GENETIC DISSECTION OF RESISTANCE TO ANTHRACNOSE IN YELLOW BEAN COLLECTION OF COMMON BEANS

(*Phaseolus vulgaris* L.)

By

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A thesis submitted to the University of Zambia in partial fulfilment of the requirements for

The degree of Master of Science in

PLANT BREEDING AND SEED SYSTEMS

SCHOOL OF AGRICULTURAL SCIENCES

DEPARTMENT OF PLANT SCIENCE

THE UNIVERSITY OF ZAMBIA

LUSAKA.

2023

DECLARATION

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ABSTRACT

Anthracnose caused by *Colletotrichum lindemuthianum* is a major disease of common bean (*Phaseolus vulgaris*) worldwide. Yellow beans are a major market class of common bean especially in eastern and southern Africa. The objectives of this study were i) evaluate the yellow bean collection for resistance to eight races of *C. lindemuthianum*, and ii) conduct genome-wide association analysis to identify genomic regions and candidate genes associated with resistance to eight races of *C. lindemuthianum*. The Yellow Bean Collection comprised of 255 diverse yellow bean genotypes was evaluated for resistance to races 5, 19, 39, 51, 81, 183, 1050 and 1105 of *C. lindemuthianum*. The Yellow Bean Collection was genotyped with 72, 866 SNPs using Genotyping by Sequencing and genome-wide association analysis was conducted using Mixed Linear Model in TASSEL. Several genotypes with superior levels of resistance to the eight races used in the current study were identified. The yellow bean genotype YBC278 was the only one among 255 genotypes that was highly resistant to all eight races. Resistance in the Yellow Bean Collection to the eight races used in the current study was controlled by major-effect loci on chromosomes Pv01, Pv03, Pv04, Pv05 and Pv07. The genomic region on Pv01, which overlapped with the Andean locus *Co-1* provided resistance to races 81, 1050 and 1105. Significant SNPs for resistance to race 39 were identified on Pv02. The genomic region on Pv04, which overlaps with known major-effect loci *Co-3*, *Co-15*, *Co-16*, *Co-y* and *Co-z*, provided resistance to races 5, 19, 51 and 183. Novel genomic regions for resistance to race 39 were identified on Pv05 and Pv07. Plant resistance genes (R genes) with NB-ARC and LRR domains, which occurred in clusters, were identified as positional candidate genes for genomic regions on Pv02 and Pv04.

DEDICATION

This thesis is dedicated to my family and my fellow scientists especially all plant breeders in the never-ending pursuit of variety development and improvement.

ACKNOWLEDGEMENTS

My deepest thanks go to my graduate advisor Dr. Kelvin Kamfwa for the invaluable advice and mentorship throughout my programme. I am grateful to my second supervisor Dr. Paul Kachapulula for critically evaluating this thesis. I would also like to extend my sincerest gratitude to Mr. Chikoti Mukuma whose knowledge and understanding in pathology was of great importance to this study. My thanks also go to Ms Swivia Hamabwe and the members of the University of Zambia Legume Breeding and Genetics team for their friendship and support throughout the course of this study. Furthermore, I am most especially grateful to the Kirkhouse Trust for sponsoring my entire study and research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

˚C = Degree Celsius

ANOVA = Analysis of Variance

ANTH = Anthracnose

ARC =APAF-1 (apoptotic protease-activating factor-1), R proteins and CED-4 (*Caenorhabditis elegans* death-4 protein)

DNA = Deoxyribonucleic Acid

eQTLs = expression quantitative trait loci

GBS = Genotyping-by-sequencing

GH = greenhouse

GWAS = genome-wide association studies

ha = hectare

hr = hour

LOD = Log of odds

LRR= leucine rich repeat *domain*(s)

m = Meter

MAS=Marker assisted selection

Masl=meters above sea level

mm = Millimeter

NB-nucleotide binding *domain*

NIL = Near Isogenic Line

PacBio = Pacific biosciences

PCR = Polymerase Chain Reaction

PDA = Potato Dextrose Agar

PK = protein kinase

QTL= Quantitative trait loci

R = Correlation coefficient

R2 = Coefficient of multiple determination

rDNA = recombinant Deoxyribonucleic Acid

RNA = ribonucleic acid

RNA-Seq = RNA sequencing

R-Genes=Resistance *genes*

RIL= Recombinant inbred line

SNP= Single nucleotide polymorphism

TIR = Toll-interleukin-1 receptor

WES = Whole-exome sequencing

CHAPTER ONE

1. INTRODUCTION

Common bean (*Phaseolus vulgaris*) is a source of food and nutritional security for millions of households in Latin America and Africa (Uebersax, et al., 2022). Beans play an important role in combating malnutrition because of their richness in protein, carbohydrates, and minerals (especially iron and zinc) (Haas, et al., 2016).

Beans have a rich diversity in traits such as seed color, seed size and seed shape (Singh, 2001). These traits have been used to classify beans in different market classes. One of the market classes is the yellow bean, which is comprised of both Andean and Middle American genotypes. Within the yellow bean market class, there are several seed type classifications based on the shade of yellow color. These include the Manteca, Canary (Canario), Mayocoba, and Njano classes (Sadohara, et al., 2021; Sadohara, et al., 2022). Various yellow bean market classes are commercially important in Latin America, the Caribbean, and Africa (Buruchara, et al., 2011; Voysest, 2012). In Africa, yellow beans are popular in Angola, Kenya, Tanzania, and Zambia (Buruchara, et al., 2011). In Zambia, the Manteca yellow landrace known as Lusaka is preferred over other bean market classes and fetches higher market prices (Sichilima, et al., 2016). In Tanzania, the Njano class is the most popular yellow seed type, and it is estimated that about 32% of total bean production there is for yellow beans, and mainly the Njano type (Sperling, et al., 2021). Yellow beans have become increasingly important bean corridors of both East and Southern Africa (Farrow & Muthoni-Andriatsitohaina, 2020).

Yellow beans are preferred to other market classes in several end-use traits. Manteca yellow beans often have shorter cooking time and higher iron bioavailability than other market classes (Wiesinger, et al., 2018; Hart, et al., 2019; Cichy, et al., 2015; Mishili, et al., 2011). Despite the consumer preference and nutritional attributes of some yellow bean market classes they have historically received less breeding efforts compared to other popular bean market classes.

Average yields for yellow bean are often lower than other bean classes. Several abiotic and biotic stresses contribute to low yields (Parker et al., 2023). In some countries, continued use

of yellow bean landraces with poor genetics and yield potential contribute to low yields. Diseases are a major contributing factor to the low yields of yellow beans. Anthracnose (caused by *Colletotrichum lindemuthianum*) is a major disease that affects yellow beans. Depending on varietal susceptibility and weather conditions (high humidity and moderate temperatures favors its spread), anthracnose can cause yield losses of up to 100% (Pastor-corrales & Tu, 1989). Anthracnose is a seed borne disease and planting infected seed is a major mode of its transmission (Ferreira, et al., 2013; Schwartz & Pastor-Coralles, 2005). This transmission mode of anthracnose makes its control/or management challenging because of the widespread use of on-farm saved seed in Africa and Latin America. Though anthracnose can effectively be controlled using fungicides, smallholder farmers who are the majority of bean growers cannot afford fungicides. Additionally, there are health and safety concerns involved in using fungicides. Development and use of resistant varieties is the most cost-effective and ecologically-sound management strategy for anthracnose.

Anthracnose resistance exists within the primary gene pool of common bean and several sources of resistance have been identified and used in breeding. Major-effect genes mainly control resistance to anthracnose in common bean, though a few minor QTL have also been identified. To date, 19 dominant major-effect anthracnose resistance genes have been identified on seven chromosomes. These genes are classified as either Andean or Middle American depending on whether they were identified in an Andean or Middle American genotype. Andean genes include *Co-1, Co-12, Co-13, Co-14, Co-15, Co-x, Co-w, Co-y,* and *Co-z* while Middle American genes include *Co-2, Co-3, Co-4, Co-5, Co-6, Co-11, Co-16, Co-17, Co-u,* and *Co-v* (Geffroy, et al., 1999; Gonçalves-Vidigal, et al., 2008; Gonçalves-Vidigal, et al., 2009; Gonçalves-Vidigal, et al., 2012). The genes *Co-1, Co-3, Co-4* and *Co-5* are multi-allelic. Some of these major genes are located in genomic regions that contain clusters of disease resistance genes that control resistance to multiple diseases (Oblessuc, et al., 2015; Campa, et al., 2017). For example, *Co-1* is located in the genomic that also contain *Pgh-1,* which is a major gene for resistance to angular leaf spot. In addition to the major genes, QTL with smaller effect on anthracnose resistance have been reported (Zuiderveen, et al., 2016).

The major challenge in breeding for durable anthracnose resistance is the broad genetic diversity of *Colletotrichum lindemuthianum*. To date, over 147 races of *C. lindemuthianum* have been reported worldwide (Padder, et al., 2017). *C. lindemuthianum* races have co-evolved with the two gene pools of common beans and are classified as either Andean of Middle American (Geffroy, et al., 1999). Andean races are virulent mostly on Andean beans while Middle American races are virulent on both Andean and Middle American beans. Because of the diversity of *C. lindemuthianum*, resistance often breaks down; therefore, there is need for continuous identification of new sources of resistance and understanding the genetic architecture of that resistance. Resistance to anthracnose of the major genes follows the gene-for-gene concept. Therefore, some genes are effective against some races, but less effective against others. It is therefore important to identify genes that are effective against specific races so as to develop varieties with effective and durable resistance for a given geographic location. Diversity panels are important genetic resources that can serve as sources of resistance and also for understanding the genetic architecture of traits such as *C. lindemuthianum*. The Yellow Bean Collection (YBC) is one of the diversity panels that have been assembled in common bean (Sadohara, et al., 2022). This panel is comprised of landraces, varieties, and elite lines of yellow beans, which are variable for several traits including anthracnose resistance. The YBC, therefore, is an excellent genetic resource for understanding the extent of anthracnose resistance and its genetic architecture in the yellow bean market class.

1.1. Statement of the problem

The major challenge in breeding for durable ANTH resistance is the broad genetic diversity of *Colletotrichum lindemuthianum*. To date, over 147 races of *C. lindemuthianum* have been reported worldwide (Padder, et al., 2017). *C. lindemuthianum* races have co-evolved with the two gene pools of common beans and are classified as either Andean of Middle American (Geffroy, et al., 1999). The genetic variability that occurs in *Colletotrichum lindemuthianum* presents a major challenge in breeding for durable resistance to anthracnose in common bean. Therefore, some genes are effective against some races, but less effective against others. It is

therefore important to identify genes that are effective against specific races so as to develop varieties with effective and durable resistance for a given geographic location. Continuous identification of new sources of resistance and understanding the genetics underlying this resistance is necessary. Although resistance to anthracnose exists in the primary gene pool of *Phaseolus vulgaris*, breeding efforts for resistance in the yellow beans are faced with the challenge of poor recovery of the yellow color and seed size when crossed with other colors or with Middle-American genotypes. This therefore necessitates the identification of sources of resistance within the yellow bean market classes and understanding the genetic architecture of that resistance in order to enhance the efforts of breeding programmes involved in the development of yellow bean cultivars.

1.2. Justification

Diversity panels are important genetic resources that can serve as sources of resistance and also for understanding the genetic architecture of traits such as *C. lindemuthianum*. The Yellow Bean Collection (YBC) is one of the diversity panels that have been assembled in common bean (Sadohara, et al., 2022). This panel is comprised of landraces, varieties and elite lines of yellow beans, which are variable for several traits including anthracnose resistance. The YBC, therefore, is an excellent genetic resource for understanding the extent of ANTH resistance and its genetic architecture in the yellow bean market class.

Quantitative trait loci (QTL) mapping using recombinant inbred lines (RILs) and genome-wide association studies (GWAS) using diversity panels are two commonly used genetic approaches to understand the genetic architecture of traits. Both approaches have previously been used to identify genomic regions for resistance to variable races of *Colletotrichum lindemuthianum* in common bean. One of the advantages of GWAS over QTL mapping is the higher mapping resolution that it offers, because of the smaller linkage disequilibrium blocks. The enhanced mapping resolution of GWAS offers opportunity to identify candidate genes for resistance to anthracnose. Additionally, GWAS using diversity panels provide the opportunity to identify minor effect QTL because they are relatively larger in size than QTL mapping populations using RILs. Minor-effect QTL are important sources of horizontal

resistance, and if put in the same genetic background with major effect genes, which offer vertical resistance, they can confer durable resistance to multiple races of *Colletotrichum lindemuthianum*. Thus, conducting Genome-Wide Association Studies (GWAS) with diversity panels can offer a powerful solution by identifying resistance genes, assessing genetic variability, and improving our understanding of the yellow color trait. These insights can ultimately lead to the development of more desirable and marketable yellow bean varieties with durable resistance to anthracnose benefiting both farmers and consumers in Zambia.

1.3. General objective

To identify sources of anthracnose resistance and understand the genetic basis of resistance to anthracnose in the Yellow Bean Collection of common beans.

1.4. Specific objectives

i. To evaluate the yellow bean collection for resistance to races 5, 19, 39, 51, 81, 183, 1050 and 1105 of *Colletotrichum lindemuthianum*
ii. To identify genomic regions and positional candidate genes associated with resistance to eight races of *Colletotrichum lindemuthianum* using genome-wide association analysis.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Introduction

Common bean (*Phaseolus vulgaris* L.) is grown and consumed globally largely due to its high nutritional profile. It is a nutrient rich grain legume important in combating malnutrition in developing countries in Africa and Latin America. It serves as an inexpensive source of protein, dietary fiber and essential micronutrients such as zinc and iron. Additionally, it serves as an income generator for the farmers and other parties involved in the value chain. Globally, the land dedicated to common bean production is approximately 33 million hectares out of which 7.9 million hectares is in Africa A larger proportion of the production in Africa is by small holder farmers (mainly women) with an estimated 5 million hectares grown. Although there has been a notable increase in bean production in some countries in Africa mainly due to an increase in the area planted, average yields still remain low as compared to countries in Latin and North America (Bucheyeki & Mmbaga, 2013) estimated at 850 kg per hectare These low yields could be attributed to various constraints such as the use of local landraces that have poor productivity. Additionally, biotic and abiotic factors such as diseases and drought stress further contribute to the low production (Beebe, et al., 2011; Wortmann, 1998). Beans do well in cool highlands typical of Eastern, Central and Southern Africa (Katungi, et al., 2009). In Zambia, common bean is mainly grown in the Northern, Muchinga, Luapula and Northwestern provinces due to their favorable conditions, though it can be grown in most parts of the country due to its wide adaptation (Wortmann, 1998).

2.2. Yellow beans

Common beans exhibit a wide variety of seed characteristics including seed size, shape, seed coat color and patterns which are used to group them into various specific market classes (González, et al., 2006). One of the important characteristics is seed coat color and of particular interest is the yellow beans. Yellow beans can be found in both the Middle-American and Andean gene pools of common bean (Voysest, 2012) and occur in various

shades and sizes (Sadohara, et al., 2021). Some yellow beans market classes of importance include the Manteca, Canario, Mayocoba and Njano (Sadohara, et al., 2021; Sones, 2015). They make up important market classes of beans in Latin America, the Caribbean and Africa. (Kilomo Trust, 2012; Voysest, 2012; Wortmann, 1998). Their popularity can be attributed to their multiple end-use and nutritional benefits to the consumers. The Manteca type for example is reported to have good taste and texture (Hosfield, et al., 1998). Additionally, they have also been reported to be generally fast cooking and to have high bio-available iron (Cichy, et al., 2015; Wiesinger, et al., 2018). This is attributed to unique polyphenolic profiles that have high levels of iron absorption promoters and low inhibitors (Hart, et al., 2019).

In Africa, yellow beans are especially popular in Angola, Tanzania, Kenya, and Zambia (Buruchara, et al., 2011) and vary in shade, shape and size giving rise to strong regional preferences (Wortmann, 1998; Sichilima, et al., 2016; Tumeo, et al., 2017). In Tanzania for example, the Njano type is preferred most by consumers (Sones, 2015) while the popular types in Angola range from cream to yellow (Wortmann, 1998). On the other hand, in Zambia, a local landrace of the Manteca type called Lusaka is highly demanded and often fetches higher market prices than other market classes of beans (Sichilima, et al., 2016). A survey was carried out by the Pan-Africa Bean Research Alliance (PABRA) and the Alliance of Bioversity International and International Centre for Tropical Agriculture (ABC) on yellow bean trading in Eastern and Southern Africa region including Tanzania, Burundi, DRC, Kenya, Rwanda, Uganda, and Zambia. The results of the study revealed that there was an increase in the popularity of yellow beans in the region and that consumer preferences were associated on a large part with palatability and faster cooking times (Birachi, et al., 2020; Sperling, et al., 2021).

2.3. Constraints of production of common bean

There are several factors that constrain the production of common bean including poor agronomic and management practices, as well as biotic and abiotic factors (Katungi, et al., 2009; Farrow & Muthoni-Andriatsitohaina, 2020). Among the abiotic factors, poor soil fertility poses a major challenge in the tropics where it is widespread. Most of the bean

growing regions in Africa and Latin America are faced with low fertility soils. Fertilizers can be used to ameliorate the effects of poor soil fertility. However, the resource poor small-scale farmers who are the majority producers of the crop cannot afford fertilizers. Another major abiotic stress is drought which has a wide global distribution. It is estimated that about 60% of the bean growing area is prone to drought worldwide. The frequency, severity and duration of drought stress are even further exacerbated by climate change. Aluminium toxicity also limits bean production especially in regions that receive excessive rainfall. It affects about 52% of bean growing areas in Eastern, Central and Sothern Africa and can account for 25-80% of crop yield losses in acidic soils.

Diseases are another major constraint to bean production after abiotic factors in Africa and can cause yield losses of up to 80-100% (Wortmann, 1998). The major diseases of beans of importance include anthracnose, angular leaf spot, rust, and root rots which are caused by fungi while bacterial diseases include common bacterial blight and halo blight. Other diseases of importance in beans include the bean common mosaic virus and bean golden mosaic virus. These diseases can be very devastating in farmers' fields and can lead to crop yield losses of up to 100% depending on weather conditions, severity of the disease and susceptibility of a cultivar (Trabanco, et al., 2015).

In addition to abiotic and agronomic challenges, pests and diseases contribute to low bean productivity and production. The main pests that attack beans include the bean stem maggot and the bean weevil. Bean stem maggot attacks beans at seedling stage and if not controlled can result in poor crop stand. Weevils are a major post-harvest pest responsible for significant production losses. Additionally, weevils reduce the quality of seed, which may results in its poor germination and poor quality for human consumption.

2.4. Anthracnose

Anthracnose caused by the fungal pathogen *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams. Scrib. is one of the most of economically important diseases in common bean production (Melotto, et al., 2000). It has a worldwide distribution and is one of the more severe among the many bean diseases (Pastor-corrales & Tu, 1989). It is more prevalent in cooler and humid climates and in regions with higher elevation. Temperatures of about 18-22^{o}C and relative humidity (RH) of at least 92% favour its occurrence (Guzmán, et al., 1995). Anthracnose is devastating in farmers' fields and can cause yield losses of up to 100% in susceptible cultivars and under favourable climatic conditions (Pastor-corrales & Tu, 1989).

2.5. Transmission and symptoms of anthracnose

Colletotrichum lindemuthianum is a hemi-biotrophic pathogen that relies on the bean plant for nutrients before killing it (Ferreira, et al., 2013). Anthracnose is a seed borne disease which poses a serious challenge for small scale farmers from Africa and Latin America who save seed from previous seasons. The major modes of transmission are through planting infected seed. It is also transmitted through diseased plant residues in which the fungal spores can over winter from the previous farming seasons. Wind, splashing water (like rain), farm tools and machinery, and animals facilitate the spread of the spores. The disease can affect all aerial parts of the plant at all growth stages (Padder, et al., 2017), with the most distinctive symptoms being lesions on leaf veins and deep sunken lesions on the pods. Other symptoms include lesions on the stems, hypocotyls, and leaf veins of young plants which lead to wilting and flagging of chlorotic leaves (Ferreira, et al., 2013). Further progression of the disease results in complete girdling and death of the plant. On the pods, the symptoms occur as rust-colored lesions that later develop into sunken cankers with dark ring borders. Severe infection on immature pods results in abortion and falling off of pods, while pods that survive produce infected seed with dark blemishes that make it undesirable to consumers (Pastor-corrales & Tu, 1989).

2.6. Control of anthracnose in common bean

There are a number of approaches that can be used to prevent and control anthracnose in common beans, and they have varying degrees of effectiveness. These include crop rotations, seed, and foliar treatment with fungicides, using uninfected seed and planting resistant cultivars. Crop rotations are an important component in reducing the continued transmission through plant residues from previous seasons. Treatment of the seed and plant foliage using fungicides provides effective control in the early stages of the disease. However, these chemicals pose a financial challenge to most small-scale farmers in developing countries. They also bring about health concerns and are damaging to the environment. Transmission through infected seed is very effective (Kelly & Vallejo, 2004), therefore planting clean seed is essential in the prevention of anthracnose. In Europe and America, effective clean seed programs have resulted in diminished cases (Pastor-corrales & Tu, 1989). However this is not a feasible option for many countries in Africa and Latin America where the disease is endemic (Ferreira, et al., 2013) and infected seed is still a mode of transmission in subsistence farming systems where seed is recycled from previous years (Melotto, et al., 2000). Of all the approaches, development, and use of cultivars with resistance to anthracnose shows the most promise. It minimizes the production costs and reduces the damage to the environment.

2.7. Co-evolution of *Colletotrichum lindemuthianum* and *Phaseolus vulgaris*

Colletotrichum lindemuthianum has co-evolved with Phaseolus vulgaris and falls into two distinct groups corresponding to the Middle-American and Andean gene pools of common bean. This has played a significant role in shaping the diversity of resistance genes and the virulence of the pathogen. The pathogen has evolved to overcome plant defenses, leading to the emergence of new races. The Middle-American races are virulent on both Middle-American and Andean bean cultivars while the Andean races are more virulent on Andean bean cultivars (Pastor-corrales & Tu, 1989; Balardin, et al., 1997). Andean bean cultivars provide effective resistance to Middle-American races (Pastor-Corrales 1996). This co-evolutionary arms race has implications for resistance management and breeding strategies.

2.8. Pathogen variability

Colletotrichum lindemuthianum exhibits high genetic variability with over 147 races reported worldwide (Padder, et al., 2017). This makes it especially challenging in breeding for effective and durable resistance. Some races are more widely dispersed across multiple countries while others are found only in specific countries (Kelly et al 1994). For consistency in naming different races, a standardized system using a set of 12 differential cultivars and their corresponding binary codes was developed for race designation. Within the system, each of the differential cultivars is assigned a binary number and the sum of the binary values of the susceptible cultivars determines the identity of the specific races of *C. lindemuthianum*. The binary naming system however has some limitations in that there is still variability among isolates of the same race (Rodriguez-Suarez et al 2005). An intra race molecular variability between isolates of race 65 has been reported (Thoazella et al 2004). In addition, race 0, which does not infect any of the 12 differential cultivars, infects other cultivars and has been reported in Mexico, France and India (Sharma, et al., 1999; Sharma, et al., 2007). Nonetheless, the binary naming system using the 12 differential cultivars is invaluable as it allows for consistent comparison of pathogenic variation and race distributions studies among research groups across several countries which would result in better strategies for resistance breeding (Padder, et al., 2017)

2.9. Host resistance

It has been reported that resistance to anthracnose in common bean follows a gene-for-gene theory in which a resistance gene in the plant corresponds with an avirulence gene in the pathogen to mediate an incompatible reaction (Flor, 1947). Resistance is triggered by a specific gene product of the plant in recognition of avirulent gene products produced by the pathogen. Specificity between *C. lindemuthianum* races and common bean cultivars is well known (Tu, 1982), with the incompatible reactions often being characterized by a

hypersensitive reaction. Hypersensitive reaction resistance is often characterized by localized cell death quickly after fungal penetration in order to prevent further spread of the infection. Plant resistance genes (R genes) confer resistance through their involvement in detecting specific effectors from the pathogen as well as playing a role in activating plant immune response to prevent further infection of the disease. Currently, over 70 R genes have been isolated from various plants (Liu, et al., 2007). The majority of the R proteins contain conserved structural motifs such as leucine-rich repeat (LRR), nucleotide-binding site (NBS), protein kinase domain (PK), Toll-interleukin-1 receptor domain (TIR) and leucine zipper (LZ) structure or other coiled-coil (CC) structures. These domains are involved in R protein interaction with pathogen effectors and activating signal transduction pathways involved in plant immunity (Liu, et al., 2007).

2.10. Genetics of anthracnose resistance in common bean

In general, resistance to anthracnose in common bean follows a qualitative mode of inheritance exhibiting a clear-cut distinction between resistant and susceptible reactions (Ferreira, et al., 2013). This resistance is controlled largely by major genes although some minor effect quantitative trait loci (QTL) have been reported to offer additional resistance. Previous studies have identified 19 dominant major-effect anthracnose resistance genes on seven chromosomes. These genes fall either in the Andean or Middle American categories depending on whether they were identified in an Andean or Middle American genotype. Andean genes include Co-1, Co-12, Co-13, Co-14, Co-15, Co-x, Co-w, Co-y, and Co-z while Middle American genes include Co-2, Co-3, Co-4, Co-5, Co-6, Co-11, Co-16, Co-17, Co-u, and Co-v (Geffroy, et al., 1999; Gonçalves-Vidigal & Kelly, 2006; Gonçalves-Vidigal, et al., 2008; Gonçalves-Vidigal, et al., 2009; Gonçalves-Vidigal, et al., 2012; Lacanallo & Gonçalves-Vidigal, 2015; Sousa & Gonçalves, 2015; de Lima Castro, et al., 2017; Gilio, et al., 2017). The genes Co-1, Co-3, Co-4 and Co-5 have multiple allele series. These major genes provide effective resistance to specific races of *C. lindemuthianum* (Mungalu, et al., 2020), while some like the *Co-4* confer broad-spectrum resistance to a wide-range of races and has been widely deployed in breeding programs (Pastor Corrales, et al., 1994). Despite

their effectiveness in providing resistance, no single gene confers resistance to all races of *C. lindemuthianum* however a combination of major genes can confer broad resistance to diverse races. The differential cultivar G2333 for example, the genotype G2333 is resistant to a wide range of races largely because it possesses three resistance genes namely the *Co-4*^{2}, *Co-5*^{2} and *Co-7* genes (Pastor Corrales, et al., 1994; Vallejo & Kelly, 2009; Young, et al., 1998). However, even with such high levels of resistance to multiple races G2333, was found to be susceptible to race 3481 (Mahuku, et al., 2002). Because of the high genetic variability of *C. lindemuthianum*, there is a need to be searching continuously for new sources of resistance.

2.11. Breeding for anthracnose resistance in common bean

The development and use common bean cultivars with resistance to anthracnose is one of the most effective ways of controlling the disease. In order to develop such varieties, identification of sources of resistance as well as understanding the underlying genetic basis of anthracnose resistance is critical. Breeding for race-specific requires identification of genes or loci providing effective resistance to particular races (Strange and Scott, 2005), which can then be deployed in breeding programmes. Historically, the majority of resistance genes identified in breeding programs have been Middle American in origin. Kelly and Vallejo (2004) reported that of the known anthracnose resistance genes, only 10% were Andean in origin. However with more recent studies, additional Andean sources of resistance genes *Co-12, Co-13, Co-14,* and *Co-15* have been identified including additional alleles of the *Co-1* locus (Gonçalves-Vidigal, et al., 2008; Gonçalves-Vidigal, et al., 2009; Gonçalves-Vidigal, et al., 2012; Sousa & Gonçalves, 2015). Identifying additional sources of resistance in both gene pools plays a critical role in reducing the vulnerability of common bean to the constantly evolving *Colletotrichum lindemuthianum*. One approach to counter breakdown of major gene resistance is to place multiple resistance genes under the same genetic background to provide a more durable resistance. For a broad-spectrum resistance, pyramiding resistance genes from the alternative gene pools may prove very useful (Young & Kelly, 1997). Minor effect loci have been reported to provide additional resistance to that of the major genes. It is critical to

add minor effect QTL in the pyramiding schemes for even more effective resistance to anthracnose.

2.12. Approaches to understand the genetic architecture of anthracnose resistance in common beans.

Genetic studies play a fundamental role in enhancing the understanding of the genetic basis of anthracnose resistance in common bean. Furthermore, understanding the molecular mechanisms underlying resistance is crucial. One of the foundational approaches in unraveling the genetics of anthracnose resistance involves quantitative trait loci (QTL) mapping using recombinant in-bred lines (RILs). A previous QTL mapping study uncovered several QTL associated with resistance on chromosomes Pv02, Pv03, Pv04, Pv07 and Pv10 (Mungalu et al). Another comprehensive QTL analysis identified several regions associated with resistance (Gonçalves-Vidigal et al. (2019). Their study underlines the importance of QTL mapping in pinpointing candidate genes and facilitating marker-assisted breeding efforts. Another approach which has emerged as a powerful tool for dissecting the genetic architecture of anthracnose resistance is through genome-wide association studies (GWAS). GWAS have revolutionized the field of plant genetics by allowing researchers to explore the entire genome for associations between genetic markers and phenotypic traits. Unlike traditional QTL mapping, GWAS offers higher resolution and the ability to identify candidate genes without prior knowledge. Advancements in high-throughput genotyping platforms have facilitated large-scale GWAS in common bean.

In Genome-Wide Association Studies (GWAS), various sequencing platforms and genotyping technologies are employed to analyze the genetic data of large populations. These platforms offer diverse capabilities to suit specific research needs. Microarrays, although not traditional sequencing platforms, enable simultaneous genotyping of a multitude of single nucleotide polymorphisms (SNPs). Illumina sequencing, known for its high throughput and cost-effectiveness, is commonly used for both whole-genome and targeted sequencing. Ion Torrent sequencing is suitable for amplicon sequencing in targeted GWAS studies. Pacific Biosciences (PacBio) sequencing provides long reads, aiding in the identification of structural variants. Oxford Nanopore sequencing offers long reads and is valuable for structural variant detection and haplotype phasing. Whole-exome sequencing (WES) is cost-effective and

focuses on protein-coding regions, while targeted sequencing panels allow customization for specific genes or regions of interest. Genotyping-by-Sequencing (GBS) is a reduced representation sequencing technique that focuses on a subset of the genome. It's cost-effective and suitable for large-scale GWAS. RNA sequencing (RNA-Seq) is used for gene expression analysis and identifying expression quantitative trait loci (eQTLs). Whole-genome sequencing (WGS) captures all genetic variation but can be costlier. Single cell sequencing techniques dissect genetic heterogeneity in certain GWAS studies, such as cancer research

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Plant material

In the current study the Yellow Bean Collection (YBC) comprised of 255 genotypes was used. Details on the composition of the YBC can be found in (Sadohara, et al., 2022). Briefly, the YBC is comprised of landraces, breeding lines and varieties with variable shades of yellow seed colors from various countries and breeding programs. The YBC consists of genotypes from both the Andean and Middle-American gene pools. The study used the cultivars G2333 and Kabulangeti as the resistant and susceptible checks respectively. The YBC and checks were evaluated for resistance to eight characterized races of *C. lindemuthianum*.

3.2. Evaluation of the YBC for resistance to anthracnose

A total of eight races 5, 19, 39, 51, 81, 183, 1050 and 1105 of *Colletotrichum lindemuthianum* were used in the current study. These eight races were characterized from isolates collected from major bean-growing regions of Zambia. Characterization was conducted using a set of 12 differential cultivars (Pastor-Corrales, 1991). The eight races used in the current study offered a broad spectrum of virulence. Inoculation of the YBC followed a protocol described in Mungalu, et al. (2020). This protocol involved planting the YBC (255 genotypes), anthracnose susceptible check (Kabulangeti) and anthracnose resistant check (G2333) on Styrofoam trays. Each tray had 60 wells and each well was 5 cm wide, 5 cm long and 5 cm deep. A completely randomized design with three replications was used. Each replication had two seedlings in a well. Therefore, a total of six seedlings per genotype were evaluated for resistance to each race. Inoculation was conducted when seedlings had developed fully expanded unifoliate leaves. Evaluation of the YBC was conducted separately for each of the eight races.

3.3. Inoculum preparation

Inoculum preparation was conducted using a protocol described in Mungalu et al. (2020). Each race was cultured on petri dishes on either potato dextrose agar (PDA) 39 g L^{-1} or modified Mathur's agar culture media made with dextrose (8 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (2.5 g L^{-1}), KH_2PO_4 (2.7 g L^{-1}), neopeptone (2.4 g $L^{-1)}$, yeast extract (2.0 g L^{-1}), and agar (16 g L^{-1}). The reason for using either PDA or Mathur's agar was because some isolates sporulated better on PDA than Mathur's agar and vice versa. The petri dishes were incubated in the dark at 23-25 ºC for 7 to 10 days for sporulation. Sporulated plates were flooded with distilled water and allowed to settle for 20 minutes. Spores where Spores were then harvested by scrapping them off from the culture using a glass rod. The harvested spore suspension was then sieved through a double-layered cheesecloth into a clean beaker. The spore suspension concentration was then adjusted to 1.2 x 10^6 spores per ml using a hemocytometer. Tween 20 was added to the inoculum for adhesion. Seedlings were thoroughly sprayed with the inoculum on both surfaces of the primary leaves and the stem using a hand sprayer. After inoculation, plants were left to air-dry for a few minutes before they were placed in a high humidity chamber (>90% humidity) for 72 hrs at room temperature. After this incubation the seedlings were removed from the humidity chamber and transferred to greenhouse benches where they stayed for 5 days for further anthracnose development. Anthracnose severity on seedlings was rated based on a 1-9 CIAT scale (Balardin, et al., 1997). The score range of 1-3 was considered as resistant and included plants with no visible symptoms or with few very small lesions, mostly on primary leaf veins, 4-6 was considered moderately resistant and included seedlings with small lesions on leaves and seedling stem, and 7-9 was considered susceptible and included dead seedlings and those with numerous small or enlarged lesions, with sunken cankers on leaves and seedling stem.

3.4. Severity score analysis

Statistical analyses on disease severity scores were conducted in SAS 9.3 (SAS Institute, 2011). Severity score data was first checked for normal distribution using PROC UNIVARIATE. Normality test results showed that severity scores for all races were normally

distributed. Analysis of Variance was conducted using PROC MIXED following the mixed model:

$Y_{ik}=\mu+\alpha_i+\gamma_k+\varepsilon_{ik}$

Where: Y_{ik} was anthracnose severity score, with genotype i, replication k; αi was the fixed variable effect of the genotype i; γ was the random variable effect of a replication; ε_{ik} was the residual associated with replication k in genotype i. γk was a random variable, which was assumed to be normally distributed with mean = 0.

3.5. Genotypic data analysis

The YBC (255 genotypes) was genotyped with 72,866 Single Nucleotide Polymorphism (SNPs) markers using Genotyping by Sequencing (GBS) at Michigan State University in the USA. Details on this genotyping can be found in Sadohara et al. (2022). Briefly, for each line, five trifoliate leaves were frozen at -80°C, subjected to lyophilization, and subsequently milled using a bench-top mill (Geno Grinder 2000, Spex Certiprep, Metuchen, NJ, USA). Genomic DNA was subsequently isolated from the milled leaves utilizing the NucleoSpin Plant II Kit (Macherey–Nagel, Duren, Germany), in accordance with the 'Genomic DNA from plant' protocol, with elution carried out in 50 µL of PE buffer. The quality of the DNA was evaluated through electrophoresis (of random samples) on 1% agarose gels, visualized by ethidium bromide staining. DNA concentration was quantified utilizing the Quant-iTTM PicoGreenTM dsDNA Assay Kit (Invitrogen, Waltham, MA, USA), and 10 ng/µL of DNA was utilized for the preparation of a Genotyping-By-Sequencing (GBS) library in accordance with Elshire et al. (2011), employing a single restriction enzyme, ApeKI. Subsequently, the library was sequenced utilizing an Illumina standard HiSeq 4000, yielding 150 bp single-end reads, at the RTSF Genomics Core located at Michigan State University. The SNP data was used in population structure, kinship and SNP-disease severity analyses.

3.6. Population structure

Correction for population structure in a diversity panel such as the YBC is necessary to avoid false positives in GWAS. Details on the population structure in the YBC can be found in Sadohara et al. (2022). In the current study, the population structure in the YBC was determined using Principal Component Analysis (PCA) in TASSEL (Bradbury et al., 2007). The first five principal components, which together accounted for 62.5% of variation in the YBC, were used as covariates in the GWAS analyses.

3.7. Kinship

Kinship (cryptic relatedness) among genotypes in a diversity panel such as the YBC can result in false positive. In the current study, kinship among genotypes was investigated and corrected for in GWAS using Identical by Descent method in the software TASSEL.

3.8. Association analysis for anthracnose resistance

SNPs significantly associated with resistance to races 5, 19, 51, 81, 183, 1050 and 1105 were identified using the following Mixed Linear Model (Zhang, et al., 2010).

$$Y = G + P + K + \varepsilon$$

Where Y the phenotype of a genotype; X was the fixed effect of the SNP; P was the fixed effect of population structure (from PCA matrix from TASSEL); K was the random effect of relative kinship (from kinship matrix from TASSEL); ε was the error term, which was assumed to be normally distributed with mean = 0.

MLM analysis was conducted in the software TASSEL 5.0. Before use in MLM, SNP data was filtered for minor allele frequency (MAF=5%), which reduced the number of usable SNPs for MLM to 55,600. The Bonferroni-corrected p-value of 8.9×10^{-7} ($\alpha = 0.05$; 55,600 SNPs)

was used as a threshold to determine the significance of the association between the SNP and severity score of a given race. The Manhattan plots for visualization of significant SNPs were made in a custom R-script.

3.9. Identification of candidate genes

Candidate genes in genomic regions associated with resistance to the eight races of anthracnose in the current study were identified from *Phaseolus vulgaris* v2.1 (Schmutz, et al., 2014) in Phytozome using JBrowse following a previously described method (Kamfwa, et al., 2015). Briefly, a gene was identified as a positional candidate gene based on two criteria: (i) it was within a genomic region of 400 kb of either upstream or downstream of the most significant SNP, and (ii) the functional role of a gene in disease resistance has been determined or proposed.

CHAPTER FOUR

4. RESULTS

4.1. Anthracnose severity score analysis

Highly significant (P<0.01) differences were observed among YBC genotypes in their reaction to races 5, 19, 39, 51, 81, 183, 1050 and 1105 of *C. lindemuthianum*. Severity scores for the YBC ranged from 1 to 9 for all eight races (Table 4.1).

Table 4.1. Means of anthracnose severity score of G2333 (resistant check), Kabulangeti (susceptible check) and 255 lines of the YBC evaluated for resistance in the greenhouse at University of Zambia, Lusaka, Zambia.

	Checks		YBC		
Anthracnose race	**G2333**	**Kabulangeti**	**Mean**	**Range**	**ANOVA**
Race 5	1.0	9.0	6.7±0.2	1.0-9.0	***
Race 19	1.0	9.0	6.3±0.21	1.0-9.0	***
Race 39	1.0	9.0	6.7±0.19	1.0-9.0	***
Race 51	1.0	9.0	5.2±0.21	1.0-9.0	***
Race 81	1.0	9.0	5.9±0.18	1.0-9.0	***
Race 183	1.0	9.0	6.4±0.2	1.0-9.0	***
Race 1050	1.0	9.0	6.5±0.19	1.0-9.0	***
Race 1105	1.0	9.0	5.7±0.21	1.0-9.0	***

YBC = Yellow Bean Collection, ANOVA = analysis of variance, *** = Signifiacant (p < 0.001)

The average severity score for the YBC to races 5, 19, 39, 51, 81, 183, 1050 and 1105 were 6.7, 6.3, 6.7, 5.2, 5.9, 6.4, 6.5 and 5.7, respectively (Table 4.1). The highest mean severity scores for the YBC were observed for Andean races 5 and 39. The frequency distribution of severity scores showed a bimodal distribution for all eight races (Fig 4.1).

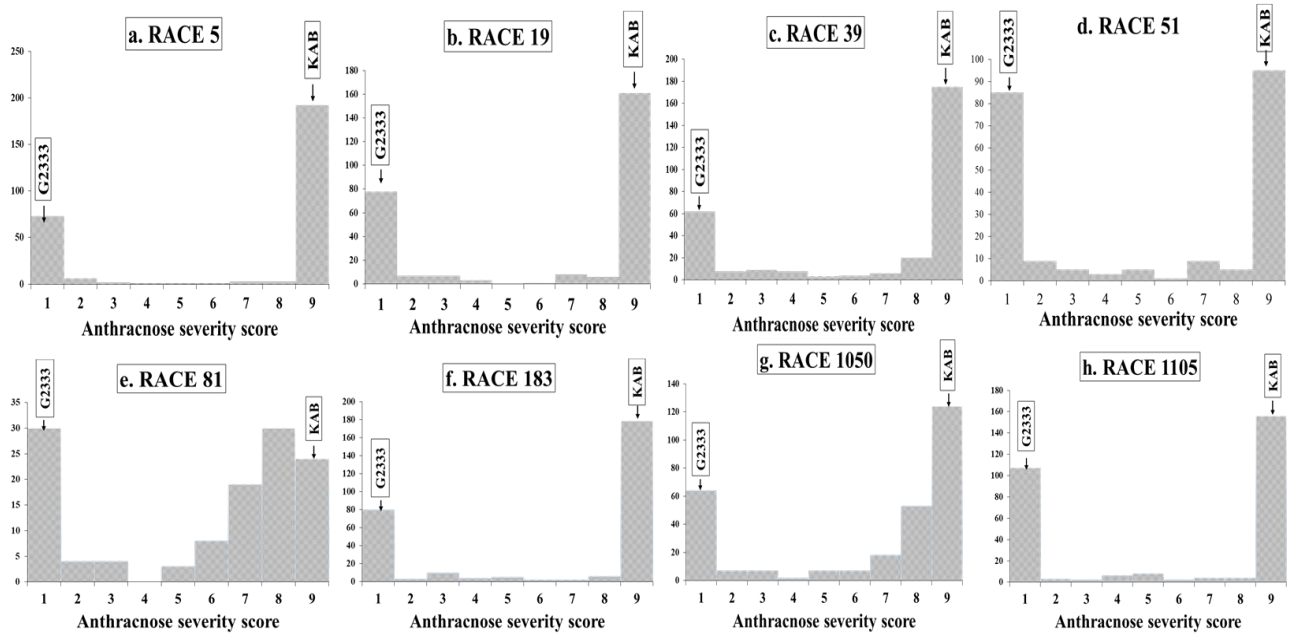

Fig 4.1. Frequency distributions of severity scores for eight races 5, 19, 39, 51, 81, 183, 1050 and 1105 of *C. lindemuthianum* incoculated on the Yellow Bean Collection, resistant check (G2333) and susceptible check (Kabulangeti).

The resistant and susceptible checks reacted to all eight races as expected. The resistant (G2333) and susceptible (Kabulangeti) checks consistently scored 1 and 9, respectively, for all eight races (Table 4.1). Of the 255 YBC genotypes evaluated, five genotypes (YBC278, YBC130, YBC173, YBC192 and YBC267) showed superior resistance to most of the eight races (Table 4.2).

The genotype YBC278 was the only one that that was highly resistant (severity score < 1) to all eight races. The severity scores for all 255 YBC genotypes for the eight races are presented in appendix 1.

Table 4.2. Mean anthracnose severity scores for the five genotypes identified from the 255 Yellow Bean Collection genotypes as being highly resistant against races 5, 19, 39, 51, 81, 183, 1050 and 1105 of *Colletotrichum lindemuthianum* after artificial inoculation in the greenhouse at University of Zambia, Lusaka, Zambia.

					Mean anthracnose scores							
YBC ID	**ID**	**Country of origin**	**Seed color**	**Genepool**	**Race 5**	**Race 19**	**Race 39**	**Race 51**	**Race 81**	**Race 183**	**Race 1050**	**Race 1105**
YBC278	SMC28	Uganda	Amarillo	Middle-American	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
YBC130	P1527538	Burundi	Green-yellow	Andean	1.0	1.0	-	1.2	1.0	1.0	1.0	1.0
YBC173	G22501	Burundi	Green-yellow	Andean	1.0	1.0	9.0	1.0	1.0	1.0	1.3	1.0
YBC267	DAB933	Uganda	Amarillo	Andean	3.3	1.0	1.0	1.0	1.3	3.5	-	1.0
YBC192	Roba-1	Uganda	Amarillo	Middle-American	1.0	1.3	-	1.0	6.8	1.0	1.7	1.0

YBC = Yellow Bean Collection; ID = identity

4.2. Genome-wide Association analysis

SNPs significantly associated with resistance to races 5, 19, 39, 51, 81, 183, 1050 and 1105 were identified on chromosomes Pv01, Pv02, Pv04, Pv05 and Pv07 (Figures 4.2 – 4.9, Table 4.3).

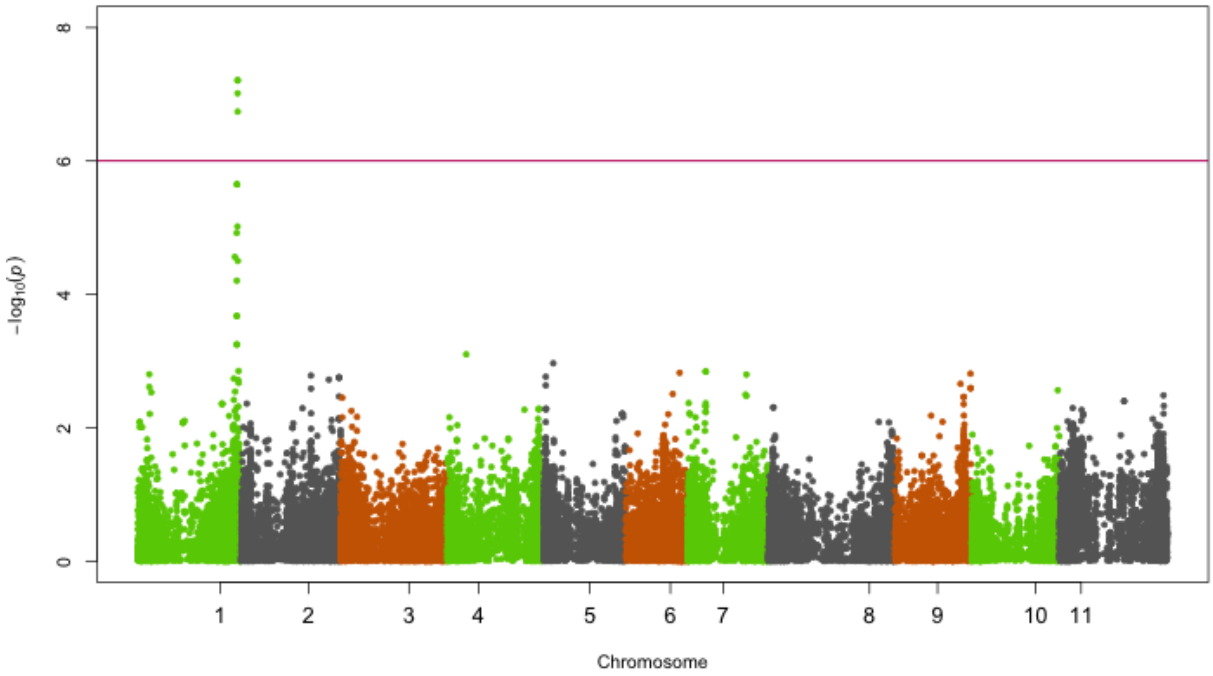

Figure 4.2. Manhattan plot showing SNPs on chromosome Pv01 significantly associated with resistance to race 81 of the Yellow Bean Collection genotypes evaluated in the greenhouse at University of Zambia, Lusaka, Zambia. The red solid horizontal line is the Bonferroni adjusted *P*-value (1.0E-06).

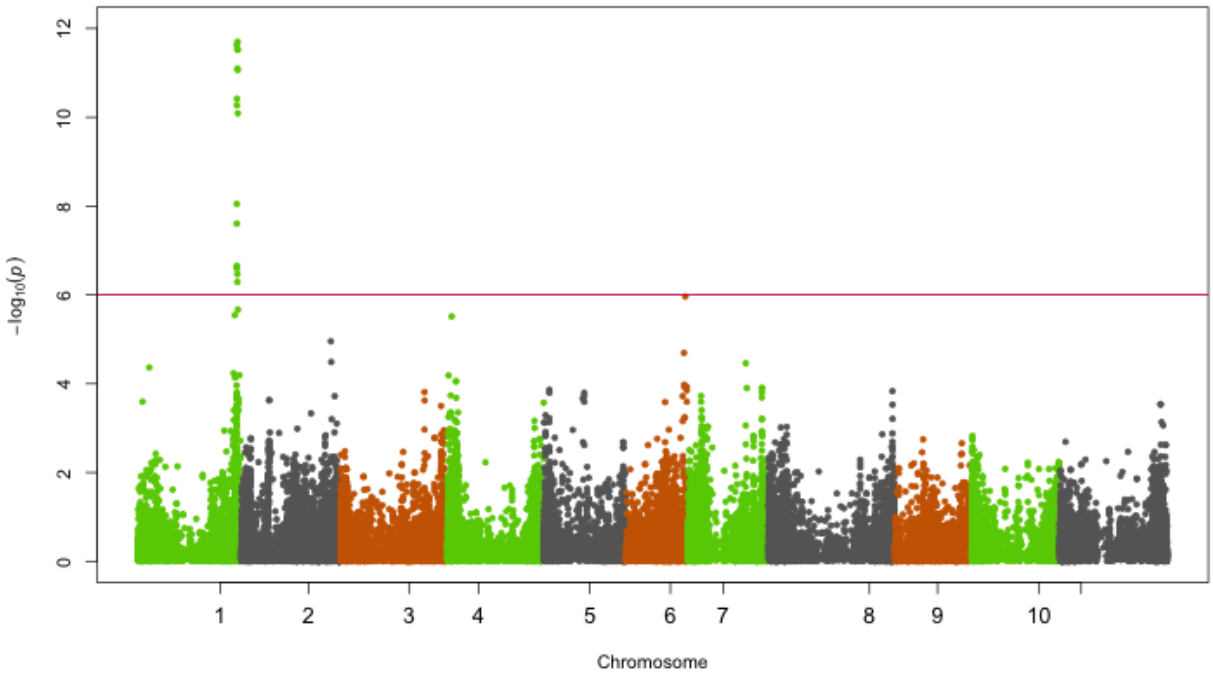

Figure 4.3. Manhattan plot showing SNPs on chromosome Pv01 significantly associated with resistance to race 1050 of the Yellow Bean Collection genotypes evaluated in the greenhouse at University of Zambia, Lusaka, Zambia. The red solid horizontal line is the Bonferroni adjusted *P*-value (1.0E-06).

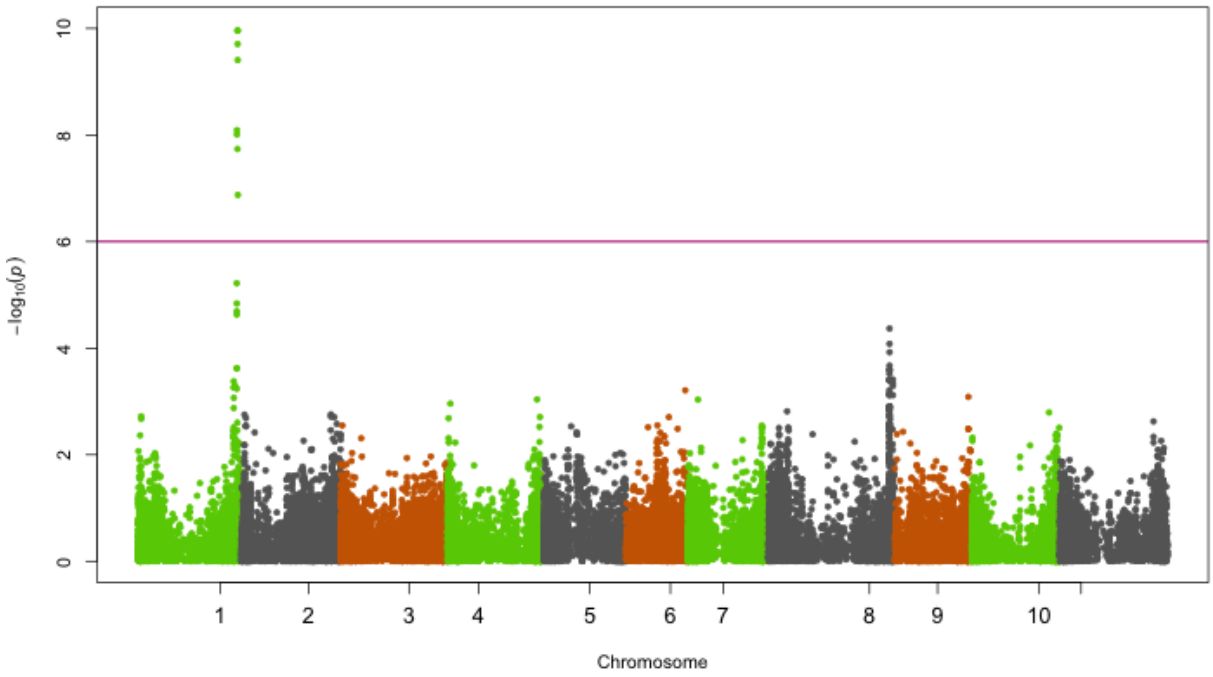

Figure 4.4. Manhattan plot showing SNPs on chromosome Pv01 significantly associated with resistance to race 1105 of the Yellow Bean Collection genotypes evaluated in the greenhouse at University of Zambia, Lusaka, Zambia. The red solid horizontal line is the Bonferroni adjusted *P*-value (1.0E-06).

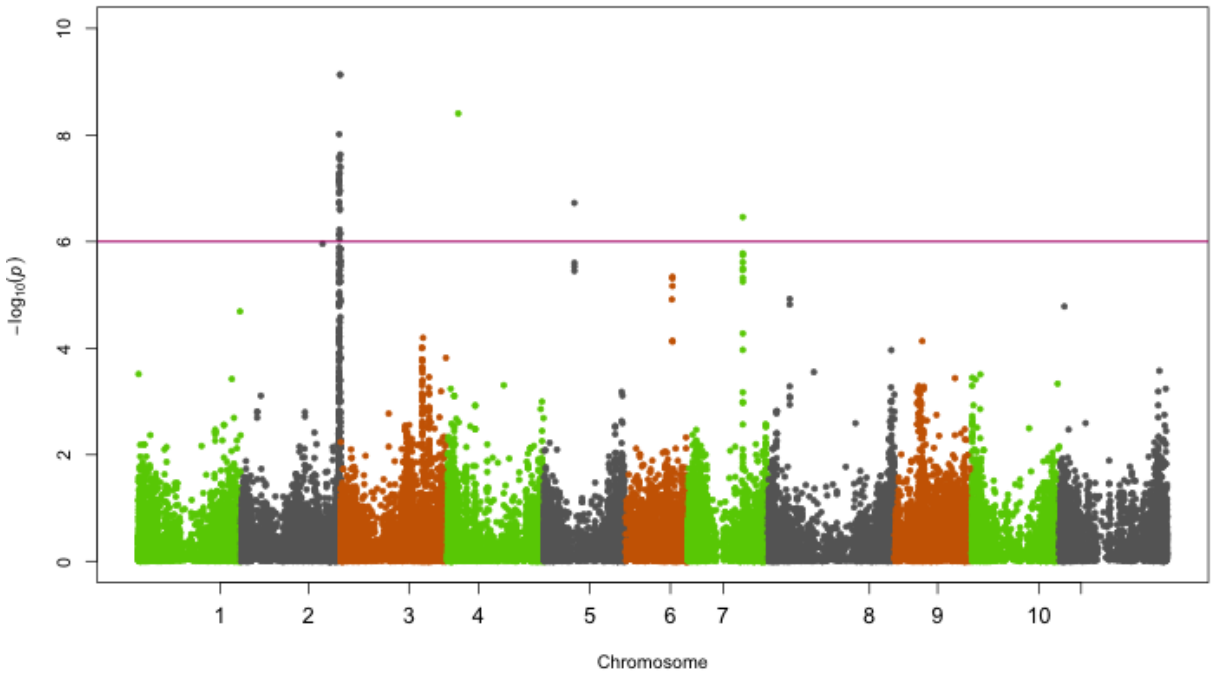

Figure 4.5. Manhattan plot showing SNPs on chromosome Pv02, Pv04, Pv05 and Pv07 significantly associated with resistance to Andean anthracnose race 39 of the Yellow Bean Collection genotypes evaluated in the greenhouse at University of Zambia, Lusaka, Zambia. The red solid horizontal line is the Bonferroni adjusted *P*-value (1.0E-06).

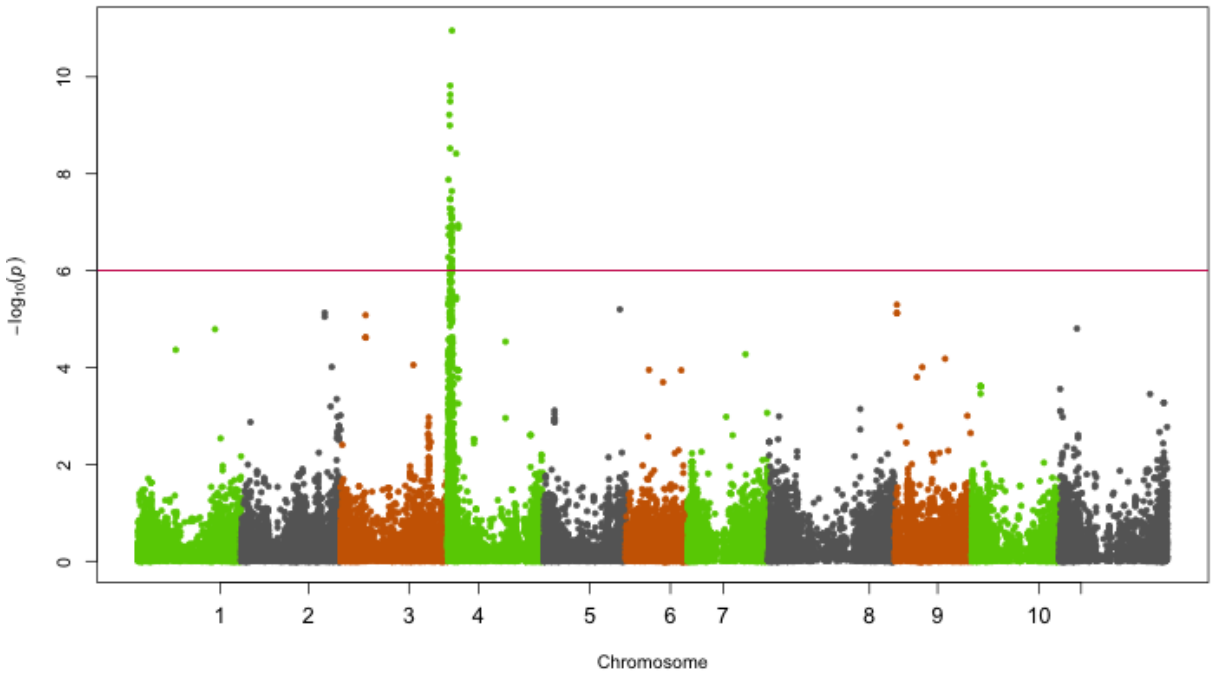

Figure 4.6. Manhattan plot showing SNPs on chromosome Pv04 significantly associated with resistance to race 19, 51 and 183 of the Yellow Bean Collection genotypes evaluated in the greenhouse at University of Zambia, Lusaka, Zambia. The red solid horizontal line is the Bonferroni adjusted *P*-value (1.0E-06).

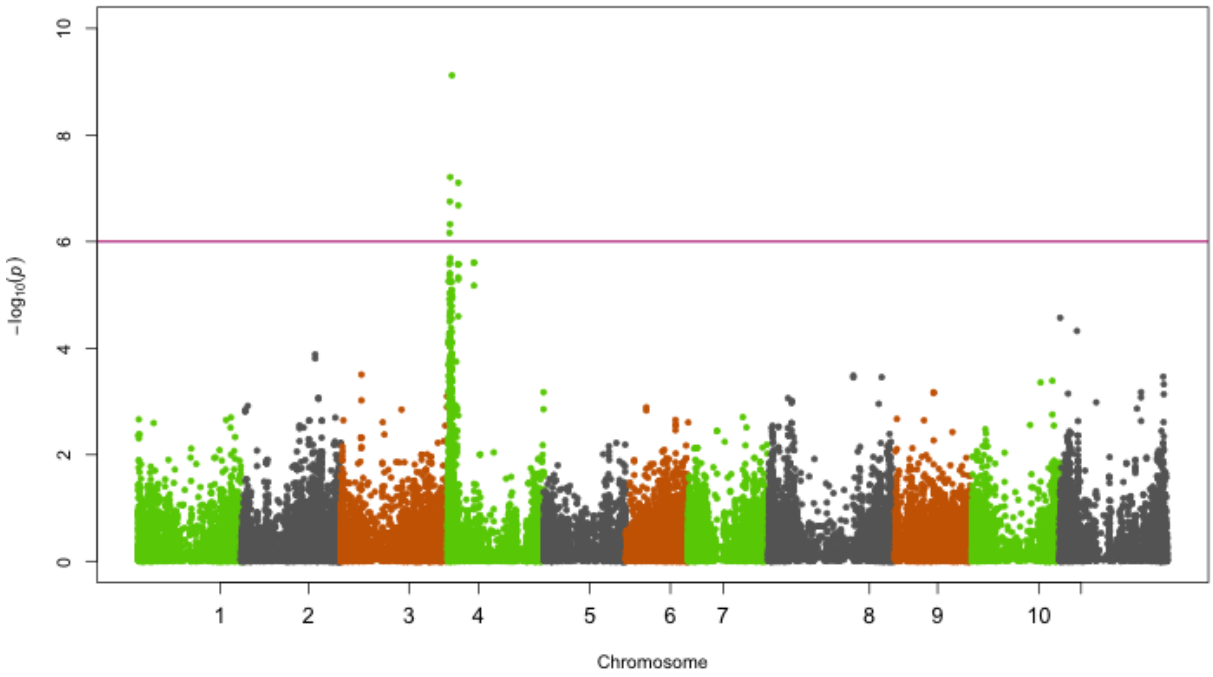

Figure 4.7. Manhattan plot showing SNPs on chromosome Pv04 significantly associated with resistance to race 51 of the Yellow Bean Collection genotypes evaluated in the greenhouse at University of Zambia, Lusaka, Zambia. The red solid horizontal line is the Bonferroni adjusted *P*-value (1.0E-06).

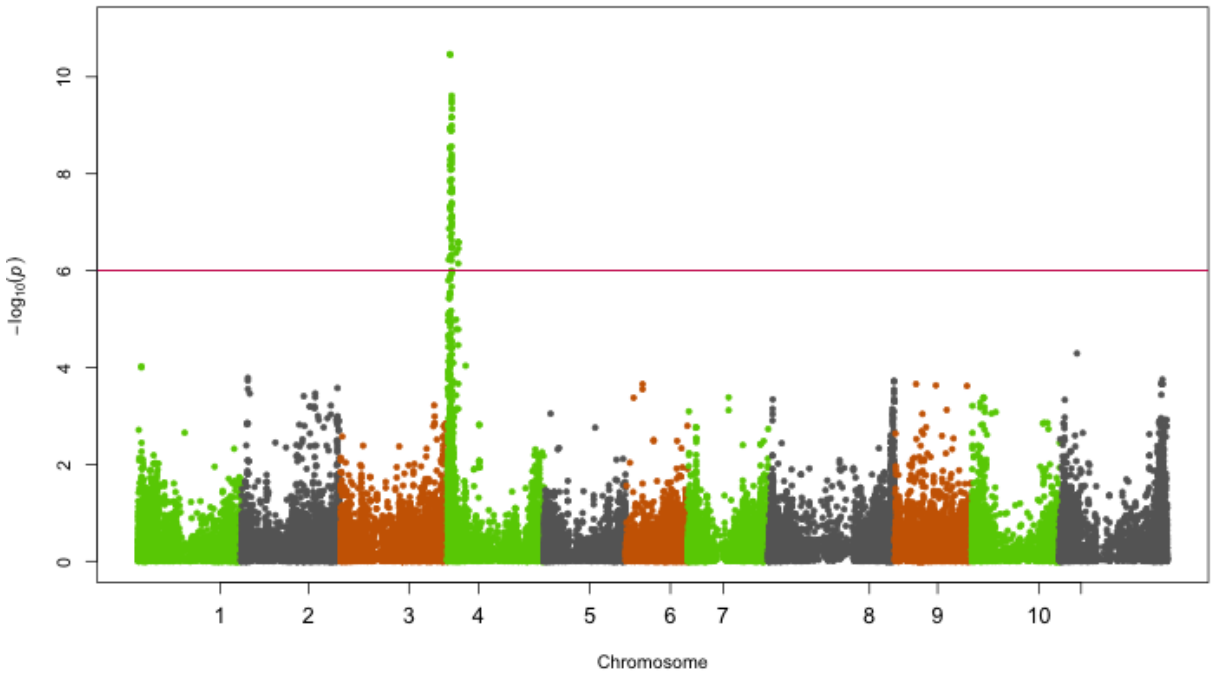

Figure 4.8. Manhattan plot showing SNPs on chromosome Pv04 significantly associated with resistance to race 183 of the Yellow Bean Collection genotypes evaluated in the greenhouse at University of Zambia, Lusaka, Zambia. The red solid horizontal line is the Bonferroni adjusted *P*-value (1.0E-06).

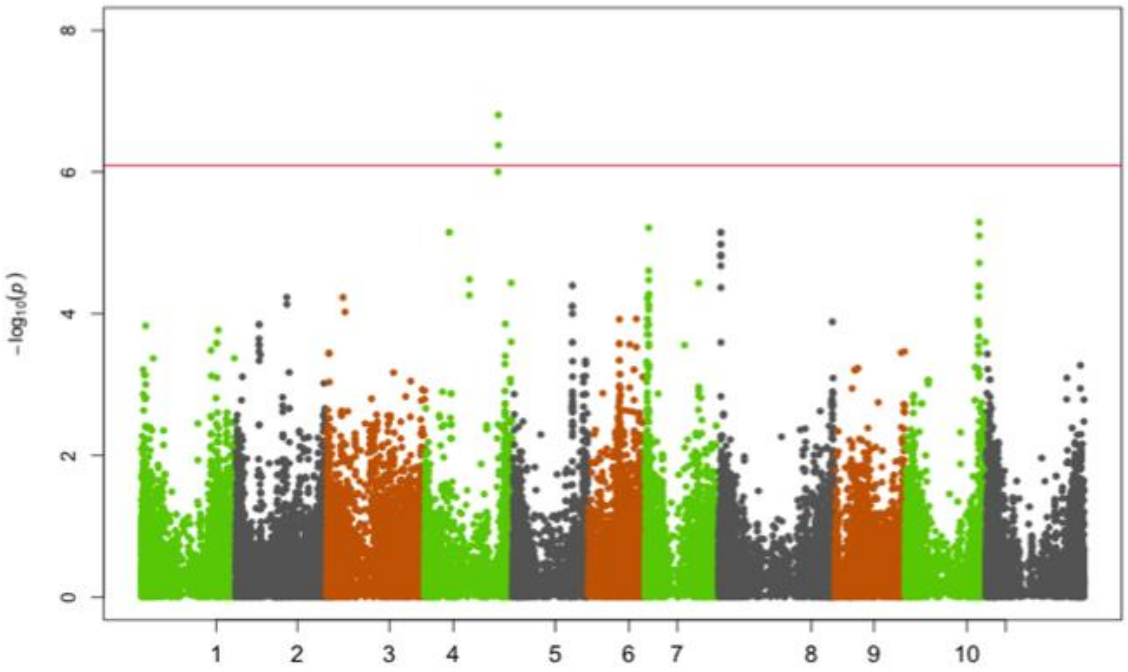

Figure 4.9. Manhattan plot showing SNPs on chromosome Pv04 significantly associated with resistance to Andean anthracnose race 5 in the Yellow Bean Collection evaluated in the greenhouse at University of Zambia, Lusaka, Zambia. The red solid horizontal line is the Bonferroni adjusted *P*-value (1.0E-06).

Table 4.3. SNPs markers significantly associated with resistance to races 5, 19, 39, 51, 81, 183, 1050 and 1105 of *Colletotrichum lindemuthianum* identified using the Yellow Bean Collection evaluated in the greenhouse at University of Zambia, Lusaka, Zambia.

Trait	Chr	SNP	Position (bp)	*P*-value	R^2 (%)
Race 5	Pv04	Chr04_40179029	4,0179,029	1.5E-06	12.6
Race 19	Pv04	Chr04_2140558	2,140,558	1.1E-11	24.7
Race 39	Pv02	Chr02_49318523	49,318,523	7.4E-10	30.1
Race 39	Pv05	Chr05_15211384	15,211,384	1.19E-07	13.3
Race 39	Pv07	Chr07_27213163	27,213,163	7.15E-07	14.2
Race 51	Pv04	Chr04_2140558	2,140,558	7.6E-10	18.8
Race 81	Pv01	Chr01_49584097	49,584,097	6.2E-08	41.2
Race 183	Pv04	Chr04_1067693	1,067,693	3.4E-11	22.5
Race 1050	Pv01	Chr01_49583965	49,583,965	1.9E-12	27.5
Race 1105	Pv01	Chr01_49584097	49,584,097	1.1E-10	21.4

Chr = Chromosome; SNP = single nucleotide polymorphism; bp = base pairs; *P*-value = level of significance; E = exponential; R^2 = proportion of variation of anthracnose resistance explained by the significant SNP

Chromosome Pv01

Several SNPs in the genomic region spanning 49,151,104 bp – 49,584,097 bp on chromosome Pv01 were significantly associated with resistance to races 81, 1050 and 1105 (Figures 4.2 – 4.4). The most significant SNP in this genomic region explained 41.2%, 27.5% and 21.4% of

the YBC variation in anthracnose severity caused by races 81, 1050 and 1105, respectively (Table 4.3).

Chromosome Pv02

Significant SNPs for resistance to the Andean anthracnose race 39 were identified on Pv02 in a genomic region spanning from 49,155,240 bp – 49,416,064 bp (Figure 4.5). The most significant SNP in this genomic region explained 30.1% of the YBC variation in anthracnose severity caused by race 39 (Table 4.3).

Chromosome Pv04

Significant SNPs for races 19, 51 and 183 were identified in separate genomic regions of Pv04 (Figures 4.6 – 4.8). The first genomic region was at the beginning (1,067,693 bp – 2,140,558 bp) of Pv04. Several SNPs in this genomic region were associated with resistance to races 19, 51 and 183. The R^2 for the most significant SNP for races 19, 51 and 183 were 24.7%, 18.8% and 22.5%, respectively. The second genomic region identified on Pv04 was at 40,179,029 bp. This region was significantly associated with resistance to race 5 and the most significant SNP at this region explained 12.6% of the variation in the YBC for anthracnose severity caused by race 5.

Chromosome Pv05 and Pv07

SNPs significantly associated with resistance to race 39 were identified on Pv05 and Pv07 (Figure 4.9). The most significant SNPs on Pv05 (15,211,384 bp) and Pv07 (27,213,163 bp) explained 13.3% and 14.2% of the variation in severity scores for race 39 (Table 4.3).

CHAPTER FIVE

5. DISCUSSION

Anthracnose caused by *C. lindemuthianum* is a major disease of common bean. The productivity of yellow beans, a major market class in several African countries, is constrained by anthracnose. In the current study, the YBC was evaluated for its reaction to races 5, 19, 39, 51, 81, 183, 1050 and 1105 of *C. lindemuthianum*, and GWAS conducted to identify genomic regions and positional candidate genes conferring resistance to these eight races.

The frequency distribution graphs for severity for all eight races showed a bimodal pattern, suggesting that major-effect genes were the basis of anthracnose resistance, which is consistent with previous description of anthracnose resistance in common bean as being controlled by major-effect genes. Identification of sources of resistance to anthracnose within the yellow bean market class would necessitate progress in the genetic enhancement of yellow beans for resistance to anthracnose. Such identification is necessary because of the challenges associated with poor recovery of yellow seed color and seed size in progenies from crosses of yellow bean with other colors or with smaller-seeded Middle American genotypes. The current study has confirmed the existence of adequate levels of anthracnose resistance to both Andean and Middle-American races of *C. lindemuthianum* in the yellow bean market class. In this study the genotype YBC278 was identified as having superior resistance to anthracnose. YBC278 was the only genotype in the YBC that was highly resistant (severity score of less than 1) to all the eight races used in the current study. This reaction was only similar to that of the resistant check G2333, a Middle-American genotype widely reported to be highly resistant to both Andean and Middle-American races of *C. lindemuthianum*. YBC278, also known as SMC28, is a Middle American bean of race Mesoamerica from CIAT-Uganda. It has an Amarillo (dark yellow) seed type and a 100-seed weight of 27 grams. The identification of YBC278 as highly resistant to both Middle American and Andean genotypes is important, as it will serve as a source of superior resistance for developing yellow bean varieties with durable resistance to anthracnose. Genotypes YBC130 and YBC267 are the other two genotypes that showed superior levels of resistance to anthracnose. YBC130 is an Andean landrace from Burundi with a green-yellow seed coat and a 100-seed weight of

approximately 43 grams. It was included in the Andean Diversity Panel as ADP0468 and is listed as PI 527538 in the USDA-NPGS gene bank. YBC267, also known as DAB 933, is an Andean bean from CIAT-Uganda of the Amarillo (dark) market category, with a 100-seed weight of 30 grams. These accessions could be used as alternative sources of anthracnose resistance for yellow bean breeding.

The genetic basis of the observed resistance in the YBC to eight races of *C. lindemuthianum* was explored using genome-wide association analysis. Genomic regions and positional candidate genes associated with resistance to the eight races used in the current study were identified on chromosomes Pv01, Pv02, Pv04, Pv05 and Pv07.

Several SNPs within a 528 kbp region (49,151,104 – 49,679,631 bp) on Pv01 were significantly associated with resistance to race 81, and the highly virulent races 1050 and 1105. This is a major-effect QTL and it appears to be the major locus *Co-1*, which confers resistance mainly to Middle American races of *C. lindemuthianum*. The SNPs most significantly associated with resistance to races 81, 1050, and 1105 were found at positions 49,584,097, 49,583,965, and 49,584,097 of Pv01, respectively. In each case, the most SNP most significantly associated with anthracnose resistance is in the gene model Phvul.001G243800, a protein-kinase recently proposed to underlie *Co-1* (Mahiya-Farooq et al. 2019, Lima et al, 2023). This is a powerful validation of the role of this gene, as well as the experimental methods employed in this study. Previous studies have shown existence of both Andean and Middle American races in East and Southern African countries including Zambia, which underscores the importance of having *Co-1* gene in Andean genotypes such as the yellow beans.

The genomic region 49,318,523 bp on Pv02 contained SNPs that were significantly associated with resistance to races 19 and 39. The identification of YBC genotypes resistant to races 19 and 39, and their associated resistance genomic regions has breeding significance to Zambia and other bean producing countries for two reasons. First, race 19 is the most prevalent race in the bean-producing regions of Zambia (Sansala et al 2023). Second, race 39 is a highly virulent Andean race (Zuiderveen et al., 2016) and it is important that yellow bean genotypes with superior resistance to race 39 are identified to serve as source of resistance for genetic

improvement of yellow beans in countries where it is a major market class. The association between race 39 and the genomic region on Pv02 (49,318,523 bp) has previously been reported (Zuiderveen et al, 2016). Eight plant disease resistance genes (R genes) including Phvul.002G323100, Phvul.002G323200, Phvul.002G323300, Phvul.002G323400, Phvul.002G323404, Phvul.002G323704, Phvul.002G323708, and Phvul.002G323712 with NB-ARC and LRR domains were identified as positional candidate genes for resistance to race 39 identified on Pv02. R genes encode receptors that play a key role in the recognition of avirulence (Avr) products expressed by the pathogen during infection, which triggers an immune response and is the basis for gene-for-gene or race-specific resistance for anthracnose observed in common bean.

The genomic region on 1.1 Mbp – 2.1 Mbp on Pv04 conferred resistance to races 19, 51 and 183. This genomic region overlaps with major-effect loci *Co-3*, *Co-15*, *Co-16*, *Co-y* and *Co-z*. The relatively large number of races whose resistance was controlled by this genomic is indicative of its significant role in the YBC as a source of broad-spectrum resistance to multiple races of *C. lindemuthianum*. The genomic region 1.1 Mbp – 2.1 Mbp identified in the current study as providing resistance to races 19, 51 and 183 was previously reported to also provide resistance to races 7, 19, 49, 55, 109, 530, 566 and 1331 (Zuiderveen et al. 2016; Mungalu et al., 2019), which underscores the importance of this genomic region for resistance to multiple races of anthracnose. Five disease resistance (R) genes including Phvul.004G015600, Phvul.004G015800, Phvul.004G015900, Phvul.004G016000 and Phvul.004G016532 with the NB-ARC and LRR domains were identified as candidate genes for resistance to races 19, 51 and 183 at the 1.1 Mbp - 2.1 Mbp genomic region on Pv04. These R genes occurred in a cluster, and it is plausible that some of them provide race-specific resistance because this genomic region provided resistance to a relatively large number of races. Given that *Co-3* has previously been reported as multi-allelic it is plausible that resistance to races 19, 51 and 183 reported in the current study may be conditioned by different alleles. In addition to overlapping with *Co-3*, the genomic region on Pv04 identified in the current study also overlaps with the telomere cluster of resistance genes for rust (*Ur-5* and *Ur-14*) (Valentini et al., 2017), angular leaf spot (*Phg-3*) (Valentini et al., 2017), powdery mildew (Binagwa et al., 2021) and halo blight QTL (HB4.2) (Tock et al., 2017), which are major diseases of beans in Africa. Given the convergence of resistance to several races of

anthracnose and other major diseases at the identified genomic region in the current study, it is a worthwhile target for Marker-assisted selection to develop common bean varieties with durable resistance to anthracnose and also multiple resistances to anthracnose, rust and angular leaf spot. The current study has identified another genomic region on Pv04 to control resistance to anthracnose. This genomic region located at the distal end on Pv04 (40.2 Mbp) is a major locus (R^2=12.6) and the basis for resistance in the YBC to race 5 (Figure 4.5). This is the first report of anthracnose resistance to race 5 at this genomic region.

Significant SNPs for resistance to race 39 were identified on Pv05 (15.2 Mbp) and Pv07 (27.2 Mbp). Given the large R^2 for the most significant SNPs on Pv05 (13.1%) and PV07 (15.5%) the two QTL had major effect on resistance to race 39 in the YBC. This is the first report of resistance for race 39 on Pv05 and Pv07. While significant SNPs for resistance to races 87 and 73 have been reported on Pv05 and Pv07 (Banoo et al, 2020), the genomic region differs from the one detected in this study. Resistance to race 39 has previously been reported on other chromosomes but not in the genomic regions on Pv05 and Pv07 identified in the current study. Zuiderveen et al. (2016) conducted GWAS using the ADP and reported resistance to race 39 only on chromosome Pv04, the same region we have also identified in the current study. Campa et al. (2014) identified *Co-2* on Pv11 as the source of resistance to race 39 in a population of RILs. The current study has not only identified a previously known genomic region on Pv04 as source of resistance to race 39 but has also identified novel major QTL on Pv05 and Pv07 as additional genomic regions for resistance to race 39.

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Five YBC accessions with superior resistance to races 5, 19, 51, 81, 183, 1050 and 1105 were identified. The accession YBC278 was notable because it is the only genotype in the YBC that showed strong resistance to all eight races of *Colletotrichum lindemuthianum* used in the current study.

The genetic architecture for resistance to eight races of *Colletotrichum lindemuthianum* in the YBC has been determined. Major-effect loci on Pv01 (Co-1), Pv02 and Pv04 (which overlaps with Co-3, Co-13, Co-15, Co-y and Co-z) controlled most of the observed resistance to eight races observed in the YBC. In addition to the identified major-effect loci genomic regions on Pv01, Pv02 and Pv04, novel genomic regions on Pv05 and Pv07 were significantly associated with the virulent Andean race 39. Clusters of R genes with NBC-LRR domain on Pv02 and Pv04 were identified as candidate genes.

6.2. Recommendations

The genotype YBC278 that has been identified in the current study as being highly resistant to eight races of *Colletotrichum lindemuthianum* should be considered for use as source of anthracnose resistance for those breeding programs aiming to develop yellow common bean varieties with durable resistance to anthracnose. There is also need to determine the genetic basis of the observed superior anthracnose resistance in YBC278 to facilitate its use as a source of resistance. Development of yellow bean varieties with durable anthracnose resistance will require pyramiding of the Andean locus Co-1 and any major effect Middle American loci such as the one on Pv04, which appears to confer resistance to a relatively large number of *Colletotrichum lindemuthianum* races.

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APPENDICES

Appendix 1: Anthracnose severity scores for 273 YBC genotypes evaluated for resistance to Races 5, 19, 39, 51, 81, 183, 1050 and 1105 of *Colletotrichum lindemuthianum* in the greenhouse at University of Zambia, Lusaka, Zambia.

YBC ID	Genepool	Race 5	Race 19	Race 39	Race 51	Race 81	Race 183	Race 1050	Race 1105	Mean
YBC001	Middle-American	9.0	.	.	.	9.0	9.0	.	9.0	9.0
YBC002	Andean	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC003	Andean	9.0	9.0	9.0	9.0	.	9.0	4.5	9.0	8.4
YBC004	Andean	.	1.0	9.0	.	1.0	3.8	1.0	9.0	4.1
YBC005	Andean	9.0	9.0	9.0	.	.	9.0	9.0	9.0	9.0
YBC006	Andean	9.0	9.0	8.8	.	.	9.0	9.0	9.0	9.0
YBC007	Andean	9.0	9.0	9.0	.	7.0	9.0	9.0	2.0	7.7
YBC010	Middle-American	9.0	.	9.0	.	.	9.0	9.0	9.0	9.0
YBC011	Middle-American	9.0	7.8	8.0	7.0	9.0	9.0	9.0	1.0	7.5
YBC012	Middle-American	9.0	9.0	9.0	9.0	.	9.0	8.0	9.0	8.9
YBC014	Middle-American	1.0	.	1.0	.	.	1.0	6.3	1.0	2.1
YBC015	Andean	9.0	3.5	1.3	.	.	9.0	9.0	1.0	5.5
YBC017	Middle-American	.	9.0	9.0	9.0	.	9.0	7.3	1.2	7.4
YBC018	Middle-American	9.0	9.0	9.0	.	.	9.0	9.0	9.0	9.0

YBC021	Middle-American	9.0	9.0	8.8	9.0	.	9.0	9.0	9.0	9.0
YBC022	Middle-American	9.0	9.0	9.0	9.0	.	1.3	9.0	9.0	7.9
YBC024	Andean	6.5	9.0	9.0	.	.	.	9.0	9.0	8.5
YBC025	Andean	1.0	1.0	9.0	9.0	.	1.0	9.0	1.0	4.4
YBC026	Andean	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC028	Andean	1.6	.	6.3	1.0	.	5.7	5.0	1.0	3.4
YBC029	Andean	1.0	9.0	1.0	1.0	6.3	9.0	9.0	1.0	4.7
YBC030	Andean	8.3	4.0	6.3	.	.	9.0	9.0	4.2	6.8
YBC034	Andean	.	.	4.0	.	.	.	9.0	1.0	4.7
YBC035	Andean	9.0	9.0	9.0	1.0	.	4.8	9.0	9.0	7.3
YBC036	Andean	9.0	9.0	9.0	.	8.0	9.0	9.0	4.8	8.3
YBC041	Andean	9.0	8.0	9.0	1.0	.	9.0	1.0	1.0	5.4
YBC045	Middle-American	9.0	.	9.0	.	7.0	9.0	9.0	9.0	8.7
YBC046	Middle-American	9.0	9.0	9.0	9.0	3.5	9.0	8.7	9.0	8.3
YBC047	Andean	1.0	1.0	1.3	1.0	.	1.0	9.0	1.0	2.2
YBC049	Middle-American	9.0	8.8	8.0	.	.	9.0	9.0	1.0	7.5
YBC050	Middle-American	9.0	1.0	9.0	9.0	8.5	1.0	6.0	1.0	5.6
YBC051	Middle-American	8.6	1.5	8.0	9.0	.	7.0	9.0	1.0	6.3
YBC052	Middle-American	9.0	9.0	9.0	.	.	9.0	.	9.0	9.0

YBC053	Middle-American	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC054	Middle-American	1.0	9.0	9.0	1.0	.	9.0	9.0	9.0	6.7
YBC055	Andean	9.0	9.0	9.0	.	.	9.0	5.3	1.0	7.0
YBC056	Middle-American	9.0	9.0	9.0	.	.	9.0	9.0	9.0	9.0
YBC057	Middle-American	9.0	2.5	1.0	.	.	8.7	9.0	1.0	5.2
YBC059	Middle-American	9.0	7.5	7.8	9.0	.	8.0	9.0	1.3	7.4
YBC061	Middle-American	9.0	9.0	9.0	.	.	9.0	8.0	9.0	8.8
YBC063	Andean	9.0	9.0	9.0	.	.	9.0	3.7	9.0	8.1
YBC064	Andean	1.0	9.0	9.0	1.7	.	9.0	1.0	1.0	4.5
YBC065	Andean	1.0	9.0	1.0	1.0	.	9.0	1.3	9.0	4.5
YBC066	Middle-American	1.0	9.0	9.0	2.0	1.0	9.0	1.0	9.0	5.1
YBC067	Andean	1.0	1.0	9.0	1.0	.	1.0	1.0	9.0	3.3
YBC068	Andean	9.0	.	.	1.0	.	9.0	.	9.0	7.0
YBC069	Middle-American	9.0	8.3	9.0	1.0	.	9.0	1.0	9.0	6.6
YBC070	Middle-American	1.0	.	9.0	1.0	1.0	9.0	2.0	.	3.8
YBC071	Middle-American	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC072	Andean	9.0	9.0	9.0	.	.	9.0	8.3	9.0	8.9
YBC073	Andean	9.0	9.0	9.0	9.0	7.5	9.0	9.0	7.0	8.6
YBC075	Andean	9.0	1.0	9.0	9.0	.	1.0	9.0	9.0	6.7

YBC076	Andean	9.0	3.7	9.0	9.0	9.0	9.0	9.0	1.0	7.3
YBC077	Andean	9.0	9.0	1.0	1.0	1.2	9.0	.	2.0	4.6
YBC078	Andean	.	1.3	3.0	.	9.0	.	9.0	1.0	4.7
YBC079	Andean	1.0	.	9.0	1.0	.	5.7	1.0	1.0	3.1
YBC080	Middle-American	9.0	9.0	9.0	9.0	.	1.0	9.0	1.0	6.7
YBC081	Andean	9.0	9.0	9.0	.	.	9.0	8.3	9.0	8.9
YBC082	Admix	9.0	9.0	9.0	9.0	.	9.0	8.3	.	8.9
YBC083	Admix	9.0	9.0	9.0	9.0	.	9.0	8.0	9.0	8.9
YBC084	Andean	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC085	Middle-American	9.0	9.0	9.0	.	.	9.0	8.5	9.0	8.9
YBC086	Andean	9.0	9.0	9.0	9.0	.	9.0	8.3	9.0	8.9
YBC088	Andean	9.0	9.0	9.0	9.0	8.0	9.0	8.8	3.5	8.2
YBC089	Andean	9.0	9.0	.	.	.	9.0	1.0	2.0	6.0
YBC090	Andean	9.0	9.0	9.0	.	.	9.0	1.0	1.0	6.3
YBC091	Andean	9.0	9.0	9.0	1.0	.	9.0	9.0	9.0	7.9
YBC092	Andean	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC093	Andean	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC094	Andean	9.0	.	4.8	.	8.7	9.0	9.0	1.0	6.9
YBC095	Andean	9.0	9.0	9.0	.	8.2	9.0	7.8	9.0	8.7

YBC096	Andean	9.0	1.0	1.0	.	1.0	.	.	1.0	2.6
YBC097	Andean	9.0	7.3	.	3.0	8.8	9.0	8.3	9.0	7.8
YBC098	Andean	1.5	9.0	9.0	1.0	.	9.0	1.3	1.0	4.5
YBC099	Andean	.	9.0	1.0	.	8.8	1.5	9.0	9.0	6.4
YBC100	Middle-American	9.0	9.0	9.0	9.0	8.3	9.0	5.0	9.0	8.4
YBC101	Middle-American	9.0	9.0	9.0	9.0	1.3	9.0	1.0	9.0	7.0
YBC102	Andean	9.0	9.0	9.0	.	2.0	9.0	5.0	9.0	7.4
YBC103	Andean	9.0	9.0	9.0	.	.	9.0	.	9.0	9.0
YBC105	Andean	9.0	9.0	9.0	9.0	.	9.0	8.3	9.0	8.9
YBC106	Andean	9.0	8.7	8.5	9.0	.	8.3	9.0	9.0	8.8
YBC107	Andean	9.0	.	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC108	Andean	9.0	9.0	9.0	.	.	9.0	8.3	9.0	8.9
YBC109	Andean	1.7	9.0	9.0	1.0	8.0	9.0	8.8	9.0	6.9
YBC111	Andean	.	1.0	9.0	.	.	1.0	3.3	9.0	4.7
YBC112	Andean	9.0	9.0	9.0	.	.	9.0	8.3	9.0	8.9
YBC113	Andean	9.0	9.0	9.0	9.0	7.5	9.0	9.0	9.0	8.8
YBC114	Andean	9.0	1.0	9.0	8.0	3.3	9.0	1.3	1.0	5.2
YBC115	Andean	9.0	1.0	9.0	9.0	5.0	1.0	8.5	1.0	5.4
YBC116	Andean	9.0	.	9.0	.	.	9.0	9.0	9.0	9.0

YBC117	Andean	1.0	9.0	9.0	9.0	.	.	1.0	1.0	5.0
YBC118	Andean	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC119	Middle-American	1.0	1.0	9.0	9.0	.	1.0	9.0	5.7	5.1
YBC120	Andean	1.0	9.0	9.0	1.0	.	9.0	3.0	.	5.3
YBC121	Middle-American	.	9.0	8.3	1.0	.	9.0	1.0	1.0	4.9
YBC122	Andean	9.0	9.0	9.0	.	.	9.0	8.3	7.8	8.7
YBC123	Andean	9.0	9.0	9.0	.	7.0	9.0	9.0	1.0	7.6
YBC124	Middle-American	9.0	.	9.0	9.0	.	1.0	6.3	1.0	5.9
YBC125	Andean	1.0	9.0	9.0	6.0	9.0	9.0	9.0	9.0	7.6
YBC126	Andean	9.0	9.0	9.0	.	8.5	9.0	3.7	.	8.0
YBC127	Andean	9.0	9.0	1.0	.	.	9.0	9.0	5.3	7.0
YBC128	Andean	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
YBC129	Andean	9.0	.	9.0	9.0	.	9.0	7.0	9.0	8.7
YBC130	Andean	1.0	1.0	.	1.2	1.0	1.0	1.0	1.0	1.0
YBC131	Andean	1.3	.	1.0	1.0	9.0	1.3	9.0	1.0	3.4
YBC132	Andean	1.0	1.0	1.0	1.7	.	1.0	5.3	4.6	2.2
YBC133	Andean	9.0	2.3	9.0	.	8.8	1.0	8.3	9.0	6.8
YBC134	Admix	9.0	9.0	9.0	8.0	.	9.0	8.8	9.0	8.8
YBC135	Andean	9.0	9.0	7.7	1.0	1.0	9.0	1.0	.	5.4

YBC136	Andean	1.0	1.0	9.0	9.0	.	9.0	8.3	1.0	5.5
YBC137	Andean	9.0	1.0	9.0	9.0	.	5.0	8.0	3.5	6.4
YBC138	Andean	1.0	.	1.0	1.0	.	1.0	2.0	1.0	1.2
YBC139	Andean	1.5	1.3	9.0	.	7.0	1.0	1.8	9.0	4.4
YBC140	Andean	9.0	1.0	9.0	9.0	7.3	1.0	9.0	1.0	5.8
YBC141	Andean	.	9.0	9.0	9.0	8.8	1.0	9.0	9.0	7.8
YBC142	Andean	9.0	9.0	9.0	.	2.0	9.0	1.3	1.8	5.9
YBC143	Andean	1.0	1.0	9.0	1.0	.	1.0	9.0	9.0	4.4
YBC144	Andean	9.0	.	8.3	.	9.0	6.3	8.5	1.0	7.0
YBC145	Andean	9.0	1.3	9.0	9.0	8.5	.	9.0	1.8	6.8
YBC146	Andean	5.7	3.8	9.0	9.0	9.0	3.0	1.0	9.0	6.2
YBC147	Andean	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC148	Andean	9.0	9.0	9.0	8.0	9.0	9.0	9.0	9.0	8.9
YBC149	Andean	1.0	9.0	8.0	1.0	1.0	1.3	4.0	1.0	3.3
YBC150	Andean	9.0	9.0	9.0	9.0	.	9.0	8.3	9.0	8.9
YBC151	Andean	9.0	1.5	9.0	9.0	8.2	1.0	7.5	9.0	6.8
YBC152	Andean	1.0	1.0	9.0	1.0	.	1.5	1.3	1.0	2.3
YBC153	Andean	1.0	9.0	9.0	1.0	.	1.3	9.0	1.0	4.5
YBC154	Middle-American	7.0	7.3	8.5	9.0	.	9.0	2.8	1.0	6.4

YBC155	Admix	9.0	1.0	9.0	9.0	8.0	1.0	8.5	.	6.5
YBC156	Andean	9.0	1.0	9.0	9.0	7.3	1.0	7.3	.	6.2
YBC157	Andean	1.3	9.0	9.0	1.0	.	9.0	1.0	9.0	5.6
YBC159	Andean	1.0	8.3	9.0	1.0	8.0	9.0	9.0	9.0	6.8
YBC160	Andean	1.0	1.0	1.0	1.0	7.0	1.0	8.0	9.0	3.6
YBC161	Andean	9.0	1.0	9.0	9.0	.	1.0	1.3	4.2	4.9
YBC162	Middle-American	1.0	6.7	5.0	1.0	8.5	9.0	9.0	1.0	5.1
YBC163	Andean	9.0	9.0	9.0	9.0	1.0	9.0	9.0	6.3	7.7
YBC165	Andean	9.0	1.5	2.3	1.0	.	8.3	9.0	1.0	4.6
YBC166	Andean	9.0	9.0	.	9.0	9.0	9.0	9.0	9.0	9.0
YBC167	Andean	1.0	9.0	9.0	7.0	9.0	3.8	9.0	9.0	7.1
YBC168	Andean	9.0	7.0	8.5	3.0	6.5	3.0	8.3	9.0	6.8
YBC169	Andean	1.0	9.0	9.0	1.0	1.0	9.0	1.0	5.8	4.6
YBC170	Andean	2.0	.	9.0	1.0	.	9.0	9.0	.	6.0
YBC171	Andean	2.8	9.0	9.0	1.0	6.7	9.0	9.0	1.4	6.0
YBC172	Andean	9.0	8.8	7.8	1.0	1.0	9.0	1.0	1.0	4.8
YBC173	Andean	1.0	1.0	9.0	1.0	1.0	1.0	1.3	1.0	2.0
YBC174	Andean	9.0	1.0	1.0	1.0	1.0	1.5	1.0	1.0	2.1
YBC175	Andean	9.0	.	9.0	.	.	9.0	1.0	1.0	5.8

YBC176	Andean	9.0	9.0	9.0	.	.	9.0	9.0	9.0	9.0
YBC177	Andean	9.0	9.0	1.0	2.2	.	9.0	1.0	9.0	5.7
YBC178	Andean	.	9.0	4.0	1.0	1.0	9.0	.	9.0	5.5
YBC179	Andean	9.0	9.0	9.0	9.0	.	9.0	1.0	9.0	7.9
YBC180	Andean	9.0	1.5	9.0	9.0	8.0	1.0	6.7	9.0	6.6
YBC181	Andean	2.0	1.0	9.0	1.3	9.0	1.0	9.0	1.0	4.2
YBC182	Andean	9.0	2.8	9.0	1.0	.	9.0	6.0	9.0	6.5
YBC183	Andean	1.0	1.0	9.0	3.0	.	1.0	1.7	9.0	3.7
YBC184	Middle-American	9.0	1.3	9.0	.	.	9.0	7.0	1.0	6.0
YBC186	Andean	9.0	3.0	8.8	9.0	.	9.0	7.8	9.0	7.9
YBC187	Middle-American	9.0	9.0	9.0	9.0	.	9.0	8.0	9.0	8.9
YBC190	Andean	9.0	1.0	8.5	9.0	8.0	3.3	7.0	9.0	6.8
YBC191	Middle-American	9.0	9.0	2.5	2.0	1.4	.	1.0	1.0	3.7
YBC192	Andean	1.0	1.3	.	1.0	6.8	1.0	1.7	1.0	2.0
YBC193	Andean	9.0	4.0	3.8	2.0	9.0	9.0	7.3	9.0	6.6
YBC194	Andean	1.3	9.0	1.0	1.0	.	9.0	1.0	9.0	4.5
YBC195	Andean	9.0	9.0	1.0	.	.	.	8.5	9.0	7.3
YBC196	Andean	1.0	9.0	3.0	7.0	.	9.0	.	1.2	5.0
YBC197	Andean	.	9.0	9.0	.	.	.	9.0	.	9.0

YBC198	Andean	1.0	9.0	.	1.0	.	9.0	1.3	1.0	3.7
YBC199	Andean	7.6	9.0	9.0	1.0	.	9.0	9.0	1.2	6.5
YBC200	Andean	9.0	.	9.0	5.0	.	9.0	9.0	9.0	8.3
YBC203	Andean	9.0	3.7	7.5	3.0	8.7	1.8	9.0	1.2	5.5
YBC204	Andean	9.0	.	9.0	9.0	9.0	9.0	8.8	9.0	9.0
YBC206	Andean	9.0	9.0	1.0	1.0	.	1.8	.	1.3	3.8
YBC207	Andean	.	.	.	2.0	.	.	.	1.0	1.5
YBC208	Andean	9.0	.	1.0	.	.	9.0	9.0	1.0	5.8
YBC209	Andean	9.0	.	3.0	4.0	.	9.0	.	7.0	6.4
YBC210	Middle-American	9.0	9.0	3.8	1.0	6.5	9.0	8.8	8.5	6.9
YBC211	Andean	9.0	9.0	1.8	7.0	7.3	9.0	9.0	5.8	7.2
YBC212	Andean	9.0	9.0	1.0	.	.	9.0	9.0	.	7.4
YBC213	Andean	9.0	9.0	1.0	2.0	7.0	9.0	.	9.0	6.6
YBC214	Andean	9.0	9.0	9.0	9.0	.	9.0	3.0	6.0	7.7
YBC215	Andean	9.0	.	1.0	.	.	9.0	8.8	9.0	7.4
YBC216	Andean	9.0	9.0	2.5	.	9.0	9.0	9.0	9.0	8.1
YBC217	Admix	1.0	.	8.3	1.0	.	4.5	7.0	5.0	4.5
YBC221	Middle-American	9.0	9.0	.	.	.	1.0	7.0	1.0	5.4
YBC222	Andean	7.8	9.0	1.0	.	1.0	9.0	9.0	1.0	5.4

YBC223	Andean	9.0	4.3	1.0	1.0	.	9.0	9.0	1.0	4.9
YBC224	Andean	9.0	1.3	9.0	9.0	.	1.0	8.0	9.0	6.6
YBC225	Andean	.	9.0	9.0	9.0	.	1.3	9.0	1.0	6.4
YBC226	Andean	.	9.0	9.0	.	.	9.0	9.0	9.0	9.0
YBC229	Andean	9.0	9.0	8.8	9.0	.	9.0	9.0	9.0	9.0
YBC230	Andean	9.0	9.0	9.0	.	.	9.0	9.0	9.0	9.0
YBC231	Andean	9.0	.	1.0	1.0	.	1.0	8.0	9.0	4.8
YBC232	Andean	9.0	9.0	9.0	9.0	.	9.0	9.0	9.0	9.0
YBC233	Andean	9.0	9.0	3.0	1.0	.	9.0	.	9.0	6.7
YBC235	Andean	9.0	9.0	1.3	1.0	.	9.0	9.0	1.0	5.6
YBC236	Andean	9.0	.	1.0	1.0	8.5	9.0	9.0	9.0	6.6
YBC237	Andean	9.0	9.0	9.0	1.3	3.7	9.0	1.0	9.0	6.4
YBC238	Andean	9.0	9.0	7.3	.	9.0	.	6.5	1.2	7.0
YBC239	Andean	.	9.0	2.8	1.0	.	7.0	.	1.0	4.2
YBC240	Andean	9.0	9.0	2.0	.	.	9.0	9.0	4.2	7.0
YBC241	Andean	.	9.0	3.8	1.0	.	9.0	9.0	1.0	5.5
YBC242	Andean	9.0	9.0	9.0	9.0	8.0	9.0	9.0	9.0	8.9
YBC243	Andean	9.0	9.0	1.0	.	.	9.0	9.0	7.8	7.5
YBC244	Andean	9.0	9.0	1.0	1.6	.	9.0	9.0	9.0	6.8

YBC245	Andean	9.0	9.0	1.0	.	5.0	9.0	9.0	9.0	7.3
YBC246	Andean	9.0	9.0	1.0	.	.	9.0	9.0	9.0	7.7
YBC247	Andean	9.0	9.0	9.0	1.0	.	9.0	1.0	9.0	6.7
YBC248	Admix	9.0	9.0	1.3	5.5	.	9.0	9.0	8.3	7.3
YBC249	Andean	9.0	.	1.7	2.0	.	9.0	.	1.0	4.5
YBC250	Admix	9.0	1.0	8.0	7.0	1.0	1.0	7.8	9.0	5.5
YBC253	Middle-American	9.0	1.0	9.0	9.0	7.0	1.0	8.0	9.0	6.6
YBC254	Admix	9.0	1.0	8.3	9.0	8.0	3.0	8.0	1.0	5.9
YBC256	Andean	9.0	.	.	9.0	1.0	8.8	1.0	1.0	5.0
YBC258	Andean	.	1.0	9.0	9.0	.	1.0	9.0	9.0	6.3
YBC259	Andean	9.0	9.0	9.0	9.0	.	8.0	1.3	9.0	7.8
YBC260	Andean	9.0	1.0	9.0	4.0	7.5	9.0	9.0	9.0	7.2
YBC262	Andean	9.0	9.0	9.0	9.0	.	9.0	1.0	9.0	7.9
YBC263	Admix	1.2	1.5	9.0	9.0	.	1.8	9.0	4.2	5.1
YBC266	Andean	8.0	1.3	9.0	5.0	.	1.0	9.0	9.0	6.0
YBC267	Andean	3.3	1.0	1.0	1.0	1.3	3.5	.	1.0	1.7
YBC268	Andean	9.0	1.0	9.0	9.0	2.0	1.0	9.0	.	5.7
YBC273	Andean	1.0	1.0	1.0	.	.	1.0	3.0	.	1.4
YBC274	Andean	9.0	.	5.7	.	7.0	9.0	9.0	9.0	8.1

YBC275	Andean	9.0	7.5	8.3	.	.	9.0	9.0	9.0	8.6
YBC277	Admix	9.0	9.0	9.0	.	1.0	9.0	1.5	1.0	5.6
YBC278	Admix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
YBC279	Andean	1.0	1.0	1.0	.	8.2	1.0	1.5	1.0	2.1
YBC280	Andean	1.0	.	6.7	1.0	1.0	1.3	1.0	5.5	2.5
YBC281	Andean	1.0	2.0	9.0	.	2.4	1.3	7.8	9.0	4.6
YBC282	Andean	1.0	1.0	1.0	.	.	2.0	1.0	1.0	1.2
YBC283	Andean	1.0	1.0	1.0	1.0	.	1.0	8.5	1.0	2.1
YBC284	Andean	1.0	1.0	1.0	1.0	9.0	1.0	9.0	1.0	3.0
YBC285	Andean	1.0	1.5	1.0	.	1.0	1.0	9.0	9.0	3.4
YBC286	Andean	.	1.0	4.0	1.6	9.0	4.8	8.3	1.0	4.2
YBC287	Andean	1.0	7.3	4.8	1.0	.	4.8	9.0	1.0	4.1
YBC288	Andean	1.0	8.8	9.0	.	.	1.0	8.3	1.0	4.8
YBC289	Andean	4.2	3.0	4.5	1.0	8.0	9.0	8.3	9.0	5.9
YBC290	Andean	1.0	9.0	5.3	.	3.0	9.0	9.0	9.0	6.5
YBC291	Andean	1.2	1.0	1.0	.	7.0	1.3	8.0	9.0	4.1
YBC292	Middle-American	1.0	1.0	1.0	5.0	7.0	1.0	.	9.0	3.6
YBC293	Admix	1.0	1.0	3.3	1.0	7.7	3.0	5.0	1.0	2.9
YBC294	Admix	1.0	1.0	1.8	1.0	8.5	3.0	8.7	9.0	4.2

YBC295	Andean	1.0	1.3	.	.	8.3	3.5	9.0	1.0	4.0
YBC296	Middle-American	1.0	1.3	1.8	1.0	7.8	1.0	8.8	9.0	3.9
YBC297	Andean	2.3	.	1.0	.	6.5	5.3	9.0	1.0	4.2
YBC299	Andean	1.0	1.0	1.0	1.0	7.7	.	1.0	9.0	3.1
YBC300	Andean	1.6	2.3	1.0	2.0	1.0	1.8	6.3	8.5	3.1
YBC301	Andean	1.0	9.0	9.0	4.0	1.0	1.8	9.0	1.0	4.5
YBC302	Andean	7.2	2.3	4.3	9.0	5.0	1.0	8.3	1.0	4.8
YBC303	Andean	1.5	1.5	3.0	3.0	.	2.0	2.0	1.8	2.1
YBC304	Andean	9.0	9.0	6.7	7.0	8.3	9.0	5.3	9.0	7.9
YBC305	Andean	1.0	7.7	2.5	1.0	8.8	9.0	9.0	8.0	5.9
YBC306	Andean	.	1.0	4.5	2.0	.	.	.	1.0	2.1
YBC307	Andean	1.0	.	9.0	1.0	.	.	8.3	1.0	4.1
YBC308	Andean	9.0	1.0	8.3	5.0	9.0	1.0	9.0	9.0	6.4

Appendix 2: Positional candidate genes associated with significant SNPs for resistance to eight races of *Colletotrichum lindemuthianum*

Chr	SNP	Position(bp)	Candidate gene	Function
Pv01	Chr01_49583965	49583965	Phvul.001G243500	SER/THR-PROTEIN KINASE-LIKE PROTEI
	Chr01_49583965	49583965	Phvul.001G243600	SER/THR-PROTEIN KINASE-LIKE PROTEI
	Chr01_49583965	49583965	Phvul.001G243700	SER/THR-PROTEIN KINASE-LIKE PROTEIN
Pv02	Chr02_49318523	49318523	Phvul.002G328300	MITOGEN-ACTIVATED PROTEIN KINASE RELATED
Pv04	Chr04_1067693	1067693	Phvul.004G008900	LRR CONTAINING PROTEIN
	Chr04_1067693	1067693	Phvul.004G008909	LRR CONTAINING PROTEIN
	Chr04_1067693	1067693	Phvul.004G008918	LRR CONTAINING PROTEIN
	Chr04_1067693	1067693	Phvul.004G009100	LRR CONTAINING PROTEIN
	Chr04_1067693	1067693	Phvul.004G009136	LRR CONTAINING PROTEIN
	Chr04_1067693	1067693	Phvul.004G009154	LRR CONTAINING PROTEIN
	Chr04_1067693	1067693	Phvul.004G009300	LRR CONTAINING PROTEIN
	Chr04_1067693	1067693	Phvul.004G009500	LRR CONTAINING PROTEIN
	Chr04_1067693	1067693	Phvul.004G009527	LRR CONTAINING PROTEIN
	Chr04_1067693	1067693	Phvul.004G009800	LRR CONTAINING PROTEIN
	Chr04_40179029	40179029	Phvul.004G116600	LEUCINE RICH REPEAT (LRR_1/LRRNT_2/LRR_8)
	Chr04_40179029	40179029	Phvul.004G116800	CYTOCHROME B561-RELATED

Pv07	Chr07_27213163	27213163	Phvul.007G160701	LRR CONTAINING PROTEIN

Chr = Chromosome: SNP = Single Nucleotide Polymorphism: bp = base pairs: SER/THR = SERINE/THREONINE: LRR = Leucine Rich Repeat