to bean anthracnose and root rot, Second RUFORUM Biennial Meeting 20 - 24 September Entebbe, Uganda.

Kour, B., Kour, G., Kaul, S. and Dhar, M. K. (2012). Screening of *phaseolus vulgaris* Cultivars Growing in Various Areas of Jammu and Kashmir for Anthracnose Resistance. *International Journal of Scientific and Research* 2(6): 2250 – 3153.

Liu, F., Cai, L., Crous, P.W. and Damm, U. (2013). Circumscription of the anthracnose pathogens *Colletotrichum lindemuthianum* and *C. nigrum. Mycologia* 105(4): 844 – 860.

Mahuku, G. S., Jara, C. E., Cajiao, C. and Beebe, S. (2002). Sources of resistance to *Colletotrichum lindemuthianum* in the secondary gene pool of *Phaseolus vulgaris* and in crosses of primary and secondary gene pools. *Plant Disease* 86: 1383 – 387.

Markell, S., Wunsch, M. and Rio, L.D. (2012). *Anthracnose of Dry Beans*. 1233 pp.

Mathur, S. B. and Kongsdal, O. (2003). Common laboratory seed health testing methods for detecting fungi. International Seed Testing Association. 425pp.

Martínez-Pacheco, M. M., Saucedo-Luna, J. A., Flores-García, R. E., Martínez-Muñoz, J. and Campos-García, J. (2009). *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib is a potential cellulases producer microorganism. *Rev Latinoam Microbiology* 51 (1-2): 23 – 31.

Mendes-Costa, M.C. and Rodrigues de Carvalho, C. (2011). Vegetative compatibility and heterokary on formation between different isolates of *Colletotrichum lindemuthianum* by using the *nit* mutant system. *Brazilian Journal of Microbiology* 42(1): 1517 – 8382.

Miklas, P. N., Kelly, J. D., Beebe, S. E. and Blair, M. W. (2006). Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica* 147(2): 105 –131.

Mohammed, A. (2013). An Overview of Distribution, Biology and the Management of Common Bean Anthracnose. *Journal of Plant Pathology and Microbiology* 4: 193 – 199.

Mohammed, A., Amare, A. and Selvaraj, H. (2014). Evaluation of various fungicides and soil solarization practices for the management of common bean anthracnose (*Colletotrichum lindemuthianum*) and seed yield and loss in Hararghe highlands Ethiopia. *Journal of Plant Breeding and Crop Science* 6(1): 1 – 10.

Munch, S., Lingner, U., Floss, D. S., Ludwig, N., Sauer, N. and Deising, H. B. (2008). The hemibiotrophic life style of Colletotrichum species. *Journal of Plant Physiology* 165: 41 – 51.

Munda, A., Radisek, S., Šuštar-Vozli, J. and Javornik, B. (2009). Genetic variability of *Colletotrichum lindemuthianum* isolates from Slovenia and resistance of local *Phaseolus vulgaris* germplasm. *Journal of Plant Diseases and Protection* 116 (1): 23 – 29.

Mwesigwa, J. B. (2009). Diversity of *Colletotrichum lindemuthianum* and reaction of common bean germplasm to anthracnose disease. Thesis submitted to the Faculty University of Makerere, Uganda. 93pp.

Nkalubo, S. (2006). Study of anthracnose *(Colletotrichum lindemuthianum)* resistance and its inheritance in Ugandan dry bean germplasm. Dissertation for Award of PhD Degree at Kwazulu-Natal University, Pietermaritzburg, South Africa, 153pp.

Ntahimpera, N., Dillard, H. R., Cobb, A.C. and Seem, R.C. (1996). Anthracnose development in mixtures of resistant and susceptible dry bean cultivars. *Phytopathology* 86: 668 – 673.

Nogueira, G. B., Queiroz, M. V., Ribeiro, R. A. and Araújo, E. F. (2013). Structural and functional characterization of the *Colletotrichum lindemuthianum nit1* gene, which encodes a nitrate reductase enzyme, *Genetics and. Molecular. Research* 12 (1): 420 – 433.

Nyambo, B. (2009). Tanzania Agricultural Sector Development Program (ASDP) Integrat ed Pest Management Plan, (IPMP) revised version. MAFS publications, Dar es Salaam, 55pp.

Opio, F., Ugen, M. A., Kyamanywa, S. D. and Mugisa-Mutitika, M. (2001). Beans. In Agriculture in Uganda, 2 (crops), National Research Organization Ed. Mukiibi, J.K. 2: 162 – 187.

PABRA, (2009). Annual Report, 1st year of New phase, Jan. 2. 2010. 21pp.

Pastor-Corrales, M. A., Erazo, O. A., Estrada, E. I. and Singh, S. P. (1994). Inheritance of anthracnose resistance in common bean accession G 2333. *Plant Disease.* 78: 959 – 962.

Pastor-Corrales, M. A., Goncalves - Vidigal, M. C., Pedro, S., Vidigal, F. and Medeiros, A. F. (2009). Common bean landrace jalo listras pretas is the source of a new Andean anthracnose resistance. *Journal of crop science* 49: 134 – 135.

Peloso, M. J., del, Cardoso, A., Vieira, C., Saraiva, L. S. and Zimmerman, M. J. O. (1989). Genetic system for the reaction of *Phaseolus vulgaris* to the BA-2 (alpha) race of *Colletotrichum lindemuthianum. Brazilian Journal of Genetics and Molecular Biology* 12(2): 313 – 318.

Pinto, J. M. A., Pereira, R., Mota, S. F., Ishikawa, F. H. and Souza, E. A. (2012). Investigating phenotypic variability in *Colletotrichum lindemuthianum* popul ations. *Phytopathology* 102: 490 – 497.

Porch, T. G. and Jahn, M. (2001). Effects of high-temperature stress on micro sporogen esis in heat-sensitive and heat-tolerant genotypes of *Phaseolus vulgaris. Plant, Cell and Environment* 24(7): 723 – 731.

Porch, T. G., Beaver, J. S., Debouck, D. G., Jackson, S. A., Kelly, J. D. and Dempewolf, H. (2013). Use of wild relatives and closely related species to adapt common bean to climate change. *Journal Agronomy* 3: 433 – 461.

Poletine, J. P., Gonçalves-Vidigal, M. C., Vidigal Filho, P. S., Gonela, A. and Schuelter, A. R. (2006). Genetic inferences in common bean differential cultivarsto *C. lindemuthianum* Race 69. *Ciencias Agrarias Londirna* 3: 393 – 398.

Raatz, S. (2013). Nutritional value of Dry Beans, Retrieve from [http://beaninstitute. com/healthbenefits/nutritionalvalueofdrybeans]. Site visited on 25/5/2013.

Richard, N. and Strange, H. (2005). Plant Disease: A Threat to Global Food Security, *Phytopathology*. 43: 83 – 116.

Rio, L. and Bradley, C. (2002). Anthracnose of Dry Beans, Fargo, N.D. Extension publication PP-1233, North Dakota State University. Fargo. 12pp.

Rocha, G.S., Pereira, L. P., Carneiro, L., Pedro, C. S., Paula J. and Carneiro, J.E.S. (2012). Common bean breeding for resistance to anthracnose and Angular Leaf Spot Assisted by SCAR molecular markers, Crop Breeding and Applied Biotechnology. *Brazilian Society of Plant Breeding* 12: 34 – 42.

Rodrigues de Carvalho, C. and Mendes-Costa, M. C. (2011). Vegetative compatibility and heterokary on formation between different isolates of *Colletotrichum lindemuthianum* by using the *nit* mutant system. *Brazilian Journal of Microbiology* 42: 1 – 42.

Sharma, P. N., Padder, B. A., Sharma, O. P., Pathania, A. and Sharma, P. (2007). Pathological and molecular diversity in *Colletotrichum lindemuthianum* (bean anthracnose) across Himachal Pradesh, a north-western Himalayan state of India. *Australasian Plant Pathology* 36: 191 – 197.

Shimray, R. A. and Ragui, R. (2014). Isolation of Seed Mycoflora and Biological Control of Anthracnose Disease of French Bean (*Phaseolus vulgaris Linn*) *Journal of Science, Research and Technology* 2(2): 3 – 12.

Silva, M. G., Pozza, E. A. and Machado, J. C. (2013). Influence of Contaminated Crop Remains and Seed Health Quality on the Intensity of Bean Anthracnose. *Journal of Agricultural Science* 5: 10 – 26.

Souza, E. A., Camargo, O. A., Jr. H. and Pinto, J. M. A. (2010). Sexual recombination in *Colletotrichum lindemuthianum* occurs on a fine scale. *Genetic Molocular Research* 9(3): 1759 – 1769.

Souza, D.A., Pereira, F.A.C., Dias, J. A., Leite, M.E. and Bosco dos Santos, J. (2014). Reaction of common bean progenies to white mold derived from recurrent selection. *Ciência Rural* 44: 4 – 7.

Stanfield, W.D. (1991). *Theory and problems of Genetics*; McGraw – Hill Companies, New York. 452pp.

Takahara, H., Huserb, A. and O'Connell, R. (2012). Two arginine biosynthesis genes are essential for pathogenicity of *Colletotrichum higginsianum* on *Arabidops Mycological Society of China* 3: (1): 54 – 64.

Tu, J.C. (1983). Epidemiology of anthracnose caused by *Colletotrichum lindemuthianum* on white bean *(Phaseolus vulgaris)* in southern Ontario: Survival of the pathogen. *Plant Disease* 67: 402 – 404.

Tryphone, M. G., Chilagane, L. A., Protas, D., Kusolwa, P. M. and Nchimbi-Msolla, S. 2013). Marker assisted selection for common bean diseases improvements in Tanzania prospects and future need. In: *Plant Breeding from Laboratories to* Fields "Edited by", *Andersen, S. B*. InTech, Rijeka. pp. 121 – 146.

Vallejo, V. and Kelly, J. D. (2009). New Insights into the Anthracnose Resistance of Common Bean Landrace G 2333. *Horticulture Journal* 2: 29 – 33.

Van Schoonhoven, A. and Pastor – Corrales, M. A. (1987). *Standard System for the evaluation of bean germplasm*. (CIAT), Cali, Colombia. 54pp.

Vidigal Filho, P. S., Goncalves-Vidigal, M. C., Kelly, J. D. and Kirk, W. W. (2007). Sources of Resistance to Anthracnose in Traditional Common Bean Cultivars from Parana. *Brazilian Journal of Phytopathology* 155: 108 – 113.

Wray, N. and Visscher, P. (2008). Estimating trait heritability. *Nature Education* 1(1): 29 – 39.

Young, R. A. and Kelly, J. D. (1996). Characterization of the genetic resistance to *Colletotrichum lindemuthianum* in common bean differential cultivars. *Plant Disease* 80: 650 – 654.

# APPENDICES

## Appendix 1: Table of results

| Growth habit | No. of isolates | Gene pool | Total no. Isolates | Percentage |
|---|---|---|---|---|
| Bush type | 25 | Andean | 32 | 78.1 |
| Semi Climber | 3 | Mesoamerica | 32 | 9.4 |
| Climber | 4 | Mesoamerica | 32 | 12.5 |

## Appendix 2: Temperatures, Rain fall and Humidity at Bashnet during field pathogenicity

| **RAINFALL,TEMPERATURE & HUMIDITY DATA- JAN-MARCH 2015** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **NAME OF THE SITE: BASHNET- LONG** | | | | | | | | | |
| | **Jan-15** | | | | | **FEBRUARY 2015** | | | |
| | RAIN FALL (mm) | TEMPE RATURE | HUMI DITY | | R/FALL (mm) | TEMPERATURE | | % RELATIVE HUMIDITY | |
| Date | | AVERAGE | MIN. | Date | | MAX | MIN. | UPPER | LOWER |
| 1 | NIL | | | 1 | 25 | 26 | 13 | 96 | 27 |
| 2 | NIL | | | 2 | 7 | 24 | 11 | 93 | 41 |
| 3 | NIL | | | 3 | NIL | 25 | 13 | 98 | 38 |
| 4 | 1 | | | 4 | NIL | 27 | 12 | 97 | 25 |
| 5 | NIL | | | 5 | NIL | 27 | 13 | 96 | 26 |
| 6 | NIL | | | 6 | NIL | 28 | 13 | 90 | 25 |
| 7 | 5 | | | 7 | NIL | 28 | 13 | 80 | 19 |
| 8 | 7 | | | 8 | NIL | 26 | 13 | 87 | 21 |
| 9 | 10 | | | 9 | 1 | 26 | 13 | 90 | 32 |
| 10 | NIL | | | 10 | NIL | 24 | 13 | 97 | 34 |
| 11 | 2 | | | 11 | NIL | 24 | 13 | 95 | 34 |
| 12 | 1.5 | | | 12 | NIL | 25 | 11 | 92 | 35 |
| 13 | NIL | | | 13 | NIL | 25 | 13 | 93 | 35 |
| 14 | NIL | | | 14 | NIL | 26 | 13 | 93 | 32 |
| 15 | NIL | 23 | | 15 | 12.8 | 23 | 13 | 96 | 46 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | NIL | | | 16 | NIL | 21 | 13 | 99 | 64 |
| 17 | NIL | | | 17 | NIL | 24 | 13 | 97 | 45 |
| 18 | NIL | | | 18 | 21.1 | 24 | 14 | 98 | 45 |
| 19 | NIL | | | 19 | 2.8 | 22 | 13 | 100 | 59 |
| 20 | NIL | | | 20 | NIL | 24 | 12 | 98 | 43 |
| 21 | NIL | | | 21 | NIL | 23 | 13 | 96 | 49 |
| 22 | 16 | | | 22 | NIL | 26 | 12 | 98 | 27 |
| 23 | NIL | 24 | 81 | 23 | NIL | 24 | 13 | 97 | 49 |
| 24 | NIL | 24 | 82 | 24 | NIL | 24 | 14 | 96 | 34 |
| 25 | NIL | 23 | 84 | 25 | NIL | 24 | 14 | 98 | 39 |
| 26 | NIL | 23 | 84 | 26 | 6 | 26 | 12 | 97 | 27 |
| 27 | NIL | 22 | 81 | 27 | 0.1 | 25 | 12 | 100 | 26 |
| 28 | NIL | 23 | 78 | 28 | 1.1 | 27 | 13 | 97 | 27 |
| 29 | NIL | 24 | 80 | | | | | | |
| 30 | 0.4 | 23 | 82 | | | | | | |
| 31 | NIL | 23 | 86 | | | | | | |

**MARCH 2015**

| | **RAIN FALL (mm)** | **TEMPERATURE (°C)** | | **%RELATIVE HUMIDITY** | |
|---|---|---|---|---|---|
| | | MAX | MIN. | UPPER | LOWER |
| 1 | 2.1 | 27 | 13 | 83 | 31 |
| 2 | 1.2 | 24 | 13 | 95 | 29 |
| 3 | NIL | 24 | 13 | 89 | 42 |
| 4 | NIL | 26 | 13 | 96 | 27 |
| 5 | 2.8 | 24 | 11 | 93 | 41 |
| 6 | NIL | 25 | 13 | 98 | 38 |
| 7 | NIL | 27 | 12 | 97 | 25 |
| 8 | NIL | 27 | 13 | 96 | 24 |
| 9 | NIL | 28 | 12 | 90 | 25 |
| 10 | NIL | 28 | 13 | 80 | 19 |
| 11 | NIL | 26 | 13 | 87 | 21 |