BREEDING FOR RESISTANCE TO *Colletotrichum lindemuthianum* (Sacc. and Magn. Scrib) IN COMMON BEAN (*Phaseolus vulgaris* L.)

BY

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THIS DISSERTATION IS SUBMITTED TO THE SCHOOL OF AGRICULTURAL SCIENCES OF THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PLANT BREEDING AND SEED SYSTEMS

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DECLARATION

I, CHILIPA LORRAINE.NK, declare that the work contained in this dissertation is entirely mine. It has never been submitted for the award of any degree or its equivalent in this University or in any other higher institution of learning elsewhere, and unless indicated, the work is entirely through my effort.

Signature: _____

Date: 18th January, 2017.

APPROVAL

This dissertation of CHILIPA LORRAINE N.K. is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Plant Breeding and Seed systems of the University of Zambia.

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ABSTRACT

Bean anthracnose caused by *Colletotrichum lindemuthianum* causes severe common bean (*Phaseolus vulgaris* L.) yield losses. Breeding for resistance is the best method to mitigate this problem. The objectives of this study were to; (1) screen and identify two common isolates of *C. lindemuthianum* from major bean growing areas in Zambia, (2) investigate the effectiveness of *C. lindemuthianum* multiple race inoculations on genotypic response in common bean and (3) determine the nature of inheritance to *C. lindemuthianum* in common bean genotypes. Seven parents were crossed in a 7 X 7 full diallel mating design. Forty-two progeny crosses together with their parents were raised in the green house, in a Completely Randomised Design (CRD) with four replications. The treatment used were (1) inoculation with race 54; (2) inoculation with race 311 and (3) a combination of inoculation of race 54 X race 311. The mean genotypic scoring were found to be 1.76, 2.62 and 3.06 for treatments 1, 2 and 3 respectively. There were significant differences ($P < 0.01$) among genotypic responses to *C. lindemuthianum* with respect to race 311 while race 54 and race 54 plus race 311(multiple inoculations) showed no significant differences among the genotypes. The *t*-test analysis revealed that multiple race inoculation (Treatment 3) had a higher disease severity expression than those of single race inoculations (Treatment 1 and Treatment 2). Multiple infection had a synergistic effect suggesting its suitability for the screening of resistant genotypes in the breeding program. With regards to treatment 2 (Inoculation with race 311), the analysis of variance revealed that general combining ability (GCA) and specific combining ability (SCA) were not significant for resistance to *C. lindemuthianum*. Only reciprocal effects were found to be significant implying that both cytoplasmic gene- and environmental effects were at play. Further on, the baker's ratio of 0.15 obtained meant that inheritance was due to non-additive gene action. Breeders should therefore use hybridization as breeding strategy, in the production of resistant beans with regards *C. lindemuthianum*, race 311.

DEDICATION

To my mother and siblings for their love and sacrifices which have made me what I am today.

To my late father who unfortunately did not live to witness his love for me to bear these wonderful fruits.

To my late daughter and son.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ACRONYMS AND ABBREVIATIONS

BSH	Broad Sense Heritability
CIAT	Centro Internacianal de Agricultura Tropical
CRD	Completely Randomized Design
F1s	Filial one generation
GCA	General Combining Ability
MAL	Ministry of Agriculture and Livestock
NSH	Narrow Sense Heritability
PDA	Potato Dextrose Agar
R	Resistance
RH	Relative Humidity
S	Susceptible
SCA	Specific Combining Ability
SCCI	Seed Control and Certification Institute
UNZA	University of Zambia
ZARI	Zambia Agriculture Research Institute

CHAPTER ONE: INTRODUCTION

1.0 BACKGROUND

1.1 General Bean Production and Importance

Common beans (*Phaseolus vulgaris.*L.) are an important grain legume in human consumption in the whole world. Due to its importance, researchers around the world for many years have been developing new improved varieties from the many useful landraces present (Broughton *et al*., 2003; Munda, 2009).

The world's annual production is about 12 million tons with Latin America being the largest producer at 5.5 million tons, Africa is second contributing 2.5 million metric tons (Broughton *et al*., 2003; Ansari *et al.*, 2004) while Zambian production stood at 50,398 metric tons (MAL, 2013). In Zambia resource poor farmers, who are mostly women, female headed, youth headed and vulnerable children headed households rely on the production of beans as an important source of income and nutrition (Akibode and Maredia, 2011; Chalwe *et al*., 2011).

Common bean have been long valued for their macro and micro nutrients, particularly their high levels of protein and complex carbohydrate content. Common bean have growth habits which vary from determinate dwarf or bush types to indeterminate climbing (Kelly, 2010) and can be grown as a vegetable crop for fresh pods and leaves, or for dry seed (Figure 1). Furthermore, common bean is important because it plays a role in improving soil fertility through nitrogen fixation. When used in crop rotation or intercropped with

Figure 1: Assorted dry bean grain (A), leaves (B) and fresh pods (C).

cereals it disrupts the life cycle of soil pathogens in an event were soil pathogens are present and supplies nitrogen to the cereal crop (Akibode and Maredia, 2011).

1.2 Constraints of common bean production

Inspite of the importance of common bean, its production in Zambia is hindered by several biotic and abiotic stresses. The productivity of common bean has been low with average yield of 0.5 tons compared to potential yield of 2 tons per hectare (MAL, 2013). The low productivity of the crop is caused by abiotic stresses such as low soil fertility, droughts, floods, poor agronomic practices and biotic stresses such as pests and diseases.

Diseases are usually triggered by use of recycled seed, unfavourable weather patterns and/or use of poor field sanitation practices among many. Common bean in Zambia has a number of disease problems which tend to have great influence on the stability of production (Msuku *et al*., 2000). Diseases that particularly affect beans include anthracnose (*Colletotrichum lindemuthianum*), common bacterial blight (*Xanthomonas campestris*) and bean mosaic virus (BCMV). *Colletorichum lindemuthianum* causes an important seed-borne disease, spreads widely and can cause great yield losses of above 80

percent due to poor germination of infected seeds (Chipili *et al.*, 2002; Miklas, 2006; Bush, 2014; Padder *et al.*, 2010). In severe instances, product quality is lowered through damaged leaves, pods and seeds which affects the value of the crop, thereby making it unfit for human consumption (Gonçalves-Vidigal *et al.*, 2007).

1.3 Strategies for management of bean anthracnose disease

A number of control measures such as use of disease free certified seed, crop rotation and field sanitation have been employed to manage *C. lindemuthianum* but to a limited success (Tesfaye, 2003; Dillard and Cobb, 1993). While, the application of fungicide may be an effective and efficient control measure but the high costs associated with this method makes it not feasible for the resource poor farmers (Bush, 2014; Mohammed, 2013). On the other hand, the use of resistant common bean genotypes to *C. lindemuthianum* have been found to be the most effective, efficient and affordable for even resource poor farmers (Munda, 2009; Kiryowa, 2010). Nkalubo (2006), reported the availability of a number of resistant sources for combating *C. lindemuthianum*. However *C. lindemuthianum* is a highly variable pathogen and no genotypes have been found to be resistant across all environments (Mahuku *et al*., 2002). Combining several resistant genes into a single line is one way of making resistant genotypes stable. However this requires a thorough understanding of the biology, ecology and diversity of the *C. lindemuthianum* races from the major bean growing regions (Mwesigwa, 2009; Mohammed, 2013). The available resistant genotypes in common bean have rarely been tested against several races of the pathogen; therefore, several of the cultivars previously described as resistant were susceptible in other environments (Mahuku *et al.*, 2002; Mwesigwa, 2009). Generally,

one bean genotype may be resistant to some races, but not to others (CIAT, 1997). Resistant genes in various cultivars differ in their effectiveness against *C. lindemuthianum* (Mwesigwa, 2009). In this regard, stable resistance can be achieved through artificial multiple inoculation of different races and screening of bean genotypes for resistant ones.

1.4 Justification

In breeding for resistance to *C. lindemuthianum* in common beans, plant breeders have employed single race inoculations or have paid attention to specific races (Gonçalves-Vidigal *et al*., 2007; Davide and Souza, 2009; Padder, 2010). However, the genetic information generated has proved a challenge in initiating for durable resistance which is effective across environments (Pastor-Corrales *et al*., 1995). It should however be noted that multiple infection by *C. lindemuthianum* races may occur, which may lead to different effects when compared to a challenge with single race infection. However, little is known on the effect of multiple inoculations of the races in breeding for resistance to *C. lindemuthianum.* On the other hand successful development of a bean genotype with durable resistance will depend on the sources of resistant genes (Pastor Corrales *et al*., 1994).

An understanding of the genetic inheritance on the basis of anthracnose resistance in beans is a prerequisite for the development of resistant genotypes and methods of breeding schemes to use. Previous studies exhibited conflicting views on the type of gene action conditioning resistance to *C. lindemuthianum* in common bean (Nkalubo, 2006; Gonçalves-Vidigal *et al*., 2007). There is therefore need to establish the nature of gene action determining resistance to *C. lindemuthianum*.

1.5 Overall objective

The overall objective of this study was to breed for resistance to *Colletotrichum lindemuthianum* in common bean in Zambia.

1.5.1 Specific objectives

The specific objectives were to:

1. Screen and identify two common isolates of *Colletotrichum lindemuthianum* from major common bean growing areas;
2. Investigate the effectiveness of *Colletotrichum lindemuthianum* multiple race inoculations on genotypic response in common bean; and
3. Determine the nature of inheritance to *Colletotrichum lindemuthianum* in common bean genotypes.

1.6 Research hypothesis

The hypotheses tested were as follows:

1. Multiple race inoculations of *Colletotrichum lindemuthianum* on common bean enhance the effect on disease severity as compared to single race inoculations.
2. The nature of gene action influencing resistance to *Colletotrichum lindemuthianum* is additive.

CHAPTER TWO: LITERATURE REVIEW

2.0 INTRODUCTION

2.1 Classification, Variability and Importance of the anthracnose pathogen (*Colletotrichum lindemuthianum*)

Common bean anthracnose is a seed borne disease and is caused by fungus (*Colletotrichum lindemuthianum* **(**Sacc. and Magn.) Scrib. *Colletotrichum lindemuthianum* is an ascomycete and produces its conidia in acervuli. This fungus belongs to the genus *Colletotrichum*, order Melanconiales, family Melanconiaceae and section Hyalosporae (Alexopoulos, 1962). The fungus is known to have races that vary from country, region and location (CIAT, 1997). Most pathogens exhibit a great variability for pathogenicity. The variability arise from sexual mechanisms like recombination of nuclear genes during sexual reproduction, mutation, or by extra chromosomal variation (Ogallo, 1991). Today, the disease is reportedly one of the most important and widely distributed throughout the world especially in cool, frequently wet and humid regions (Pastor-Corrales and Tu, 1989; Singh and Schwart, 2010).

In Zambia, the disease anthracnose of common bean caused by *C. lindemuthianum* pathogen was first recorded in the Northern, Copperbelt, Northwestern and Luapula provinces in 1986 (Greenberg *et al*., 1986; Zulu 2005). The Pathogen exhibit great variability and in the year 2005, Zulu characterised and recorded 14 races. In another study done by kachapulula in the year 2010, a total of 17 races were recorded. The incidence and severity of anthracnose disease in Zambia is highest in region 3 which exhibits cool

weather, high humid and high rainfall followed by region 2 and 1 which exhibits low humid and rainfall (Zulu, 2005; Kachapulula *et al.*, 2010).

2.2 The Life cycle of bean anthracnose

Common bean anthracnose is characterised by black lesions, usually sunken, caused by certain imperfect fungi that produce asexual conidia. The fungi reproduce by forming microscopic conidia hyaline, oblong to dumbbell shaped, one celled, straight and ends rounded in shape (Zulu, 2005). These conidia are often used for identification of the pathogen found in various parts of the world. The pathogen has a sequential biotrophic and necrotrophic infection process to invade and colonize the plant hosts (Gonzáles *et al.*, 2015). The asexual conidia of *C. lindemuthianum* are dispersed mainly through rain splashes, insects, farm workers and their tools. The conidia quickly attach to the aerial parts of the bean plant, germinate and form germ tube which grow and causes indentation to occur in the cell wall (Gupta and Paul, 2002). Then the infection tubes penetrate the host tissue, through the cell wall and forms infection vesicles. In the early stage of infection proteins are released by the infection vesicles which suppress the host defence system. These proteins suppress any hypersensitive response from the host (Mohammed, 2013). The host plant stops to grow normally while unlimited growth and development of the fungus occurs. The fungus hyphae move freely and penetrate the cell wall and the membranes. The *C. lindemuthianum* reaches maximum development at right moisture content of not more than 100 RH and temperatures of not more than 27^{o}C (Niks and Lindhout, 2006). The fungus then matures and produces conidia for continued survival.

2.3 Major symptoms associated with bean anthracnose

Disease symptoms produced by all races are identical. Initial symptoms of anthracnose infection are dark brown lesions along the leaf veins on the underside of the leaves. Leaf petioles and stems may also show the above symptoms. Symptom of pod infection results in small, reddish, elongated spots. Older spots are sunken and have brown to reddish-brown borders. The fungus penetrate through the pod wall causing discoloration and distortion of the seed, at this stage of the disease development, the fungus can also penetrate the seed coat and become firmly established within the seed. Symptoms of seed infection are marked by dark, sunken lesions that extend through the seed coat.

2.4 Breeding for resistance

The use of resistant varieties leads to a reduction in common bean production costs especially pesticide cost and lowers the quantity of pesticides or their residues released into the environment (Bediako, 2012; Mohammed, 2013). In addition, the cost of insecticides and proper application equipment is beyond the economic means of the majority of resource poor farmers who grow the crop. *Colletotrichum lindemuthianum* which causes a seed-borne disease can be best combated through breeding for disease resistance (Dillard and Cobb, 1993; Nkalubo, 2009). However, the development of resistant variety is not easy for breeders as most pathogen exhibits a great variability for pathogenicity which mostly overcomes the resistance in the released cultivars. Genetic resistance can be created based on the known races by introducing the right genes to host (Haciwa, 1991; Ogallo, 1991).

2.4.1 Inoculation and evaluation of resistance to *C. lindemuthianum*

The success of any breeding program for disease resistance highly depends on the development of a suitable, reliable inoculation and screening technique. Various inoculation methods can be used to introduce a given quantity of *C. lindemuthianum* inoculum to the bean plant and subjecting the plant to suitable conditions for infection and disease progression. Previous studies have involved both artificial and natural inoculations when testing for resistance in the genotypes of interest (Zulu, 2005; Nkalubo, 2006; Silvério da Rocha, 2012). It should be noted that effective studies on races of *C. lindemuthianum* require controlled inoculation environments so as to minimise possibilities of interference from other pathogens present in the surrounding environment. In most studies single race inoculations have been employed when screening for resistance to *C. lindemuthianum* in beans (Niks and Lindhout, 2006; Monroy-Barbosa and Bosland, 2010). It remains to be established if multiple race infection has a noticeable effect as compared to single races infection. This is important because it may affect the genetic information obtained in terms of nature of inheritance and selection of resistant genotypes.

Inspite of the different methods used in different conditions, the pathogen inoculum must be applied in right quantities so as to initiate development of the *C. lindemuthianum.* Inoculation on differential cultivars is one of the methods for race identification through their variation in resistance levels (Wagara *et al*., 2005; Mwesigwa, 2009; Arunga, *et al.*, 2010). This procedure is extremely useful for phytopathological as well as breeding purposes and it requires strict control of the number of spores and incubation conditions. On the other hand, this method may result in misclassifications of races because of the subjectivity of symptom evaluation (Mesquita, 1998). It is of importance therefore to use

standard methods of screening techniques to allow results obtained in time and space to be comparable (Niks and Lindhout, 2006).

2.4.2 Inheritance of resistance to *C. lindemuthianum*

Resistance is a dynamic character that can be inherited. In recent studies the inheritance of resistance to bean anthracnose has been reported. It is now recognized that disease resistance in common bean anthracnose shows inheritance which is governed by one or few major genes (Singh, 2012). The nature of inheritance of anthracnose resistance genes depends among other factors, on the tester genotype used as the susceptible parent, the isolate or race of the pathogen used for inoculation, and the stage at which the host plant is inoculated (Pastor - Corrales *et al*., 1994).

Resistance to *C. lindemuthianum* is qualitatively inherited and both additive and non-additive gene action have been reported, for instance additive effects were important for race 31 while non-additive effects were more important for race 89 of *C. lindemuthianum* (Nkalubo, 2006; Gonçalves-Vidigal *et al*., 2007; Nkalubo *et al*., 2009). In other studies it was deduced that resistance to race 69 is predominately conditioned by additive and epistatic gene action (Nkalubo, 2006; Poletine *et al*., 2006). Variations in the results seen in different studies, may arise from differences in the genetic backgrounds of the genotypes used in the study.

CHAPTER THREE: MATERIALS AND METHODS

3.0 Experimental sites and materials used

Seven parents (Table 1) selected on the basis of varying levels of resistance to *C. lindemuthianum* and farmer preference, were mated in a full diallel to generate 42 progeny offsprings in the screen house at the Seed Control and Certification Institute (SCCI) in Chilanga (26º26'N, 15º55'E; altitude 1,227 m). Crossings were done either in the early mornings or evenings as described by Tumwesigye (1988). After successfully crossing, tags (indicating the cross and date) were affixed to the raceme beneath the pollinated bud (Figure 2). The pods with identification tags were harvested at physiological maturity, dried and threshed. The seven parents and 42 progenies were later challenged for resistance to single and multiple inoculation of isolates to *C. lindemuthianum* and then evaluated for their disease severity responses in the screen house at University of Zambia (28º 21'S, 15º 22'E; altitude 1, 262 m).

Table 1: Bean genotypes used in a 7 x 7 full diallel at the Seed Control and Certification Institute during the 2014/15 cropping season

Parent	Source	Reaction of parents to *C. lindemuthianum*	References
G2333	CIAT	R	(Pastor-Corrales, 1991)
PI-207-262	CIAT	R	(Pastor-Corrales, 1991)
AB136	CIAT	R	(Pastor-Corrales, 1991)
Perrymarrow	CIAT	S	(Pastor-Corrales, 1991)
Kabulangeti	SCCI	S	(Zulu, 2005)
Solwezi	SCCI	S	(Zulu, 2005)
Mbala	SCCI	S	(Zulu, 2005)

Key: Disease severity damage scoring, R = Resistant; S = Susceptible.
Source: (Van Schoonhoven and Pastor - Corrales, 1987).

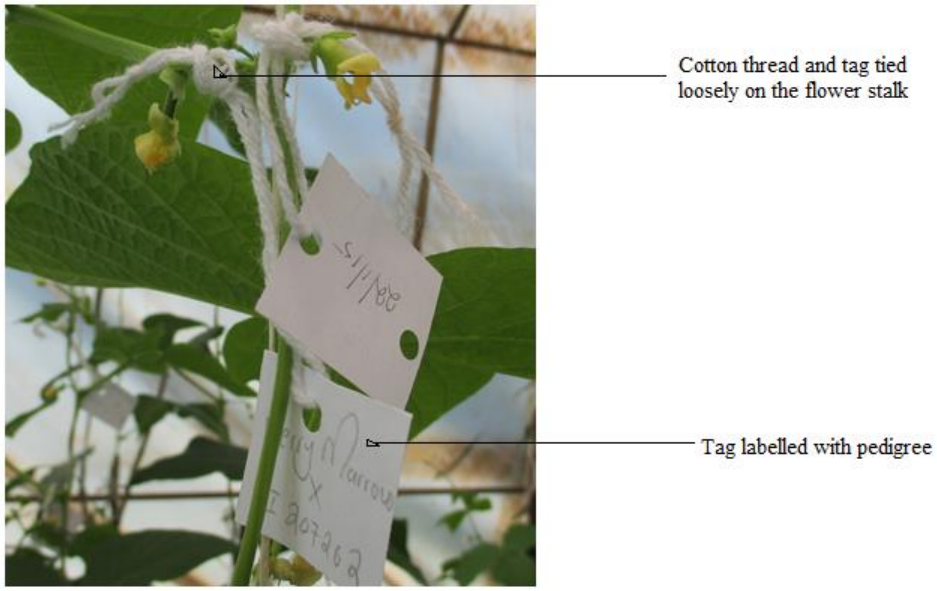

Figure 2: A cross between parents Perry Marrow and PI-207-262 with a cotton thread and tag labelled showing pedigree of the cross affixed loosely beneath the pollinated bud.

3.0.1 Collection and preparation of diseased bean plant materials

The diseased plant materials were collected during the rainy season in March 2015 from Musa in Kasama district, Northern Province and Kawiko in Mwinilunga district, North-Western province (Table 2 and Figure 3). The areas selected for disease collection were purposely chosen being the naturally moderate to high disease severity bean growing areas (Figure 3). The areas selected for disease sample collection represent a larger representative of the races found in the major bean growing area. That is to say races found in Luapula and North-western provinces are similar (Zulu, 2005) and the other race was collected from the Northern part of Zambia. Only bean plants which had pronounced anthracnose symptoms from naturally infected farmers' fields were collected. The materials were then placed in brown bags which were labelled and taken to UNZA Plant Pathology laboratory.

Table 2: Anthracnose disease collection sites used in the study

Province	District	[a] GPS co-ordinates		
		Latitude	Longitude	Altitude(m)
Northern	Kasama	10°20.016	31°13.326	1294
Northwestern	Mwinilunga	11°45.076	24°24.042	1379

Key: a = Global Positioning System

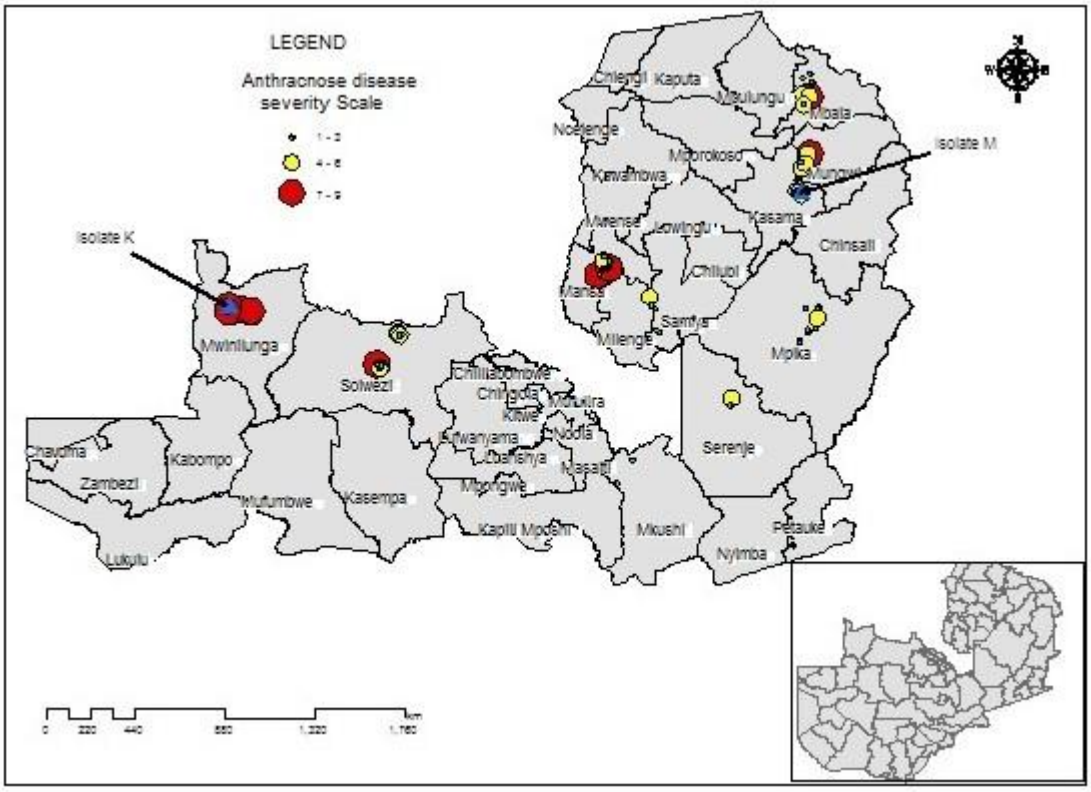

Figure 3: Map of Zambia showing major common bean growing areas and distribution of disease severity in the provinces.

Key: Actual points from where bean anthracnose disease samples were collected in Musa, Kasama (Isolate M) and Kawiko in Mwinilunga (Isolate K). Anthracnose disease severity scale (1 - 3) resistant; (4 - 6) Intermediate susceptible and (7 - 9) susceptible

Source: (Van Schoonhoven and Pastor - Corrales, 1987).

3.0.1.1 Preparation of culturing media

Two culturing media Potato Dextrose Agar (PDA) and bean pod agar were used. The preparation (PDA) was done by weighing 39 g of PDA to which up to 1000 ml of distilled water was added. A magnetic stirring plate was then used to stir the suspension after which the media was autoclaved for 15 minutes at 121^{o}C. Thereafter the media was allowed to cool, poured onto sterile petri dishes and left to solidify.

Bean pod agar preparation involved washing of the bean pods and cutting off of their two ends (remnants sepals and style). Thirty, approximately 120 mm bean pods were then

placed upright in each of the six 1000 mls conical flasks, which were later sealed with balls of cotton and aluminium foil. The conical flasks with the cut bean pods were then autoclaved for 15 minutes at 121^{o}C and allowed to cool.

3.1 Assessment and identification of races used in the study

3.1.1 Isolation and inoculum preparation of *C. lindemuthianum* pathogen

The isolates used in the study where collected from Musa in Kasama (Isolate M) and Kawiko in Mwinilunga (Isolate K) and were extracted from well-developed lesions of naturally anthracnose infected bean pods and leaves (Figure 4) at UNZA laboratory. Isolation of *C. lindemuthianum* pathogen from the pods and leaves involved cutting of 10 mm pieces of infected tissue. The 10 mm pieces were sterilised with 5% sodium hypochlorite for 5 minutes, rinsed in water and dried using paper towel before plating.

Figure 4: Symptom of anthracnose disease on bean pods (A) and on underside of the leaf (B).

Placement of sterilised pieces with specific isolates on PDA was done in the laminar flow hood and the petri dishes were placed in the incubator, in an upside down arrangement. Seven days after the initial culturing and incubation, the fungus was viewed

microscopically (Figure 5) and then sub-cultured on bean pod media. Treatments 1 (Isolate M) and treatment 2 (Isolate K) had each two conical flasks which contained 30 bean pods of approximately 120 mm in length. The two conical flasks for treatment 1 were both inoculated with approximately 15 mm pieces of agar plugs with actively growing fungus of isolate M and the other two conical flasks for treatment 2 were both inoculated with approximately 15 mm pieces of agar plugs with actively growing fungus of isolate K. The conical flasks were then kept in the incubator at 23^{o}C for fifteen days. On the fifteenth day the mycelia plugs in each conical flask for the two treatments were flooded with 200 mls of distilled water and mixed thoroughly using an electric shaker and a magnetic steering bar. After ten minutes of mixing the suspension in each of the flasks was filtered through a triple layer of muslin. The inoculum in each of the two flasks for each treatment was mixed. Using a haemocytometer, the concentration of the condia suspension was standardised by adjusting it to 1.2 X 10^{6} condia ml^{-1}. Furthermore for the inoculum to stick properly onto the leaves or any part of the plant, one drop of Tween-20 (Polyethylene glycol sorbitan monolaurate) was added per 100 mls of inoculums for each treatment and mixed thoroughly before inoculations. Only one litre inoculum was used for inoculations.

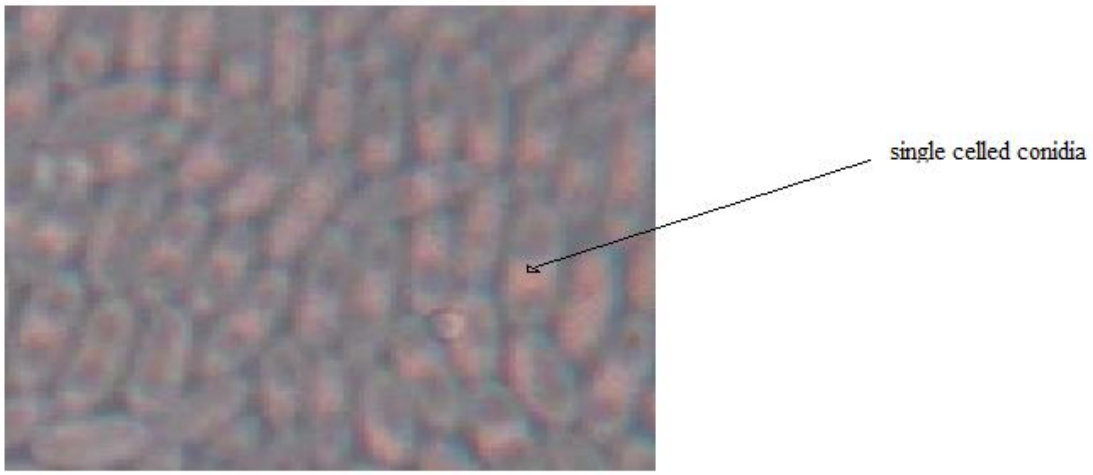

Figure 5: Microscopic view of *Colletotrichum lindemuthianum* single conidia , a bean anthracnose disease pathogen.

3.1.2 Race identification using binary designation system

The phenotype for the races were identified by inoculating the two isolates Musa and Kawiko (Isolate M and Isolate K) on thirty days old differential cultivars grown in plastic pots in the green house at the University of Zambia in May 2015. The experiment was laid out as a Completely Randomised Design (CRD) with four replications. During the inoculations the pathogenic isolates; Treatment 1 (Inoculation with isolate M) and Treatment 2 (Inoculation with isolate K) were handled separately. The genotypes were incubated and maintained in different mist chambers for 96 hours, at 23°C and 90 - 100% relative humidity. Disease assessment of the differential cultivars reaction to infection was done seven days after inoculum inoculation. Each plant was scored visually for the disease symptoms using a 1 to 9 scale (Figure 6 and Appendix 1). Three distinct plant reactions were considered using binary system that is Resistant (R) phenotype was assigned to plants with no or limited symptoms (Scores 1 to 3); whereas plants graded 4 to 6 was considered intermediate and scores of 6 to 9 were considered to be susceptible (S). A, B and C represent disease lesions on the petiole, leaves and stem respectively. Using the

binary designation system, the differential cultivars were assigned to a specific order of one to twelve each with a binary value if susceptible (Table 3). The isolates were then assigned a cumulative numerical value for each susceptible differential cultivar (Pastor - Corrales, 1991).

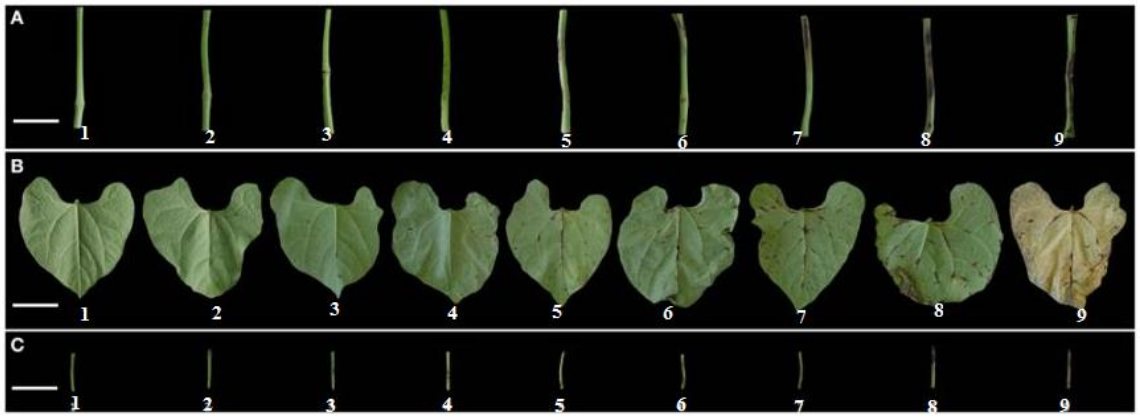

Figure 6: Standard plate for disease damage scoring scale (1-9) to evaluate the reaction of beans germplasm petioles, leaves and stem to *Colletotrichum lindemuthianum* (anthracnose pathogen).

Key: R = Resistant (1 - 3); Intermediate susceptible (4 - 6); S = Susceptible (7 - 9).

Source: (Van Schoonhoven and Pastor-Corrales, 1987).

Table 3: Binary designation system: showing anthracnose differential series

Differential cultivars	Host genes	Binary number	*Isolate C = Race 2311
Michellite	Co-11	1	S (1)
Michigan.D.R. kidney	Co-1	2	S (2)
Perry Marrow	$Co\text{-}1^3$	4	S (4)
Cornell 49242	Co-2	8	R
Widusa	$Co\text{-}1^5$	16	S
Kaboon	$Co\text{-}1^2$	32	S
Mexico 222	Co-3	64	R
PI- 207-262	$Co\text{-}4^3$,Co-9	128	R
TO	Co-4	256	S (256)
TU	Co-5	512	R
AB136	Co-6, co-8	1024	R
G2333	$Co\text{-}4^2$,$Co\text{-}5^2$, Co-7	2048	R (2048)

Designation of race was obtained by adding the binary values of susceptible reaction (S) of all the twelve differentials cultivars to a given isolate; * Example; Isolate C = race 2311 obtained by adding (1+2+4+256+2048), (Pastor - Corrales, 1991).
Key: R = Resistant (1 - 3); Intermediate susceptible (4 - 6); S = Susceptible (7 - 9).
Source: (Van Schoonhoven and Pastor - Corrales, 1987).

3.2 Assessment of multiple inoculation of identified *C. lindemuthianum* races

The isolates M and K used in section 3.2.1 were identified as race 54 and race 311, respectively, and were then utilised in this study. Seven parents and their 42 progeny crosses generated in section 3.1 were grown in plastic pots in the green house at the University of Zambia in May 2015. The treatments used were: Treatment 1 (Inoculation with single race 54), Treatment 2 (Inoculation with single race 311) and Treatment 3 which constituted inoculation with multiple race. This was achieved by adding 500 mls each of race 54 and race 311 to constitute a final volume of 1000 mls. The experiment was laid out as a Completely Randomised Design (CRD) with four replications. The

inoculation of the specific treatment was done thirty days after genotype emergence, the genotypes were inoculated with single and multiple race using a one litre hand sprayer until run off. Each treatment was inoculated on one specific set of genotypes and placed in different mist chambers. The plants were then incubated and maintained in mist chambers for 96 hours, at 23°C and 90 - 100% relative humidity. Disease assessment of the genotypes' reaction to infection for each respective treatment was done seven days after inoculum inoculation. Four individual genotypes for each of the seven parents and the 42 progeny crosses were scored visually for the disease symptoms using a 1 to 9 scale as in section 3.2.2. Three distinct plant reactions were considered using binary system that is Resistant (R) phenotype was assigned to plants with no or limited symptoms (Scores 1 to 3); whereas plants graded 4 to 6 was considered intermediate and scores of 6 to 9 were considered to be susceptible (S).

3.3 Data analysis

Identification of race 54 and race 311 was done using binary designation system (Pastor - Corrales, 1991) computed from the phenotypic disease severity reaction for each of the differential cultivars to the two isolates. Analysis of variances (ANOVA) was used to determine the genotypic responses of common bean to different races of *C. lindemuthianum*. Mean disease severity score comparisons among treatments (Single race and multiple race infection) were done through student *t*-test using Microsoft office (Excel). Diallel, Griffings (1956) method I fixed model I analysis was done using ANOVA and Regression analysis in Gen Stat Discovery Edition version 17 (Payne *et al*., 2012). The relative contribution of GCA to SCA were analysed using baker's ratio (Baker 1978), computed as $[(2\delta^2gca/ (2\delta^2gca + \delta^2sca)]$. Narrow sense and Broad sense heritability for

resistance to *C. lindemuthianum* was determined by appropriate formulas (Acquaah, 2007). Narrow-sense heritability (h^2) which measures the proportion of additive variance in the overall variance was estimated as follows:

$$h^2 = 2\delta^2gca / (2\delta^2gca + \delta^2sca + \delta^2e),$$

While, broad-sense heritability (H^2) which measures the proportion of both additive and dominance variances in the overall variance, was estimated as follows:

$$H^2 = 2\delta^2gca + \delta^2sca / (2\delta^2gca + \delta^2sca + \delta^2e)$$

Where: δ^2gca = Variance component due to GCA;

δ^2sca = Variance component due to SCA;

δ^2e, environmental or error variance;

Means were separated using least significant differences ($LSD^{0.05}$).

CHAPTER FOUR: RESULTS

4.0 Screening and identification of two common isolates of *C. lindemuthianum* from major bean growing areas

The two isolates used in this study, one collected from Musa (Isolate M) in Kasama and another from Kawiko (Isolate K) in Mwinilunga districts of Zambia were identified as race 54 and race 311 respectively (Table 4).

Table 4: Binary designation system: Presenting how computation for identification of Race 54 and 311 was done

Differential cultivars	Host genes	[c] Binary number	Reaction of differential cultivars to;	
			[d] Isolate K	Isolate M
Michellite	Co-11	1	R	S (1)
Michigan.D.R. kidney	Co-1	2	S (2)	S (2)
Perry Marrow	$Co\text{-}1^3$	4	S (4)	S (4)
Cornell 49242	Co-2	8	R	R
Widusa	$Co\text{-}1^5$	16	S (16)	S (16)
Kaboon	$Co\text{-}1^2$	32	S (32)	S (32)
Mexico 222	Co-3	64	R	R
PI- 207-262	$Co\text{-}4^3$, Co-9	128	R	R
TO	Co-4	256	R	S(256)
TU	Co-5	512	R	R
AB136	Co-6, Co-8	1024	R	R
G2333	$Co\text{-}4^2$, $Co\text{-}5^2$,Co-7	2048	R	R
Race			54	311

[c] Binary number = the sum of binary numbers of bean cultivars with susceptible reactions gives the race denomination.
Key: R = Resistant (1 - 3); S = Intermediate susceptible (4 - 6); S = Susceptible (7-9) from the disease severity scale.
[d] Isolate K = Race 54, which is virulent on Kaboon (32), Widusa (16), Perry marrow (4) and Michigan, D.R Kidney (2) results from adding the binary numbers 32+16+4+2.
Source: (Pastor-Corrales, 1991)

Out of the 12 differential cultivars screened for *C. lindemuthianum* infection to race 54 and race 311 inoculated singly, differential cultivars Michellite, Cornell 49242, Mexico 222, PI-207-262, TO, TU, AB 136 and G2333 were resistant to *C. lindemuthianum* isolate M while Michigan D.R. Kidney, Perry marrow, Widusa and Kaboon were susceptible. On the other hand, differential cultivars Cornell, Mexico, PI-207-262, TU, AB 136 and G2333 were resistant to isolate K. while differential cultivars Michellite, Michigan D.R. Kidney, Perry marrow, Kaboon, Widusa and TO were susceptible to isolates K.

4.1 Effectiveness of *C. lindemuthianum* multiple race inoculations on genotypic response in common bean

There were significant differences ($P < 0.01$) for disease severity score among genotypes for treatment 2 only (Inoculation with single race 311) (Table 5).

Table 5: Mean squares for the F1s genotypic analysis for their reaction, to single and multiple inoculation of *Colletotrichum lindemuthianum* pathogen in common bean evaluated in 2014/15 cropping season at the University of Zambia

Source of Variation	d.f	Disease severity for *Colletotrichum lindemuthianum* races		
		Race 311	Race 54	[e]Multiple inoculated
Replication	3	18.58	0.82	5.92
Genotypes	48	4.94**	2.56	3.60
Error	144	2.65	2.28	3.29

**** Significantly different at P < 0.01 probability levels e = Multiple inoculation, involved inoculation of the same bean genotype with a mixture of inoculum for race 54 and race 311, each contributed 500ml**

The genotypic means of all the genotypes in the study with respect to inoculation with race 311 were computed (Table 6). Results showed that parent AB136 with a disease

severity mean score of 1.25 had the lowest genotypic mean performance and Perry marrow with a disease severity mean score of 4.0 had the highest. The parent Kabulangeti showed intermediate mean performance for disease severity with a score of 3.75. The F1's progeny crosses, [G2333 X Solwezi], [AB136 X G2333], [Kabulangeti X AB136], [Mbala X G2333] had the lowest mean disease severity score of 1 while [Solwezi X AB136] had the highest disease severity mean scores of 5.25. The rest showed intermediate susceptible genotypic mean performance to disease severity with scores ranging from 3.5 - 5. The mean genotypic disease severity reaction for inoculation with single race 54, race 311 and multiple inoculation with *C. lindemuthianum* were 1.76, 2.62 and 3.06 respectively. The use of student *t*-test for evaluating response among the races (Table 7), indicated that the disease severity mean score of 1.76 for treatment 1 (Inoculation with single race 54) was significantly ($P < 0.001$) lower than the mean severity score of treatment 3 (Inoculation with multiple race). Similarly the mean severity score of 2.62 for treatment 2 (Single race inoculation with race 311) was significantly ($P < 0.01$) lower than the mean severity score of 3.06 for treatment 3, inoculation with multiple race. The mean severity score of 2.62 for treatment 2 (Inoculation with single race 311) was significantly ($P < 0.001$) different to the mean severity score of 1.76 for treatment 1(Inoculation with single race 54). From the student *t*-test it is evident that the single race inoculation and multiple inoculations exhibited different degree of pathogenicity virulence with multiple race infection being the most virulent.

Table 6: Bean genotypic means for anthracnose severity measured from *Colletotrichum lindemuthianum* race 311 inoculations on the parents and their F1 progenies evaluated in 2015 at the University of Zambia

Genotypes	[f] Disease severity score
G2333	2.00
PI-207-262	3.00
AB136	1.25
Solwezi	2.75
Kabulangeti	3.75
Mbala	3.00
Perry Marrow	4.00
G2333 X PI-207-262	1.25
G2333 X AB136	2.50
G2333 X Solwezi	1.00
G2333 X Kabulangeti	3.75
G2333 X Mbala	2.00
G2333 X Perry Marrow	3.75
PI-207-262 X G2333	3.00
PI-207-262 X AB136	3.00
PI-207-262 X Solwezi	1.50
PI-207-262 X Kabulangeti	2.50
PI-207-262 X Mbala	1.50
PI-207-262 X Perry Marrow	2.25
AB136 X G2333	1.00
AB136 X PI-207-262	2.50
AB136 X Solwezi	4.00
AB136 X Kabulangeti	2.75
AB136 X Mbala	5.00
AB136 X Perry Marrow	4.25
Solwezi X G2333	3.50
Solwezi X PI-207-262	2.25
Solwezi X AB136	5.25
Solwezi X Kabulangeti	1.25
Solwezi X Mbala	3.00
Solwezi X Perry Marrow	3.50
Kabulangeti X G2333	3.00
Kabulangeti X PI-207-262	1.75
Kabulangeti X AB136	1.00
Kabulangeti X Solwezi	1.25
Kabulangeti X Mbala	2.25
Kabulangeti X Perry Marrow	2.50
Mbala X G2333	1.00
Mbala X PI-207-262	2.75

Mbala X AB136	2.50
Mbala X Solwezi	2.50
Mbala X Kabulangeti	4.25
Mbala X Pery Marrow	1.50
Perry Marrow X G2333	1.50
Perry Marrow X PI-207-262	1.50
Perry Marrow X AB136	3.25
Perry Marrow X Solwezi	4.75
Perry Marrow X Kabulangeti	2.50
Perry Marrow X Mbala	2.75
LSD (P < 0.05)	2.27

LSD- Fishers Protected Least Significant Difference test performed at P < 0.05,
f = Anthracnose disease severity rating scores on foliage (1 - 9) 1 - 3 resistant, 4 - 6 intermediate susceptible, 7 - 9 susceptible.
Source: Van Schoonhoven, A. and Pastor-Corrales, M.A (1987)

Table 7: Disease severity mean comparisons among single races (race 54 and race 311) and multiple race inoculations (combination of race 54 and race 311) evaluated in the 2014/15 cropping season.

MDSC Comparisons	Student t-test (P-Value)
Race 311(2.62 ^{x}) vs Race 54 (1.76 ^{y})	0.001
^{e} Multiple inoculation (3.06 ^{z}) vs Race 311(2.62 ^{x})	0.01
Race 54 (1.76 ^{y}) vs ^{e} Multiple inoculation (3.06 ^{z})	0.001

MDSC = Mean disease severity score; x = mean genotypic score of race 311; y = mean genotypic score of race 54; z = mean genotypic severity score for multiple inoculations.
e = Multiple infection, involved inoculation of the same common bean genotype with inoculum for both race 54 and race 311

4.2 The nature of inheritance to *Colletotrichum lindemuthianum* in common bean genotypes

With regards to inoculation with single race 311, the mean squares for GCA and SCA effects were not significant while reciprocal effects were significant (P < 0.01), (Table 8). The lowest significant reciprocal effect paired crosses had a magnitude of 1.13 and -1.13 for [G2333 X Perry Marrow] and [Perry Marrow X G2333] respectively.

Table 8: Mean squares for a 7 x 7 diallel analysis for the parents and their F1 progenies reaction to *Colletotrichum lindemuthianum* evaluated in 2014/15 season

Source of Variation	d. f	Disease severity for *Colletotrichum lindemuthianum* races		
		Race 311	Race 54	[e] Multiple infection
Replication	3	18.58	0.82	5.92
Genotype	48	4.94**	2.56	3.60
GCA	6	0.87	-	-
SCA	21	0.97	-	-
Reciprocal	21	1.56**	-	-
Error	144	0.66	2.28	3.29

**** Significantly different (P < 0.01) probability levels respectively; e = Multiple infection, involved inoculation of the same common bean genotype with inoculum for both race 54 and race 311**

The paired crosses for [Mbala X AB136] [AB136 X Mbala] and [Solwezi X G2333] [G2333 X Solwezi] had the highest significant reciprocal effects with magnitude of 1.25 and -1.25 (Table 9). Furthermore, crosses between [Solwezi X G2333], [G2333 X Perry Marrow], and [AB136 X Mbala] recorded significant positive reciprocal effects (Table 9). Heritability (h^2) estimates for *C. lindemuthianum*, race 311 was based on the diallel analysis (Table 10). The Baker's ratio for disease severity scores was 0.15 while the narrow sense and broad sense heritability estimates were 0.03 and 0.22 respectively. From the study it was clear that non additive effects were important for resistance to race 311 of *C. lindemuthianum* (Table 10).

Table 9: Estimate of the reciprocal effects for disease severity obtained from full 7 X 7 diallel crosses evaluated for the parents and F1 progenies reaction to *Colletotrichum lindemuthianum* race 311 in 2014/15 cropping season

Progeny cross	Reciprocal Effect
G2333 X PI-207-262	-0.875
PI-207-262 X G2333	0.875
G2333 X AB136	0.75
AB136 X G2333	-0.75
G2333 X Solwezi	-1.25*
Solwezi X G2333	1.25*
G2333 X Kabulangeti	0.375
Kabulangeti X G2333	-0.375
G2333 X Mbala	0.5
Mbala X G2333	-0.5
G2333 X Perry Marrow	1.125*
Perry Marrow X G2333	-1.125*
PI-207-262 X AB136	0.25
AB136 X PI-207-262	-0.25
PI-207-262 X Solwezi	-0.375
Solwezi X PI-207-262	0.375
PI-207-262 X Kabulangeti	0.375
Kabulangeti X PI-207-262	-0.375
PI-207-262 X Mbala	-0.625
Mbala X PI-207-262	0.625
PI-207-262 X Perry Marrow	0.375
Perry Marrow X PI-207-262	-0.375
AB136 X Solwezi	-0.625
Solwezi X AB136	0.625
AB136 X Kabulangeti	0.875
Kabulangeti X AB136	-0.875
AB136 X Mbala	1.25*
Mbala X AB136	-1.25*
AB136 X Perry Marrow	0.5
Perry Marrow X AB136	-0.5
Solwezi X Kabulangeti	0
Kabulangeti X Solwezi	0
Solwezi X Mbala	0.25
Mbala X Solwezi	-0.25
Solwezi X Perry Marrow	-0.625
Perry Marrow X Solwezi	0.625
Kabulangeti X Mbala	-1
Mbala X Kabulangeti	1
Kabulangeti X Perry Marrow	0

Perry Marrow X Kabulangeti	0
Mbala X Perry Marrow	-0.625
Perry Marrow X Mbala	0.625
Standard error	0.57

*** Significant different (P < 0.05)**

Table 10: Estimate of the genetic parameters and their ratios for parents and their F1s in a 7 x 7 full diallel observed for the trait of resistance to *Colletetrichum lindemuthianum,* race 311 in beans evaluated in 2014/15 cropping season

Parameter	Value
h^2ns	0.03
H^2 bs	0.22
Ratio 2δ2gca / (2δ2gca + δ2sca)	0.15

h^2, Narrow sense heritability; H^2, Broad sense heritability; ratio $2\delta^2$gca / ($2\delta^2$gca + δ^2sca), Baker's ratio; δ^2gca, variances for general combining ability; δ^2sca, variance for specific combining ability

CHAPTER FIVE: DISCUSSION

5.0 DISCUSSION

The screening and identification of two *C. lindemuthianum* isolates prevailing in major bean growing area of Zambia was done. The identification of each of the isolates was confirmed by phenotypic reactions displayed by the 12 international differential cultivar set. The two isolates from Musa in Kasama (Isolate M) and Kawiko (Isolate K) in Mwinilunga districts of Zambia were identified as race 54 and 311 respectively (Table 4). The identified race, 54 is close to race 53 which was earlier characterized by the same method (Use of binary number designation system) (Zulu, 2005). Being that the isolates where obtained from the same area in Musa of Kasama district in Zambia, the slight race number difference could probably be due to the disease scoring art which is subjective. On the other hand, race 311 was not any close to any of the race formerly designated (Zulu, 2005). This could mean existence of a new race of *C. lindemuthianum* which could be as a result of introduction through movement of diseased seed from one part of the region to another (Nkalubo, 2006) or mutations which may alter the genetical make up of an already existing race hence change in virulence (Agrios, 2005). There were significant difference ($P < 0.01$) for disease severity mean score among the genotypes (Table 5). The genotypic responses considered were only for race 311 whose treatment exhibited significant differences among genotypes (Table 6). Lack of significance in genotypic response to race 54 treatment 1 and multiple races inoculation treatment 3 was probably be due to difference in virulence among *C. lindemuthianum* races used in the study. A number of studies have shown that same genotypes have different reactions to races due

to differences in race virulence (Agrios, 2005; Gonçalves-Vidigaletal *et al*., 2007; Mwesigwa, 2009). This was evident from the different virulence spectrum exhibited by the two isolates (K and M) on the differential cultivars set (Table 4), (Niks and Lindhout, 2006). The difference in the two isolates indicate existence of different *C. lindemuthianum* races in Zambia. There is evidence of different *C. lindemuthianum* races existing in Zambia and around the world (Zulu, 2005; Niks and Lindhout, 2006; Nkalubo *et al*., 2009; Kachapulula *et al*., 2010; Gonçalves-Vidigaletal *et al*., 2007; Miklas *et al*., 2006). There were significant differences among genotypes with regards to disease severity caused by *C. lindemuthianum* race 311. Genotypes G2333, PI206262, AB136 exhibited high levels of resistance to race 311 which correlated with other studies where they were screened with race 521 and 25 (Alzate- Marin *et al*., 1999 a; Gonçalves-Vidigal *et al*., 2007; Kachapulula *et al*., 2014).

On the other hand Solwezi, previously reported susceptible through the binary designation system by Zulu (2005) was found resistant in this study, with a mean score rating of 2.75 (Table 6). This implies that cultivars may respond differently to *C. lindemuthianum* infection depending on the conditions understudy. There were differences among treatments with regards to the nature of reaction of the genotypes to the inoculum infection (Table 7). From these results it was deduced that multiple race infection had higher disease severity expression than those of single race infection. Other researchers found out that when one or multiple physiological race(s) of *Phytophthora capsici* in *Caspicum annuum* was inoculated on a single plant, the effect and extent of disease infection did not differ (Monroy-Barbosa and Bosland, 2010). While other researchers reported synergistic effects resulting in increased disease symptoms (Aliyu *et al*., 2012). Results in this study

are similar to those found by Aliyu *et al*., 2012, who established that multiple infection of Blackeye Cowpea Mosaic Virus and Cowpea Yellow Mosaic virus in cowpea were more virulent than those of single infection. Therefore due to these synergistic interactions in common bean, multiple race infection should be used to screen for resistant to *C. lindemuthianum*. However where a particular race is prevalent in a locality, breeding for single race resistance can be taken as a priority.

Further analysis, with regards to inoculation with single race 311 treatment 2, revealed that reciprocal effects were significant ($P < 0.05$) while both GCA and SCA were not. Similarly, significant reciprocal effects for days to flowering in common beans were recorded (Arunga *et al*., 2010). There were significant negative reciprocal effects from crosses between [Perrymarrow X G2333] and [Mbala X AB136] for this trait (Table 9). The significant reciprocal effects suggest that, the genes controlling resistance were cytoplasmic and had influence on the inheritance of resistance to *C. lindemuthianum* (Singh, 2012). Reciprocal effects are important for consideration of qualitative traits because they can be used to specify which of the parents should be used as male or female in specific crossings (Cruz and Regazzi, 1997; Senbetay *et al*., 2015). This implies that seeds for reciprocals should not be mixed with the seed for the direct crosses since there is cytoplasmic influence on the trait expression.

The heritability (h^2) estimates varied with the type of the estimate as indicated by broad and narrow sense heritability (Table 10). Both broad and narrow sense heritability were

estimated and found to be low with values of 0.22 and 0.03 respectively. Contrary to this study, previous workers found high broad sense heritability for resistance to *C. lindemuthianum* (Senbetay *et al*., 2015). The low broad sense heritability estimates observed in this study could be attributed to lower variance component of SCA effect as compared to environmental effect computed (Table 8). The low heritability value could have been due to the cytoplasmic inheritance of the *C. lidemuthianum* resistance trait to single race 311, treatment 2 and probably due to the parental germplasm which could be from the same gene pool. Furthermore, Low narrow sense heritability from diallel analysis (0.03) of the trait suggests that there is little or no response to selection for resistant trait to *C. lindemuthianum,* race 311. Baker's ratio of 0.15 meant that non-additive gene action conditioned the resistance to this trait. These results are similar to the findings of Goncalves-vidigal *et al*., (2007), who reported non-additive gene action being more important for race 89 in resistance inheritance study of common bean.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.0 Conclusion

The two isolates from Musa in Kasama (Isolate M) and Kawiko in Mwinilunga (Isolate K) were identified as race 54 and 311, respectively. With regards to single race and multiple race inoculation, inoculation with multiple race was more virulent than single race inoculation. Therefore, multiple race inoculation should be employed in the common bean breeding programs so as to come up with resistant genotypes which are stable in several environments. There were no significant differences among the genotypes inoculated with single race 54 and multiple race a combination of race 54 and 311. However, there were significant differences ($P < 0.01$) with regards to inoculation with single race 311 and Baker's ratio of 0.15 indicated that non-additive gene action conditioned the resistance to *C. lindemuthianum* trait, implying that there was little or no response to selection as supported by the low narrow sense heritability. In this scenario, with regards for resistance breeding to *C. lindemuthianum,* race 311, hybrid production is the best breeding scheme to employ as compared to pedigree or single seed decent method.

6.1 Recommendations

The genotypic response to the pathogen caused by *C. lindemuthianum* was not significant with single and multiple race inoculations treatments 1 (Inoculation with race 54) and 3 (Inoculation with a combination of race 54 and 311) (Table 5) respectively. In this regard, further works should be done with other germplasm to gain more insight on the genotypic responses. Other new races can also be evaluated.

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Appendices

Appendix 1: Scoring scale (1-9) used in evaluating the reaction of bean genotypes to *Colletotrichum lindemuthianum* (anthracnose pathogen)

Rating	Category	Description	Comments
1 2 3	Resistant	No visible symptoms or very light (5 – 10%)	Germplasm useful as parent or commercial variety
4 5 6	Intermediate	Visible and conspicuous symptoms resulting only in limited economic damage (10 – 60%)	Germplasm can be used as commercial variety or source of resistance to diseases
7 8 9	Susceptible	Severe to very severe symptoms causing consideration yield losses or plant death (60 – 100%)	Germplasm in most cases not useful as parents or commercial varieties

Source: Van Schoohoven, A. and Pastor – Corrales, M.A (1987)