ELUCIDATED EFFECT OF COMMON BEAN ANTHRACNOSE AND RESISTANCE BREEDING IN SOUTH AFRICA

by

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DEDICATION

To my dearest mother, SZ Mpayazi and the Mbhede family, for all those lengthy periods of time I was away from home you never gave up on me, you kept pushing me. Thank you for all the sacrifices, patients and understanding.

Declaration

I **Army Makweya Payazi** hereby declare that the dissertation, with the title: **ELUCIDATED EFFECT OF COMMON BEAN ANTHRACNOSE AND RESISTANCE BREEDING IN SOUTH AFRICA ,** which I hereby submit for the degree of **MSc Agriculture** at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other institution.

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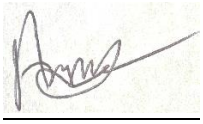
Student signature: 

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ARC	Agricultural Research Council
ARC-GC	Agricultural Research Council-Grain Crop
CBB	Common bacterial blight
CIAT	International Centre for Tropical Agriculture
CTAB	Cetyl trimethylammonium bromide
CV	Coefficient of Variation
DAFF	Department of Agriculture, Forestry and Fisheries
DF	Degrees of freedom
DNA	Deoxyribonucleic acid
GWAS	Genome-Wide Association Study
LSD	Least significant difference
PCR	Polymerase Chain Reaction
PDA	Potato dextrose agar.
R	Resistant
RAPDs	Random Amplified Polymorphic DNA
RSS	Red Speckled Sugar
S	Susceptible
SCAR	Sequence Characterised Amplified Region
SNP	Single Nucleotide Polymorphism
SW	Small white

ABSTRACT

Common bean (*Phaseolus vulgaris* L) is an important nutritionally rich crop and most affordable especially for the poor in the developing world. Existing limitations to production potential include diseases and of particular interest is anthracnose caused by the highly variable fungus *C lindemuthianum* that can be best managed through resistant breeding. Therefore, it was the purpose of this study to identify and evaluate the distribution of pathogenic races, phenotypically evaluate genotypes, and develop resistant inbred lines with the aid of SCAR makers.

During the first study, thirty-two isolates were collected in three major common bean production provinces in South Africa and evaluated in a glasshouse study at ARC-GC. The 12 CIAT differential cultivars were spray-inoculated and evaluated. Eight races of *C. lindemuthianum* were identified and were races 3, 6, 7, 81, 83, 89, 263 and 323. Only landraces AB 136, G 2333, Kaboon, TU and PI 207262 showed complete resistance.

In the second study, two separate field survey trials consisting of 51 germplasm and 26 commercial common bean cultivars were evaluated for reaction to anthracnose in South Africa. The trials were conducted in RCBD with 3 replications, at Potchefstroom and Cedara. A scale of 1-9 was used for disease severity evaluation. A total of 92% genotypes were resistant to anthracnose race 6 in Potchefstroom, on the contrary, in Cedara only 49.35% of genotypes were immune to four *C. lindemuthianum* races, which were later identified as 7, 81, 83 and 89. Only 25 germplasm were resistant in both locations.

The third study seek to validate a total of five SCAR markers. Three previously reported races 7, 81, and 89 of anthracnose were used to evaluate 26 genotypes and two F_2 populations. The selection of these races was based on the field reaction of genotypes. The F_2 generations were developed with a special interest in the *Co-4* locus and associated alleles because of high

resistance to anthracnose races. Twenty-three of the genotypes showed complete to partial resistance and only SW1, RS 7, and Teebus were susceptible. Makers SAS13, SBB14, SY20, and SCO8 were all loci specific, however, they were not allele specific. SBB12 was loci and allele specific as a single distinct band was detected in PI 207262 for *Co-9*.

Overall, the study has shown the presence of a wide geographic distribution of *C. lindemuthianum* races, diverse genetic resources, and linkage between the SCAR markers and *Co* races of interest for selection breeding in South Arica.

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1.1 GENERAL INTRODUCTION

This research study outlines issues relating to the control and management of anthracnose, a seed-borne fungal disease, which hampers the production of common beans globally. In this study, the variability of anthracnose existence in South Africa and its potential in affecting common bean production are investigated. As well as exploring the existing genetic materials that could potentially provide better management of the disease in South Africa. In the study, use of DNA Molecular markers to evaluate sources of anthracnose resistance is tested in two geographic regions with contrasting climatic conditions for the control of the disease in South Africa. The study is divided into several sections. This section (chapter 1) provides an orientation and background to the study, problem statement and rationale including the aim/objectives and hypothesis of the study. A brief literature review on the taxonomy of the fungus and the economic importance of anthracnose including its symptoms, prevalence and epidemiology is provided in the next section (chapter 2), which also discusses the current control measures available for the disease. The next three sections (chapters 3, 4 & 5) are research chapters that present and discusses the study findings in relation to the specific objectives and the last section (chapter 6) provides the general discussion and recommendations.

1.2 BACKGROUND

Common bean (*Phaseolus vulguris* L.) is regarded as a temperate to cool tropic crop, despite nativity; common bean is widely adapted and grown in all parts of the world except for Antarctica (Allen *et al.,* 1989). As much as they are adapted in different continents, common beans originated in the highlands of Mexico and South America (Beebe *et al.,* 2001; Mamidi *et al.,* 2011). Common bean is one of the most important grain crops produced in most countries

in the world, and by far the most important leguminous crop in South Africa (Department of Agriculture, Forestry and Fisheries (DAFF), 2012) and in most developing countries (Zuiderveen *et al*., 2016), mainly in Latin America, Africa and Asia (Berglund and Brucher, 1976; Broughton *et al.*, 2003; Mohammed, 2013). Moreover, common beans are a major staple source of food in Latin America and eastern and southern African countries (Broughton *et al.*, 2003). An estimated 23 million metric tonnes (MT) of common bean are produced globally annually, with Africa and Latin America contributing 7 million metric tonnes (Broughton *et al.,* 2003). Common bean is rich in protein and provides for an alternative source over meat with 20-30% protein in dry mass (Hassan and Alabdalall, 2010), thus providing the much-needed nutrition for over 100 million poor people in rural and poor urban communities in Africa at an affordable price. South Africa produces about 75% of the nationally consumed common bean annually in the country (DAFF, 2012), having a deficit of 25%, which is imported from other countries. In South Africa, among the nine provinces, Mpumalanga and Free State are the major producing provinces (DAFF, 2014).

However, in South Africa, several factors hinder the production of common beans. These factors adversely affect the common bean potential maximum yield, and this include hindrances such as drought, poor soils and a vast number of diseases affecting the bean crop (Kaser, 1976; Kyamanywa, 1997; Opio *et al.,* 2001: Graham and Vance, 2003; Blair *et al*., 2010). Common bean diseases are diverse, and the pathogens can easily adapt to an area where they are not yet declared as a major constraint to the production of common beans (Broughton *et al.,* 2003). One of the major diseases threatening common bean production is anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cavi (Graham and Ranalli, 1997), and is no exception. Anthracnose is a seed-borne fungal disease that causes symptoms on the above ground parts of the plant, i.e., leaves, pods, and seeds, thereby causing

yield reduction and poor seed quality (Schwartz *et al.,* 1981: Markell *et al.,* 2012). The disease has been recorded in major production regions around the world especially in the tropics and temperate regions (Edington, 1994; Mahuku *et al*., 2002; Kimani *et al*., 2005; Mohammed, 2013; Teran *et al*., 2013). The negative impact of anthracnose under favourable conditions is detrimental and can lead to yield losses of up to 100% (Mahuku *et al*., 2002; Schwartz *et al*., 2005; Mohammed, 2013). In South Africa, anthracnose is extensively distributed with significant prevalence in the KwaZulu-Natal Province due to the cool humid conditions that favours development of the diseases (Edington, 1994).

1.3 RATIONALE OF THE STUDY

South Africa is self-insufficient with a staple crop such as common bean, this precludes for providing cheapest nutritional food for poor South Africans at the lowest prices possible. Further, there is prospect for potential job creation, the country's ginormous challenge, which is depended on agricultural growth according to the national development plan (NDP) is also inhibited or drastically reduced. Anthracnose is a well-documented and heavily researched common bean disease, especially in developed countries, where more focus was on environmental conditions, pathogen variability and adaptability and resistant breeding. However, the success in effectively controlling the seed-borne disease has been over the years below average for assuring sustainability. The control of the disease using resistance breeding has provided success that cease to exist due to the pathogen ability to circumvent resistance over a period. Recently, gene pyramiding is considered one of the most effective approaches in managing the disease because of its causal organism high variability. Despite the prevalence of the disease, limited literature is available in South Africa on anthracnose of common bean especially data detailing existing and ongoing pathogenic variability, potential yield and quality losses, and specified control strategies for areas with frequent and infrequent favourable

climatic or environmental conditions. Throughout the country, glimpses of anthracnose have been recorded at varying severity levels, and it has been demonstrated by recent work at the Agricultural Research Council Grain Crops Institute (ARC-GC) that all good yielding commercial cultivars lack the genetic composition for anthracnose resistance. This is further influenced and supported by the erratic performance of some cultivars under different production regions. The inconsistency is not only based on environmental conditions but also on the pathogen variability or a combination of both.

It is objectively acceptable to use disease free seeds and resistant cultivars by small-scale farmers to control anthracnose, however, its success would entail intensive investment in every production season on seed. Because of this, disease free seed does not justify such a decision, and on the contrary, the use of resistance cultivars is inconsistent since there is almost none. In addition, the common practise of saving seed from previous seasons further challenges the success of small-scale farmers in successfully producing quality beans that can sell at premium in the markets. Therefore, the challenge of improving disease resistance for existing cultivars is of great significance as this will ascertain ease in managing anthracnose for small-sale farmers and have the disease epidemic maintained over a long period. This will be achieved through the recovery of yield potential of commercial cultivars, minimises production losses, reduce production costs, stabilise yields and minimise the spread of the seed borne disease (Singh, 1992).

1.4 RESEARCH PROBLEM

Globally, the control and management of anthracnose is proving to be challenging. However, several control measures exist for anthracnose, ranging from crop rotation, clean or certified seeds, foliar fungicide, seed treatment, resistance cultivars, physical, cultural, biological, and integrated methods (Markell *et al*., 2012; Mohammed, 2013). From the abovementioned

control measures, the most important and effective control method is genetic resistance, which offers the least expensive long-term investment in successfully controlling the disease especially for small-scale farmers (Pastor-Corrales *et al*., 1995; Goncalves-Vidigal *et al*., 2007; 2009; Mohammed, 2013). However, periodic evaluation of cultivars is advised due to the presence of different races of anthracnose, and pathogen ability to adapt and cause resistance breakdown (Mohammed, 2013). Currently, over one hundred races of anthracnose have been reported from different production regions of the world (Zuiderveen *et al*., 2016), with regular, sporadic, and new developments in different areas, and this makes the control of the disease very difficult for both farmers and breeders.

With the support of molecular techniques together with plant morphology, seed protein, genetic composition (DNA) and isozymes it has been made clear that two gene pools of the bean plant exist (Gept *et al*., 1986; Beebe *et al.,* 2001; Mamidi *et al.,* 2011), namely the Andean and Mesoamerica (Gept *et al*., 1986; Singh *et al*., 1991; Gept and Debouck, 1991). The two gene pools are phenotypically differentiated by their seed size. The Andean gene pool is characterised by medium to large seeds (>80 g per 100 seeds) whereas the Mesoamerica are small seed (15-25 g per 100 seeds) to medium (45-55 g per 100 seeds) (Singh *et al*., 1991; DAFF, 2014). Because of the glimpse promises shown by resistance breeding over the years in ascertaining successful control of anthracnose in a much more affordable approach, it is of paramount importance to evaluate sources of resistance factoring the differences of the two groups of differentials with incorporating DNA-Molecular markers especially in regions with significant disease prevalence such as the KwaZulu-Natal Province in South Africa. DNA-Molecular markers allow for multiple advantages, ranging from better understanding of the fungus structure and its population, as well as identifying specific resistance genes of interest (Padder *et al*., 2017), thereby saving time through accelerating the breeding process. There are

several markers reported to be yielding significant results in mapping resistance for anthracnose, namely SSR (Simple sequence repeats), RAPD (random amplified polymorphic DNA) and SNP (Single nucleotide polymorphism). Nevertheless, costs and transition in technology makes it even more challenging and interesting in selecting a marker of choice that will serve a proposed purpose (Padder, *et al*., 2017). Notably, only a single study has evaluated the sources of anthracnose resistance with incorporating SCAR DNA-Molecular markers in South Africa.

The proposition of the study was on the basis that the research knowledge that will be acquired will contribute to the better management of anthracnose through awareness according to the disease ability to change genetic structure and induce continual yield losses and degrading the quality of common bean in South Africa. Finally, provide a channel for redirecting research activities to benefit common bean production in South Africa.

1.5 RESEARCH QUESTIONS

The study was an attempt to answer a main question together with specific questions related to the distribution, management, and control of anthracnose on common bean production in South Africa.

1.5.1 Main research question

How has anthracnose *C lindemuthianum* advanced in South Africa, and what implications does this have in successfully controlling the disease using resistant cultivars?

1.5.2 Specific research questions

- Different *C. lindemuthianum* races exist sporadically under different production regions in South Africa and do they have different effects on common bean production?

- Are South Africa's common bean commercial cultivars susceptible to multiple races of anthracnose?
- Does the evaluated germplasm from ARC-GC have sufficient resistant genes for the development of anthracnose resistant cultivars?
- Can the validated SCAR markers give and locate the sources of resistance from different germplasm and the specific genes involved in controlling resistance to *C. lindemuthianum* races?

1.6 AIM AND OBJECTIVES

1.6.1 Aim of the study

The overall aim of the study was to investigate the status of common bean anthracnose as a potential destructive epidemic in South Africa under different production regions, phenotypically and genetically evaluate selected germplasm for resistance and develop resistant-inbred lines using locally sourced genetic materials as a long-term solution for ascertaining increased yields, thus increasing profits for small-scale famers.

1.6.2 Specific objectives

- To survey anthracnose disease and determine common bean yield losses in both low and major production localities.
- To identify *C lindemuthianum* different races and evaluate pathogenicity using isolates collected from different production areas.
- To assess the genetic diversity existing in the collection of common bean germplasm available for use in developing new inbred lines.
- To develop resistant inbred lines using backcross and evaluate these lines using SCAR's markers.

CHAPTER 2: LITERATURE REVIEW

2.1 COLLETOTRICHUM LINDEMUTHIANUM TAXONOMY

Before eventually assenting to the principled nomenclature, several synonyms were used (Zaumeyer and Thomas, 1957) and confusions in the process resulted (Mohammed, 2013). The fungus, *C lindemuthianum,* is an imperfect stage causing bean anthracnose belonging to the genus *Colletotrichum,* family; Melanconiales, class; Deutoromycetes, Sub Class; Coelomycetidae, order; Melanconiales, Division; Amastigomycota, Subdivision; Deuteromycotina, and Kingdom; Myceteae. The pathogen has a perfect stage *Glomerella cingulate* that is rarely found in culture nor nature (Zaumeyer and Thomas, 1957).

2.2 HISTORY OF INFECTION AND GEOGRAPHIC DISTRIBUTION OF ANTHRACNOSE

Anthracnose occurs in all major common bean production regions of the globe with varying impact in these areas due to climactic conditions variability (Hagedorn and Inglis, 1986; Mohammed, 2013; Padder *et al*., 2017). In 1875, for the first time Lindemuth described anthracnose from a sample of plant specimen obtained in Germany and reported it (Thaung and Walker, 1957; Walker, 1957; Zaumeyer and Thomas, 1957). The disease was common in North America, Italy, France, and Germany (Zaumeyer and Thomas, 1957). Between 1912 and 1920, anthracnose was considered the most important disease of common bean causing poor germination, poor emergency, and very poor yields (Zaumeyer and Thomas, 1957). Later, numerous anthracnose reports followed from the European countries, Latin America, Asian countries, Australia, New Zealand, East and West Indies, and African countries (Zaumeyer and Thomas, 1957). Anthracnose has occurred in all production regions apart from areas with a prohibitive climate such as in the Antarctica (Zaumeyer and Thomas, 1957).

Anthracnose is caused by a hemibitroph fungus, *C. lindemuthianum* (Hagedorn and Inglis, 1986). Due to the pathogen's hemibitrophic activity it is capable of surviving season to season on either dead plant materials, alternative bean crop and the harvested seed (Hagedorn and Inglis, 1986; Mohammed, 2013). As much as more than one sources of disseminating the disease exist, seeds play a dominant role in spreading the disease internationally (Mohammed, 2013).

2.3 ECONOMIC IMPORTANCE

A good, if not excellent, yielding cultivar is a product that every farmer aspires for, however, if the yield is reduced and seed quality is compromised by discolouration, the farmers returns are compromised as well. Anthracnose is a seed-borne disease that causes shrunken and blemished seeds that are not appealing to the consumers' eye (Mohammed, 2013), with the ability of causing poor germination and heavily reduced yield (Zaumeyer and Thomas, 1957; Sharma *et al*., 2008). Therefore, the economic importance of anthracnose is of grave concern for the farmer. Losses caused by anthracnose vary throughout production regions and production seasons due to favourable weather conditions (del Rio *et al*., 2003). The earlier the infection, under favourable condition, is the more likely that the loss will be severe (Sharma *et al*., 2008).

Over the years in developed countries, anthracnose has been on the decline (Hagedorn and Inglis, 1986). However, large common bean production is from developing countries where the technology and related resources are poor (Hagedorn and Inglis, 1986; Yesuf and Sangchote, 2005). This includes sub-Sahara African countries, whereby an estimated annual common bean yield loss due to anthracnose was 69,800 and 328,000 tonnes, respectively (Kimani *et al*., 2005). In south Africa alone an estimated 20 to 30 kg/ha of common bean is lost due to anthracnose (Farrow and Muthoni, 2020). The use of previous season's seed for

planting exacerbates the losses induced by anthracnose (Mohammed, 2013), as infected seed serve as primary inoculum for the next season (Singh and Schwartz, 2010).

2.4 SYMPTOMS

Anthracnose infects the seed, leaves, stems with petioles and pods. Seedlings from infected seed show brown to black blemishes and sunken lesion in cotyledons thereby causing premature stunting (Zaumeyer and Thomas, 1957; Tu, 1988). Leaves affected by anthracnose show sunken and elongate lesions extending on the veins and petioles on the abaxial side of the leaf (Figure 2.1 A) (Hagedorn and Inglis, 1986), and as the disease progresses, dark brown to black discolouration occurs on both abaxial and adaxial sides of the leaf (Zaumeyer and Thomas, 1957; Hagedorn and Inglis, 1986; Tu, 1988). Heavy infestation on petioles can cause leaves to fall (Zaumeyer and Thomas, 1957). Stem symptoms are characterized by circular reddish brown to black border with greyish black interior lesions (Mohammed, 2013). The most obvious anthracnose symptoms are on the pods. Pods have small, reddish brown or purplish to black blemishes that are circular (Figure 2.1 B) (Zaumeyer and Thomas, 1957; Hagedorn and Inglis, 1986; Tu, 1988; Mohammed, 2013) with a diameter of between 1 to 10 mm (Zaumeyer and Thomas, 1957; Hagedorn and Inglis, 1986; Mohammed, 2013).

Figure 2. 1. Common bean symptoms. Abaxial side ***(A)*** and pod ***(B)***.

Infected seed have brown to light chocolate-coloured spots on the seed coats (Tu, 1984), depending on disease severity, symptoms may extend to the cotyledons (Tu, 1984). The symptoms on the seed can easily be confused with the symptoms caused by bacterial blight organism causing common bacterial blight and halo blight (Zaumeyer and Thomas, 1957). However, the presence of yellow bacterial deposits under the seed coat caused by common bacterial blight (Zaumeyer and Thomas, 1957), and the presence of the brick-red to dark brown colour on the seed affected by anthracnose differentiates the two bacterial blights diseases symptoms from anthracnose (Hagedorn and Inglis, 1986).

2.5 HOST RANGE

Colletotrichum has a wide range of host of horticultural crops; however, the success of *C. lindemuthianum* is fundamentally limited to *Phaseolus* species (*Phaseolus lunatus* L.), scarlet runner beans (*P. coccieus*) and tepany beans (*P. acutitolius* var. latifolius L.) (Zaumeyer and Thomas, 1957). Nevertheless, *C. lindemuthianum* have been isolated on other leguminous crops, these includes legumes such as Mung bean (*Vigna radiate*), cowpea (*Vignaung viculata*), kudzu beans (*Dolichos bitloris* L.), and broad beans (*Vicia faba* L.), soybean (*Glycine max*), pea (*Pisum sativum*) and black gram (*Vigna mungo*) (Mohammed, 2013).

2.6 EPIDEMIOLOGY

2.6.1 Pathogenicity

The pathogenic variability complexity of *C. lindemuthianum* is the challenge that breeders face in breeding for resistance to the disease. *C. lindemuthianum* variability has over the years resulted in resistance breakdown of commercial cultivars (Ishikawa *et al*., 2008; Mota *et al*., 2016). A better understanding of the pathogen developments and abilities to adapt under different conditions is what could lead to the better management of the disease. The first report documenting variability of *C. lindemuthianum* was in the 19th century (Barrus, 1911). On the

report, the pathogen variability based on inoculating different bean cultivars of the *vulgaris* species was illustrated, where plants reacted differently. It was subsequently decided to categorize the isolates into two distinct physiological races, namely alpha and beta. As years went on several different races were reported in various parts of the world and were found to be distinct from the alpha and beta, these included the gamma (Burkholder, 1923).

However, the standardization of *C. lindemuthianum* races identification provided a fixed system that has resulted in a better and consistent way to categorize the pathogen races all over the world (Pastor-Corrales, 1991). The widely used standardized system comprise of 12 bean cultivars (Table 2.1) (Pastor-Corrales, 1991). From the 12 differential cultivars, eight belong to the Mesoamerica and four to the Andean region. Each differential has a binary number and the sum of the cultivars with susceptible reactions gives the number of a specific race (Pastor Corrales, 1991). Currently, about 1590 isolates of *C. lindemuthianum* inoculated on 12 bean differential cultivars have resulted in the identification of 182 races worldwide thus depicting a high pathogenic variability in pathogen population (Padder *et al.*, 2017).

2.6.2 *C. lindemuthianum* races in South Africa

Using the global standard nomenclature for race identification and categorizing of the pathogen races, it is possible to unbiasedly identify and compare the frequent occurrence of races in various parts of the country and the world as well. According to Muth (2008) and Mohamed (2003), several pathotypes were identified in South Africa in different production and research areas (Table 2.2).

Table 2. 1. CIAT Common bean Anthracnose differential cultivars

Differential cultivar	Origin	Resistance Gene	Binary value	Seed type/Colour	Growth habit
AB 136	Mesoamerican	*Co-6, co-8*	1024	Small red	IV
Michelite	Mesoamerican	*Co-11*	1	Small white	II
Michigan Dark Red Kidney	Andean	*Co-1*	2	Large dark red kidney	I
Perry marrow	Andean	*Co-13*	4	Medium white	II
Cornell 49242	Mesoamerican	*Co-2*	8	Small black	II
Widusa	Mesoamerican	*Co-15, Co-9*	16	Medium white	I
Kaboon	Andean	*Co-12*	32	Large crème	II
Mexico 222	Mesoamerican	*Co-3*	64	Medium white	I
PI 207262	Mesoamerican	*Co-43, Co-9*	128	Small tan	III
To	Mesoamerican	*Co-4*	256	Medium carioca	I
Tu	Mesoamerican	*Co-5*	512	Small black	III
G 2333	Mesoamerican	*Co-42, Co5, Co-7*	2048	Small red	IV

Growth habit: I= determinate; II= indeterminate bush, erect stem; III= indeterminate bush, weak stem, and prostrate branches; IV= indeterminate climbing habit. CIAT (1987)

Adapted from Mohamed (2003) and Kelly and Vallejo (2004)

Table 2. 2. *C. lindemuthianum* races reported in South Africa

Pathotype or race	***Location***	***Year***
89	Cedara	2007
7	Cedara	2007
81	Makhathini/Cedara	2003
263	Arnot/Delmas	2003
83	Cedara	2007
6	Kranskop/Potchefstroom	Unspecified
3	Delmas, Mlondozi and Syferbult	Unspecified
323	Delmas/Cedara	Unspecified
390	Kranskop/Potchefstroom	Unspecified
593	Bethlehem/Pietermaritzburg	Unspecified

Adapted from Mohammed, 2003 and Muth 2008.

2.6.3 Source of inoculum

The infected plant material that is left in the field as residues or bean straws serve as secondary source of infection (Tu, 1984). However, seed plays a critical role in introducing a source of the disease since it can keep the pathogen propagules within and inactive for an exceptionally long period of between 3 and 5 years (Tu, 1984; Tu, 1988). This is evident with the African small-scale farmers, where the re-use of seed is common after each season (Tu, 1984; Tu, 1988).

2.6.4 Optimum conditions for disease development

Cool and wet weather conditions favour the development of anthracnose. A minimum temperature of 13^{o}C and a maximum of 26^{o}C are considered sufficient to cause anthracnose (Tu, 1988), and 17^{o}C is regarded as optimum (Tu, 1988). Despite temperature playing a critical role in ascertaining the development of anthracnose, moisture or wet conditions play an even bigger role as it allows for the disease to develop on temperature above 26^{o}C. According to Tu (1988), anthracnose spread readily at temperature above 26 to 32^{o}C under wet conditions.

Relative humidity of 92% and above further contributes to the development of the disease (Tu, 1988).

2.6.5 Mode of entry

The most common mode of entry of *Colletotrichum* species in the host plant is direct penetration through the cuticle, and infection through wounds, natural openings, and stomatal cells, although they are not a prerequisite (Bailey *et al*., 1992). *Colletotrichum* species normally use appressoria as a prerequisite for penetration of host cuticles (Bailey *et al*., 1992). According to O'Connell *et al*. (1992) the penetration peg of *C. lindemuthianum* has the ability and strength to exert sufficient force for penetrating the plant cuticle. Attachment of spores to the plant surface signals the successful initiation of infection, however, adhesion is important in ascertaining the desired contact of spores with the plant surface for the desired period for penetration of the cuticle either mechanically or enzymatic (Bailey, 1991). As the conidia adheres to the plant tissues it germinates, produce germ tubes, and the appressoria (O'Connell *et al.,* 1985). In the epidermal layer the pathogen develops intramural network of hyphae thereby killing the host cell tissue around the infected area and rapidly spread as the plant undergoes physiological changes without showing immediate detrimental changes to the host plant (O'Connell *et al*., 1985; Dron and Bailey, 1999). The rapid spread is characterised by the development of inter and intracellular hyphae (Dron and Bailey, 1999). As soon as the damage starts showing, the pathogen produces thin hyphae causing widespread degradation of cell wall and tissue cells (O'Connell *et al*., 1985). Then acervuli with a water-soluble gelatinous matrix are formed by the mycelium of which it ruptures the host cuticle resulting in dark brown lesions (Mercer *et al*., 1975; Sindhan and Bose, 1981).

2.6.6 Survival

C. lindemuthianum remains viable in plant debris post harvesting in the field and on the seed as well (Nicholson and Moraes, 1980). The fact that *C. lindemuthianum* is hemibiotrophic, it only means its life span has an extension, since it can be a partial parasite and a saprophyte. However, environmental factors play a crucial role in determining the pathogen variability or longevity (Tu, 1984; Dillard and Cobb, 1993). According to Tu (1983), *C. lindemuthianum* longevity proved to be longer under dry conditions compared to moist conditions. *C. lindemuthianum* remained viable for 5 years under dry cool conditions and over if kept under dry environment at 4^{o}C (Tu, 1983). On the contrary, the findings of Tu (1983) under wet conditions showed that even a heavily infested field *C. lindemuthianum* could lose its viability the following season if exposed to moisture for longer. The decrease in pathogen viability under wet soil conditions can be attributed to the loss of mucilaginous water-soluble of the matrix (Nicholson and Moraes, 1980). The pathogen can survive low temperatures of -15 to -20^{o}C for a limited period as a dormant mycelium (Nicholson and Moraes, 1980).

2.6.7 Dissemination

Several factors play a vital role in disseminating the seed-borne causal organism, with some limited to short distances and some spreading the pathogen beyond boarders to other countries. Infected seed is the most common and effective way of disseminating the pathogen to new areas where the pathogen was previously not documented (Zaumeyer and Thomas, 1957), especially over long distances. The pathogen spores are enclosed in a sticky water-soluble gelatinous substance, from which they are only released through exposure of the substance to water which exposes the spores to rain, wind, human, machines, or insects (Zaumeyer and Thomas, 1957). According to Tu (1989), an average of 10 mm rain splash can spread the spores as far as 3-4, 6 metres; however, its success is depended on wind speed. Insect pests also play

a vital role in spreading the pathogen through contact with the masses of spores released from the mucilaginous substances, thereby adhering to their bodies, and transporting the spores to nearby common bean fields (Zaumeyer and Thomas, 1957).

2.6.8 Disease cycle

The seed-borne anthracnose disease cycle is initiated by the introduction of infected seed in the field, making it the primary source of infection together with infected plant debris. The infected plant debris allows for the pathogen to overwinter and under favourable conditions it gets viable and infects plant foliage through dissemination by either wind or water. The infected debris acts as secondary source of infection (Padder et al., 2017).

2.7 ANTHRACNOSE DISEASE CONTROL MEASURES

2.7.1 Cultural control method

Post harvesting of common bean, fields should be ploughed to allow for the burial of plant debris in the soil, and this practise should be applied to nearby fields that were previously infected by anthracnose (Buruchara *et al.,* 2010), preferably using mouldboard plough (Ntahimpera *et al.,* 1997). Sanitation helps in reducing the sources of infection from the different working materials such as leather, rubber, painted metal and denim. Anthracnose seed-borne nature implies that the seed should be kept free of infection. This can be achieved by avoiding seed produced under wet and humid conditions as they harbour the fungus inside their seed coat (Mohammed, 2013; Zaumeyer and Thomas, 1957), and the sowing of seed sourced from previously infected fields with anthracnose (Zaumeyer and Thomas, 1957).

Interestingly, the use of mixed cultivars can further reduce losses caused by anthracnose. According to Tu (1989), and recently by Mohamed (2003), mixing susceptible cultivars with resistant cultivars can limit the free spread of the disease in the field. Mohamed (2003) recorded

a significant yield increase of 63% at a mixture ratio of 4:1 resistant and susceptible cultivars, respectively. Besides the success of cultivar mixture crop rotation has been a successful control measure for several diseases. A two a year non-host crop rotation cycle could reduce the development of bean anthracnose due to the reduction of initial infection that arises from the initial inoculum source (Buruchara *et al*., 2010; Mohammed, 2013). Another cultural practise of interest is avoiding the use of overhead irrigation as it creates a cool micro-climate and disperses the pathogen spores' masses on foliage (Bush, 2009; Mohammed, 2013).

2.7.2 Physical control method

The use of hot water for killing the fungus within the seed has been attempted with varying results. The seed may be injured during the process of soaking (Zaumeyer and Thomas, 1957), and if left for longer the development of bacterial compounds can results and accumulate the seed thereby killing the embryo (Zaumeyer and Thomas, 1957). Nevertheless, Bush, 2009 reported that soaking the seed at 18 to 22°C for 15 h followed by another soaking at 47°C for 25 min can kill the fungus in infested seeds without reducing germination. Soil solarisation is another method of control that has been a success. Covering the soil with transparent plastic sheeting for one month before sowing, reduced both severity and incidence (Mohammed *et al*., 2013)

2.7.3 Biological control method

The use of antagonistic microorganisms to successfully control *C. lindemuthianum* or any other pathogen is based on reducing initial inoculum. According to Young and Kraus (1984), the use of microorganisms to control *Colletotrichum* species has been a success. Protection against bean anthracnose was achieved when susceptible bean leaves were treated with *Trichoderma harzianum* in a liquid medium or in a spore suspension (Bigirimana *et al*., 2000; Bailey *et al*.,

2002). Also rubbing infected seeds with cultures of *T. harzianum, T. viridae, T. hamatum* and *Gliocladium virens* for 15 min and drying them overnight before sowing significantly inhibited infection of *C. lindemuthianum* and increased seed germination (Padder and Sharma, 2010). The successes of these bio-agents were through, mycellial growth inhibition, toxic volatile metabolite production and inhibition of spore germination (Anitha and Murugesan, 2001). Other microorganisms include *Bacillus subtilis* that produces antifungal and antibacterial compounds that proved to be promising in controlling *C. lindemuthianum* (O'Connell *et al.,* 1985).

Plant extracts such as Neem (*Azadirachta indica*) effectively inhibited both germination of conidia and mycelia growth of *C. lindemuthianum* (Onifade, 2000). In addition, seed treatment and field spray using the extracts of *Lawsonia inermis* significantly improved seedling emergence and reduced incidence of bean anthracnose (Onifade, 2000).

2.7.4 Chemical control method

Seed and foliar treatments play a crucial role in reducing or effectively manage anthracnose of common bean (Conner *et al*., 2004). Tu (1989) showed that chemical control for anthracnose lies at early stage of plant growth. On the contrary Conner *et al*. (2004) demonstrated that late infections and full season control of the disease can be achieved with the use of chemicals. The use of 0.525 percent sodium hypochlorite, followed by chlorine dioxide and chloroxylenol was found to be the most effective in combating the pathogen in different working materials (Buruchara *et al.,* 2010). Furthermore, several chemicals of both seed and foliar treatments have been reported to be effective in combinations based on compatibility (Tu 1988; Conner *et al*., 2004; Gillard *et al*., 2012). According to Mohamed (2003) the following combinations are effective in managing anthracnose, Benlate (500 g a.i./kg WP) as a seed dressing at a rate of 2 g/kg seed, difenoconazole (250 ml a.i./EC) at a rate of 87.5 g a.i./ha as a foliar spray and

Benlate (500 g a.i./kg WP) seed dressing at a rate of 2 g/kg seed followed by foliar spray of difenoconazole (250 ml a.i./EC) at a rate of 87.5 g a.i./ha. The two combinations reduced disease severity and incidence and increased the yield per plot and 100 seed weight; however, the chemicals were applied at 20-day intervals after 14 days of planting (Mohamed, 2003). This makes chemical control success to be almost impossible to developing countries small-scale farmers because of the cost and the level of technical aspects involved.

2.7.5 Host resistance

Both the Andean and Mesoamerican germplasms encompass resistance genes for *C. lindemuthianum,* and over the years all over the world resistant cultivars have been developed (Singh and Schwartz, 2010). Nevertheless, Mesoamerican bean types are highly susceptible to races commonly found in Mesoamerican beans, whereas the Andean bean type is avirulent to most of both the Andean common races and the Mesoamerican (Balardin *et al.,* 1997). Although the current resistance level from different cultivars is sufficient, introgression and pyramiding of resistance from distant market classes, races, and gene pools of the cultivars and landraces of the primary gene pool may be essential to achieve high levels of durable resistance (Singh and Schwartz, 2010). However, the pathogen variability poses a threat on the success of breeding by frequently developing new virulent races or through the introduction of new races in regions that have conducive conditions for the development of that pathogen race. Such an impact can be significant in areas where planted cultivars are susceptible. For instance, the *Are*-gene (*Co-2*) sourced from Cornell 49242 was believed to be a non-race specific gene in suppressing anthracnose of common bean caused by several races. However, with time, and introduction of cultivars with the resistant gene in new areas resistance was broken (Edington, 1994; Pastor-Corrales *et al*., 1995; Liebenberg, 2009). The kappa and jota are classical example of races that overcame the *Are*-gene resistant gene (Edington, 1994). On the contrary host

variability has enabled the *A* and *Are* genes to resist the alpha, lambda, and epsilon anthracnose races (Basset, 1996).

Reducing the effect of this drawback is not dependent on incorporating multiple resistance genes in a single cultivar, of which is not an easy task within the practices of conventional breeding nor molecular aided breeding (Padder *et al*., 2017). The present genes in the 12 set of differential cultivars do not provide all known resistant genes, some of the known resistant genes are found in different cultivars, therefore revising the current differential set of cultivars would help in developing a new set that consist of all known resistant genes (Padder *et al*., 2017).

2.8 Conclusion

The understanding of the distribution of the highly variable *C. lindemuthianum* can only be achieved through continuous screening of differential cultivars and superior genetic resources available in South Africa. The superior newer resistant sources need to be evaluated in distinct environments in the targeted production regions to expose these sources to the prevailing pathogenic races to identify *C. lindemuthianum* resistant and high yielding genotypes. *C lindemuthianum* resistance breeding could be enhanced by incorporating phenotyping and maker-assisted breeding techniques.

CHAPTER 3: PATHOGENIC VARIABILITY OF *COLLETOTRICHUM LINDEMUTHIANUM* ON COMMON BEAN IN SOUTH AFRICA

3.1 ABSTRACT

Pathogenic variability of *Colletotrichum lindemuthianum,* a fungal pathogen that causes anthracnose disease on common bean (*Phaseolus vulgaris* L.) was evaluated in a glasshouse study at Agricultural Research Council-Grain Crops (ARC-GC) in South Africa. A total of 32 isolates were collected in three provinces of South Africa, namely KwaZulu-Natal, Mpumalanga, and North West. The isolates were collected from different fields of common bean at research stations and small-scale farmers' fields. Inoculum developed from mixing different isolates was used to spray-inoculate the 12 CIAT differential cultivars used to identify *C. lindemuthianum* pathogen races. Inoculation was conducted at the trifoliate developmental stage of the plants at 14 days from date of planting. Using the CIAT binomial system, from the 32 isolates, eight races of *C. lindemuthianum* were identified. These were races 3, 6, 7, 81, 83, 89, 263 and 323. All eight races were previously identified in South Africa and no new races were identified in the current study. Only race 7, 81, 83 and 89 were found in the more humid locations of the province of KwaZulu-Natal. Races 7, 81 and 89 are internationally recognized and show characteristics of the races reported in Brazil. Another race of importance is race 6 which has been reported in other Southern African countries. The most important landraces were AB 136, G 2333, Kaboon, TU and PI 207262 as they showed complete resistance from the isolates. Additionally, Cornell 49242 was one of the landraces of importance as it showed glimpses of anthracnose that faded overtime. A total of six genotypes can be successfully used to improve anthracnose resistance and these include G 2333, which can be used to improve the current genotypes used for the control of anthracnose in South Africa. This will ensure stability

in the long term since the pathogen *C. lindemuthianum* is highly variable and widely distributed in South Africa.

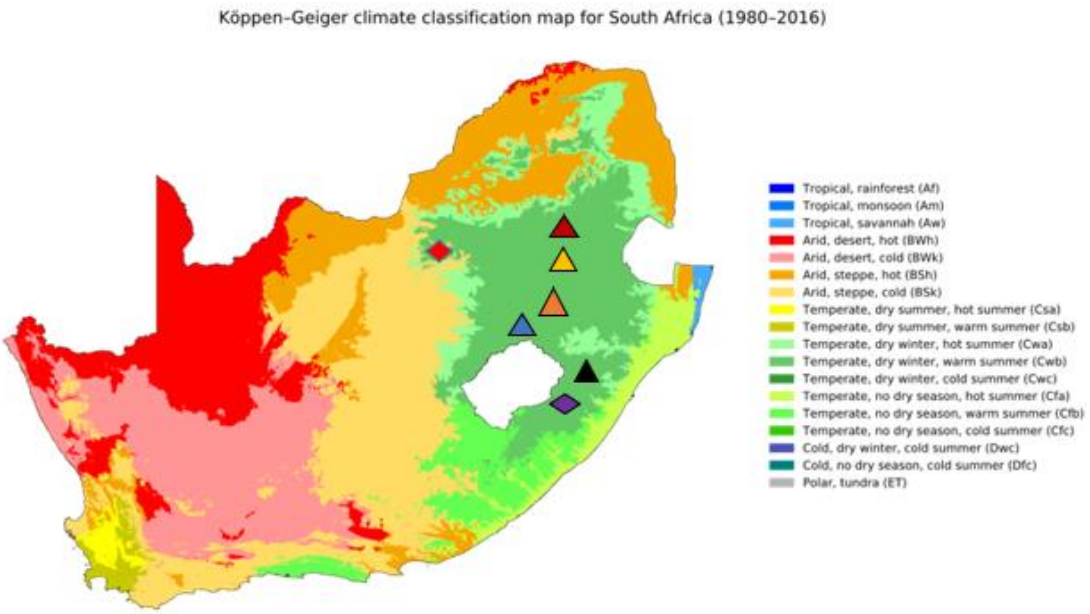
3.2 INTRODUCTION

Anthracnose disease of common bean (*Phaseolus vulgaris* L.) is a fungal seed-borne disease caused by *Colletotrichum lindemuthianum.* The disease is economically important because it causes seed impurity, rendering the seed unmarketable while under favourable conditions can directly and significantly reduce yield (Mohammed and Sangchote 2007; Markell, Wunsch and del Río, 2012). Anthracnose infects all aboveground parts of the plant including the seed. Due to the seed-borne nature of the disease, under favourable climatic conditions poor germination and emergency can be expected. Furthermore, the ability of anthracnose to heavily infest the foliage part of the plant easily causes poor seed quality (Pastor Corrales *et al*., 1995). This can cause heavy yield losses that can reach as high as 100% (Holliday, 1980; Shao and Teri, 1985; Talamini *et al*., 2006; Damasceno, Silva, Souza, and Ishikawa, 2007), especially when a susceptible cultivar is planted in places with high humidity (Diaz and Lopez, 1986).

Anthracnose symptoms include the abaxial vein blackening that extends to the petiole and stem (Allen and Lenné, 1998). On the pod, the pathogen causes brick-red to purple circular lesion that appears as the pod desiccates. The raised halo edge has many acervuli containing masses of conidia (Tu, 1988). Occurrence of anthracnose is common in production areas with cool and humid conditions. South African climate is divided into Mediterranean, arid, semi-arid, temperate, tropical, and subtropical (Kottek et al, 2006), and production of common beans is distributed throughout the country. However, major production is limited in provinces with temperate, tropical climates and dry winter, this includes Free State, Mpumalanga, KwaZulu-Natal, and North West (Fig. 3.1). Edington (1994) confined the significance of anthracnose in KwaZulu-Natal Province due to the prevalence of cool humid environmental conditions.

However, the variability and virulence of the pathogen is certainly of significance, considering its occurrence in majority of common bean production localities' environmental conditions in South Africa. The *C. lindemuthianum* pathogenic variability is great to circumvent cultivar resistance in each location or season (Ayonoadu, 1974; Menzes and Dianes, 1988).

Understanding the pathogenic variation of anthracnose in South Africa will contribute to the dry bean industry, especially breeding to ensure genetic gains achieved are sustained, improved and resources are allocated as required according to anthracnose geographical distribution. For instance, some races of anthracnose are limited to either Mesoamerican or Andean group, therefore the resistant group can be selected for breeding against the identified races in a particular region. Better understating of anthracnose variability will further enable researchers to devise management strategies that enables optimal management of anthracnose according to the distribution of the pathogen or pathogen races in the country. Furthermore, there has not been a study that evaluates pathogen distribution throughout South Africa except for selected locations that were presented by Mohammed (2003) and Muth (2009). Therefore, the purpose of this study was to identify the pathogenic variability of selected *C. lindemuthianum* isolates from major common bean production regions in South Africa.



(https://upload.wikimedia.org/wikipedia/commons/thumb/c/c8/Koppen-Geiger_Map_ZAF_present.svg/1235px-Koppen-Geiger_Map_ZAF_present.svg.png)

= *Delmas* = *Ermelo* = *Balfour* = *Bethlehem* = *Greytown*

= *Potchefstroom* = *Cedara*

Figure 3. 1. South African climate and common bean production regions where C. lindemuthianum races were identified.

3.3 MATERIALS AND METHODS

3.3.1 Pathogen isolates collection and isolation

A total of 32 isolates were collected in various common bean production fields in South Africa where incidences of anthracnose disease were observed. Isolates were collected in the 2018 and 2019 common bean growing seasons. The various fields were spread-out in four major common bean producing provinces. The provinces and locations included Mpumalanga (Delmas, Balfour and Ermelo), KwaZulu-Natal (Cedara and Grey town), Free State

(Bethlehem) and North West (Potchefstroom) (Table 3.2). The isolates were sampled from a wide range of commercial cultivars and populations of recombinant inbred lines (RINL). The isolate materials were sourced from various plant tissues such as leaves, pods, and seeds.

The isolation of the pathogen from the diseased plant tissue was conducted under sterile conditions at Agricultural Research Council Grain Crop Institute (ARC-GC) laboratory in Potchefstroom, North West Province. Small pieces of tissue showing typical anthracnose lesions were cut out using a scapula, rinsed with 70% ethanol, and hydrated in distilled water for two mins. The isolates were plated and cultivated in Petri-dishes of potato dextrose agar (PDA). The plates were placed in an incubator at a temperature of 23^{o}C for conidia germination, and a waiting period of 7 days was allowed. The end results of colonies in the plates were identified by their morphological characteristics as described by Sutton (1980). Purification of the colonies was achieved by repeating the plating of the pathogen's conidia with pure characteristics of *C. lindemuthianum*, of which after 24 h single conidia were transferred to PDA plates and incubated at room temperature for 10-12 days (Schwartz *et al*., 1981; Pastor-Corrales and Tu, 1989).

3.3.2 Plant material

The twelve set of anthracnose differential cultivars recommended by CIAT (1988) were used for the study. The material was sourced from the ARC-GC common bean breeding and germplasm maintenance programme, of which they were previously sourced from CIAT, Cali, Colombia (Table 3.1).

3.3.3 Inoculum, Inoculation and Evaluation

Inoculum was prepared by extricating spores from single conidial isolates by gently scraping the culture colonies using a sterile spatula. The detached spores were mixed with distilled water

and filtered through cheesecloth. The final concentration was adjusted to 1.2 x 10^6 spore/mL with the aid of a haemocytometer.

Seeds of the twelve differential cultivars were planted in 5 cm-diameter plastic pots and placed in trays. The pots were filled with *Culterra soil mix* and a cover of *Hygrotech* medium grade vermiculite was added for regulated aeration. The plants were grown in a glasshouse with a temperature of 18^{o}C – 26^{o}C regulated by air condition. A total number of 10 sterilized trays with a set of 12 differential cultivars were kept and maintained in the glasshouse. Each differential cultivar was replicated 3 times in a single pot and tray for each distinguished inoculum based on location and unique morphological characteristics. The seedlings were inoculated 14 days after planting (DAP) using an atomizer with an adjusted inoculum solution of 1.2 x 10^6 spore/mL until runoff. The inoculated seedlings were placed in a dew chamber for3 days at ± 2^{o}C with relative humidity of greater than 90% and a room temperature of 20^{o}C. The plants were then transferred to the glasshouse for evaluation. Disease severity rating was done 7 days after inoculation using a scale of 1-9 (Table 3.1.) were 1 is resistance and 9 is susceptible (Schoonhoven and Pastor Corrales, 1987).

Table 3. 1. Common bean Anthracnose disease severity rating scale (Schoonhoven and Pastor Corrales, 1987)

Disease Rating	Symptoms
1-2	No visible disease symptoms.
3-4	Presence of very few and small lesions, mostly on the primary vein of the lower leaf surface or on the pod, which covers approximately 1% of the surface area.
5-6	Presence of several small lesions on the petiole or on the primary and secondary veins of the lower leaf surface. On the pods, small (less than 2 mm in diameter) round lesions, with or without reduced sporulation, covered approximately 5% of the pod surface area.
7-8	Presence of numerous enlarged lesions on the lower side of the leaf. Necrotic lesions can also be observed on the upper leaf surface and on the petioles. On the pods the presence of medium-sized (larger than 2 mm in diameter) lesions are evident but also some small and large lesions with sporulation and that cover approximately 10% of pod surface area may be found.
9	Severe necrosis on 25% or more of the plant tissues are evident because of lesions on the leaf, petioles, stems, branches, and even on the growing point, which often results in death of most of the plant tissues. The presence of numerous, large, sprouting, sunken cankers can result in pod malformation, low seed number, and death of the pod.

3.4 RESULTS AND DISCUSSION

All 32 *C. lindemuthianum* isolates sourced in the 2018 and 2019 seasons from four provinces (North West, KwaZulu-Natal, Mpumalanga, and Free State) (Table 3.2) and from seven major common bean production locations showed signs of variability. A total of eight races (3, 6, 7, 81, 83, 89, 263 and 323) were identified from the 32 isolates (Table 3.3).

Table 3. 2.. Collection of *C. lindemuthianum* isolates from different South African geographical locations

Province	**Location**	**GPS Coordinates**		
		Latitude	**Longitude**	**Altitude (m)**
Kwa-Zulu Natal	Cedara	29°32'51,70″ S	30°16'00,03″ E	1115
	Greytown	29°02'38,33″ S	30°35'53,17″ E	1092
North West	Potchefstroom	26°44'04,21″ S	27°04'43,52″ E	1350
Free State	Bethlehem	28°13'41,70″ S	28°16'59,77″ E	1717
Mpumalanga	Balfour	26°37'31,40″ S	28°35'27,24″E	1639
	Delmas	26°08'06,62″ S	28°40'29,53″ E	1569
	Ermelo	26°30'37,37″ S	29°59'02,82″ E	1697

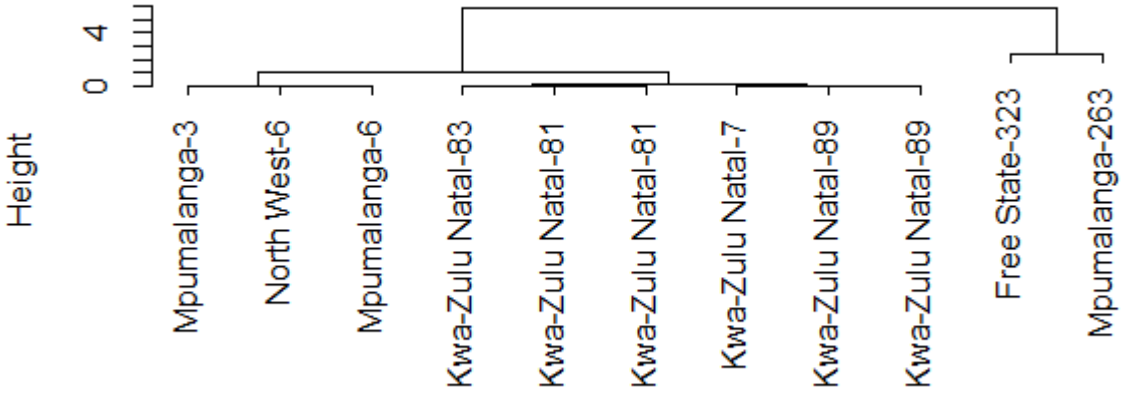


Figure 3. 2: R-Studio dendrogram hierarchal clustering according to *Colletotrichum lindemuthianum* races distribution between provinces.

Three race groups were identified according to their distributions between provinces (Fig 3:2). Races 7, 81, 83 and 89 were concentrated in KwaZulu-Natal while races 3 and 6 were identified in Mpumalanga and North West. The third group were races 323 and 263 that infected TO which contains the significant *Co-4* gene and alleles were found in Free State and Mpumalanga. Races 81 (56.25%) and 89 (9.38%) were both identified from the total isolates sourced in KwaZulu-Natal. Both races were identified in two locations in KwaZulu-Natal (Cedara and Greytown). In Mpumalanga, races 3, 6, and 263 were identified in all three locations (Delmas, Balfour and Ermelo), except for race 3 that was only identified in Balfour. In the North West and Free State provinces, only one race in each was identified, race 6 and race 323, respectively. Michelite was the most susceptible genotype on all races except for race 6. Only AB 136, G 2333, Kaboon and PI 207262 exhibited complete resistance (Table 3.3). Cornell 49242 was only mildly susceptible to race 89 in both Cedara and Greytown isolates.

Table 3. 3. *C. lindemuthianum* races identified and disease ratings in 32 isolates from 7 locations in South Africa.

	Isolates from different locations										
Differential cultivars	**CI1**	**CI2**	**CI3**	**CI4**	**GI1**	**GI2**	**PI1**	**BI**	**BAI**	**EI1**	**DI1**
AB 136	R	R	R	R	R	R	R	R	R	R	R
Michelite	S	S	S	S	S	S	R	S	S	R	S
Michigan Dark Red Kidney	S	R	S	R	R	R	S	S	S	S	S
Perry marrow	S	R	S	R	R	R	S	R	R	S	S
Cornell 49242	R	R	R	S	S	R	R	R	R	R	R
Widusa	R	S	S	S	S	S	R	R	R	R	R
Kaboon	R	R	R	R	R	R	R	R	R	R	R
Mexico 222	R	S	S	S	S	S	R	S	R	R	R
PI 207262	R	R	R	R	R	R	R	R	R	R	R
To	R	R	R	R	R	R	R	S	R	R	S
Tu	R	R	R	R	R	R	S	R	R	R	R
G 2333	R	R	R	R	R	R	R	R	R	R	R
Total binary value	7	81	83	89	89	81	6	323	3	6	263
No of Isolates	2	9	5	2	1	9	1	1	3	1	1

CI1=Cedara isolate 1, CI2=Cedara isolate 2, CI3=Cedara isolate 3 and CI4=Cedara isolate 4. GI1=Greytown isolate 1 and GI2=Greytown Isolate 2. PI1=Potchefstroom. BI=Bethlehem isolate. BAI=Balfour isolate. EI=Ermelo isolate. DI=Delmas isolate. R= Resistant. S=Susceptible

Based on the study findings, the existence of anthracnose in South Africa varies. This variability present in South Africa is significant as it emphasises that *C. lindemuthianum* is adapted to various conditions around the world as previously documented by Ishikawa *et al*. (2008) and Mota *et al*. (2016). With eight races identified, races 81 and 89 populations found in the more humid areas of KwaZulu-Natal proved to be pathogenic against the Mesoamerican genotypes. Races 81 and 89 were both found in the two locations (Cedara and Greytown) based in KwaZulu-Natal and infected over 65% of the total isolates indicates their importance, especially race 81 that was isolated in 56% of the total isolates. Race 81 in South Africa was first reported by Koch (1996) and emphasized by Muth (2009), this shows that it is an important

race and could easily be widespread in abundance, particularly in KwaZulu-Natal. Considering that it only infects Mesoamerican differential cultivars it is important that resistant germplasm be incorporated in breeding programs especially for cultivars that are planted in KwaZulu-Natal, specifically the Andeans. Race 89 resembled characteristics previously reported by Muth (2009), however, the race virulence on Cornell 49242 lapse overtime when culture is purified further. Although only isolated in Cedara samples race 83 is also of importance because it affects both the meso-American and Andean genotypes. In Potchefstroom, where the environmental conditions are less humid and warmer, only race 6 was identified, which was first identified by Mohammed (2003). Interestingly, race 6 was also identified in Delmas where the environmental conditions are also humid. Race 6 was one of the important races as it was reported in other African countries such as Tanzania and Zambia (Allen and Buruchara, 1995).

It has been previously reported that anthracnose races have circumvented multiple resistance from different sources because of its variability as documented by Fouilloux (1979) and Menezes (1985). In South Africa, there's sufficient genetic material that can be used to provide resistance to anthracnose. The most important material being AB 136, G 2333, Kaboon and PI 207262, since they showed complete resistance in all production regions including KwaZulu-Natal. Another germplasm of importance is Cornell 49242 that proved to be resistant over repeated cycles in sub-culturing against race 89. G 2333 is one genotype that has been documented as the most effective material in controlling anthracnose because of its dominant gene that can easily be transferred to susceptible varieties (Poletine *et al*., 2000).

3.5 Conclusion

The study results demonstrated a variable pathogenic distribution of anthracnose in South Africa with eight races identified. Five completely resistant genotypes (AB 136, G 2333, Kaboon, PI 207262 and Cornell 49242) were identified and can be successfully used to improve anthracnose resistance, especially G 2333. The use of these genotypes in resistant breeding will

ensure stability in a long term because of the different resistance genes and alleles in the different germplasm and this also makes it possible to breed for race specific resistance and genetic pyramiding.

CHAPTER 4: FIELD EVALUATION OF COMMON BEAN GERMPLASM AND COMMERCIAL COMMON BEAN CULTIVARS FOR REACTION TO ANTHRACNOSE IN SOUTH AFRICA.

4.1 ABSTRACT

A field survey was conducted to evaluate the reaction of common bean (*Phaseolus vulgaris* L.) germplasm and commercial common bean cultivars to common bean anthracnose caused by *Colletotrichum lindemuthianum* in South Africa. The trials were conducted in two locations with contrasting climatic conditions, specifically Potchefstroom and Cedara. Two separate trials were conducted per location for both germplasm and cultivars. A total of 51 germplasm varieties and 26 commercial common bean cultivars were evaluated. The Potchefstroom trial was evaluated under inoculated conditions with purified race 6. A scale of 1-9 was used for disease severity evaluation where 1 is resistance and 9 susceptible. There was a direct association between disease and location. A total of 70 (92%) of the evaluated genotypes were resistant to anthracnose race 6 in Potchefstroom, on the contrary, in the more humid Cedara only 38 (49.35%) of genotypes were immune to 4 *C. lindemuthianum* races, which were later identified. Over 65% of the evaluated commercial cultivars were susceptible, where; only 5 were highly susceptible and 14 moderately susceptible. Only 25 germplasm were resistant in both locations, among them were landraces with resistance to other important common bean diseases found in South Africa, such as rust (caused by *Uromyces appendiculatus)*, common bacterial blight (caused by *Xanthomonas campestris pv phaseoli*) and angular leaf spot (caused by *Phaeoisariopsis griseola*). There was a significant variation on yield between locations and very weak correlation between yield and disease severity within and between locations.

4.2 INTRODUCTION

Annually, a total of 12 million tons of common bean (*Phaseolus vulgaris* L) is produced globally (Broughton *et al*., 2003). The developing world, Latin America and Africa are the largest producers and heavy consumers of common bean at 5.5 million and 2.5 million tons,

respectively (Broughton *et al*., 2003). The importance of common bean is of high value as most multipurpose serving crops. Common bean is a affordable nutrients rich staple crop in developing countries of Africa and Latin America (Broughton *et al*., 2003). The crop is rich in nutrients such as calories, minerals, fibre, protein, folates, iron, calcium, magnesium, and potassium content and has a low glycemic index (Gept *et al*., 2008). Beyond its consumption value, common bean is an important rotational crop specifically with heavy feeding crops such as maize, since it fixes atmospheric nitrogen and make it available for uptake by other plants in intercropping systems (Dakora and Belane, 2019) and successive crop.

In South Africa, the production of common bean is spread throughout the country because of the crop's adaptability under various production conditions, making it feasible for production to occur throughout the year in some locations (DAFF, 2012). The production around the country is divided into spring and summer as well as autumn and winter. Generally, the spring and summer seasons are the main seasons of production specifically at commercial level, with the autumn and winter seasons being for subsistence production. However, local production does not maintain the national annual demand for common bean consumption (DAFF, 2012), resulting in higher market prices and making South Africa a net importer of the crop. Production challenges of common bean include low yield per hectare for small-scale and subsistence farmers. Such issues are attributed to factors such as hostile climate, poor soil fertility, damages resulting from insects and diseases, and the inability to afford inputs to control insects pest and diseases.

Anthracnose, caused by *Colletotrichum lindemuthianum* is one of the major seed-borne fungal diseases infecting common beans. Anthracnose occurs worldwide, with greater losses induced in temperate and subtropical climate than tropical climate (Pastor-Corrales and Tu, 1989; Singh and Schwartz, 2010). Anthracnose development is favoured by relatively cool and humid conditions. The disease development strives better on temperature between 13-26 ^{o}C and

requires abundant moisture or frequent rainfall (Schwartz *et al*., 2005), but not wet conditions. The disease infects all above ground parts causing the seed to be unmarketable and therefore, directly reduced yield.

Several control measures are applied in controlling anthracnose, this includes cultural practices, use of disease-free seed, application of fungicides, and resistance breeding. Resistance breeding is regarded as the most effective method of control for seed-borne diseases such as anthracnose (Dillard and Cobb, 1993). However, in the instance of anthracnose, resistance breeding has proved to be insufficient due to the pathogenic variability of the disease, which is further exacerbated by dissemination of the pathogen through infected seeds especially when infected seeds are replanted. However, because of race specificity, resistant genotypes can be better bred for known specific races in separate locations (Ogallo, 1991). Resistance breeding for common bean involves the use of genetic material sourced from germplasm from either Andean or Mesoamerican gene pool bean seed. The landraces from these regions possess sufficient resistance genes that can offer durable resistance for specific anthracnose races from specific locations where common beans are produced (Haciwa, 1991). The variability nature requires that germplasm and cultivars be evaluated under different production regions to allow for best selection of resistant landraces that can be used to improve susceptible germplasm and local cultivars. The best possible method of identifying resistant sources is to expose the potential sources of resistance to all dominant pathotypes over different production areas to eliminate highly susceptible genotypes (Beebe and Pastor Corrales, 1991). Therefore, the purpose of this study was to evaluate selected germplasm and cultivar materials available in South Africa for anthracnose under field conditions in two localities characterized by contrasting climatic conditions.

4.3 MATERIALS AND METHODS

4.3.1 Plant materials

A total of 51 selected germplasm varieties and 26 commercial cultivars (20 red sparkled sugar (RSS) and 6 small white (SW)) were evaluated in the 2018/19 common bean growing season at two localities (Table 4.1 and 4.2), Potchefstroom (North West Province) and Cedara (KwaZulu-Natal Province). The germplasm material was made up of Andean and Mesoamerican gene pool material including the 12 differential cultivars of anthracnose. For this study, the term genotype will be used to refer to both germplasm varieties and South African commercial common bean cultivars.

4.3.2 Study sites, inoculum, and inoculation

The trials were conducted in two localities with different climatic conditions, Potchefstroom and Cedara. Both localities are research stations for the Agricultural Research Council (ARC). ARC-GC is located in Potchefstroom in the North West province of South Africa, about 120 km west-southwest of Johannesburg, with geographic coordinates of 26º44'04,21″ S latitude and 27°04'43,52″ E longitude at an altitude of 1350 m. Cedara is a substation for ARC-GC located in KwaZulu-Natal province of South Africa, at 29°32'51,70″ S and 30°16'00,03″ E at an altitude of 1115 m. Potchefstroom climatic conditions is characterized by less humid and warm conditions. The long-term (2007-2017) growing season average monthly temperature is 21.42ºC, 58.70% relative humidity and 91.28 mm of rainfall (Table 4.1). On the contrary, in Cedara the climatic conditions are characterized by humid and cool conditions. The long-term monthly average temperature is 19.45ºC, relative humidity high of 72.25% and monthly rainfall of 94.84 mm.

Table 4. 1. Locality seasonal weather (From 2018-2019)

(Source: Agricultural Research Council – Soil, Climate and Water)

	Cedara			Potchefstroom		
Monthly Averages	**Long-term growing season**	**2018/2019 January - December**	**Growing season (2018/2019)**	**Long-term growing season**	**2018/2019 January - December**	**Growing season (2018/2019)**
Temperature (ºC)	19.45	17.01	19.41	21.42	19	22.26
Relative Humidity (%)	72.25	66.77	72.66	58.70	51.58	55.4
Rainfall (mm)	94.84	66.69	90.21	91.28	41.25	59.94
High Temperature (ºC)	25.51	23.99	25.26	28.33	26.91	29.63
Low Temperature (ºC)	13.39	10.83	13.56	14.51	10.2	14.9
High Relative Humidity (%)	96.75	93.24	94.43	86.15	78.77	82.5
Low Relative Humidity (%)	47.75	43.85	50.88	31.26	24.39	28.29

Long-term=2007 to 2017. Growing season 2018/2019=November to December.

Potchefstroom.

The trial in Potchefstroom was grown and treated with artificial inoculum because of prevailing climatic conditions. Isolates from the 2017/2018 season in Potchefstroom were used as source of inoculum for *C. lindemuthianum*. Using the method described by Sutton (1980), the pathogen was isolated and cultured on potato-dextrose agar (PDA) plates. Purification and multiplication of the pathogen were also done. The final suspension was concentrated and adjusted to 1.2 x 10^6 spore/mL with the aid of a haemocytometer and stereo microscope. Inoculation was conducted twice during the vegetative stage (V4- Third trifoliate leaf: from the full unfolding of the third trifoliate to the appearance of the first floral bud or raceme) and twice during early reproductive stage (R5- Pre-flowering: from the appearance of the first floral bud or raceme to the opening). Inoculation was conducted using a knapsack sprayer at 0.4 mpa pressure.

Cedara

Due to the prevailing conducive climatic conditions in Cedara the trials in this location were subjected to natural inoculum in the field. Although the trial field is under rotation with crops such as sorghum, maize and groundnuts, it was however selected based on the previous seasons' practices such as zero disease treatment and known post-harvest presence of anthracnose infected common bean stubble. Moreover, the seed used was multiplied in Cedara and uncertified thus an increased probability of contamination. No additional artificial inoculation was done to initiate disease infection and development.

4.3.3 Trial Design

In both localities, the trials were separated between germplasm and cultivars. The design was made up of a randomized completely block design (RCBD) with three replications per set. Pathways spacing between plots measured 2 m and between adjacent plots 0.9 m. For both germplasm and cultivar each plot consisted of 4 rows that were 5 m long (2 harvestable middle-rows). Spacing between rows and plants was 0.9 and 0.13 m, respectively. The trials had two rows of side borders with two block borders for every replicate.

4.3.4 Data collection and analysis

Disease severity was recorded 14 days after inoculation at the vegetative stage during the trifoliate stage in Potchefstroom. The evaluation was also conducted at pod development reproductive stage. Since there was no inoculation in Cedara, data were collected late in the vegetative stage and late during the developmental stage. Every rating was a numerical summary which was a representative of the whole plot. Ratings were done using the International Centre for Tropical Agriculture (CIAT) scale category of 1-9, where 1 is high resistant and 9 highly susceptible. Yield data were also collected for every plot after harvest

and threshing. Yield data was collected by weighing two middle rows yield of four for every plot (kilogram per two rows (7.5 m^2). The data were subjected to one-way analysis of variance (Anova) using Genstat 18th edition and R-Studio statistical analysis software's. Mean disease severity score comparisons between locations were conducted through Pearson's Chi-squared test and t-test on Genstat 18th edition and the Fischers protected LSD was used to separate data means at 95% confidence interval and was used to determine the phenotypic reaction of common bean to different races of *C. lindemuthianum.*

4.4 RESULTS AND DISCUSSION

The 2018/2019 common bean production season was characterised by drought in Potchefstroom with average monthly rainfall of 59.94 mm well below the ten year-long (2007-2017) monthly average of 91.28 mm between November and April. Moreover, during the 2018/2019 growing season months (November to April), a monthly temperature average of 22.26^{o}C and a monthly relative humidity average of 59.94% were recorded, of which was just under a degree Celsius higher than the ten-year long-term average in the same period. In Cedara, there was sufficient rainfall at 90 mm monthly between November and April 2018/19, however, it was 4 mm less compared to the ten year-long monthly average. Moreover, the monthly relative humidity average of 72.66% was slightly higher in Cedara in the same period (November to April 2018/19) making it more conducive for disease development, especially when compared to Potchefstroom conditions.

Symptoms reflecting anthracnose were identified on susceptible genotypes. Infected plants showed signs of anthracnose on the plants' tissues from stem, leaf, petiole, and pod (Fig. 4.1 A and B).

Figure 4. 1 Anthracnose symptoms on common bean cultivar Teebus. ***A***-symptoms on leaf veins and petioles. ***B***-symptoms on pods.

Only race 6 was used in Potchefstroom for inoculation because was the only race identified from the locally (Potchefstroom) sourced isolates. This is because the aim was to identify anthracnose races that occur in a specific area either Potchefstroom or Cedara and conduct the experiments under the local available races. In Cedara, the experiment was conducted under natural occurring *C. lindemuthianum* because of the conducive climatic condition. Races 7, 81, 83 and 89 were later identified from the different positive plants as the composition occurring in Cedara. In both locations, Potchefstroom and Cedara, significant ($P < 0.05$) differences between genotypes and *C. lindemuthianum* were observed.

A total of 74 (96%) of the evaluated genotypes were resistant to anthracnose race 6 in Potchefstroom. On the contrary, in Cedara only 36 (49%) (Table 4.2 and 4.3) of genotypes were immune to anthracnose (all 4 races (7, 81, 83 and 89) identified at a later stage in this study). No germplasm was recorded as highly susceptible in Potchefstroom, only one was regarded as moderately susceptible. However, in Cedara, only 6 of 51 germplasm were highly susceptible to races (7, 81, 83 and 89). Only two commercial cultivar was susceptible to anthracnose in Potchefstroom. In Cedara, 19 (73.30%) of the commercial cultivars were susceptible. Of the 19 cultivars, 5 were highly susceptible and 14 moderately susceptible. Only 7 cultivars and 28 germplasm varieties were resistant in both locations (between 1 and 3).

Among the germplasm were landraces for other important common bean diseases found in South Africa, such as rust (*Uromyces appendiculatus*), common bacterial blight (*Xanthomonas campestris pv phaseoli*) and angular leaf spot (*Phaeoisariopsis griseola*). These included AB 136, DOR 710, Don Timoteo, PI 207262, A 55, Tu, Montcalm, Red Mexican, G 2333, TO, Cornell 49242, Cal 143, CNC, Amendion, Flor De Mayo, GN 1140, Cerillos, PC 50, and Puebla 152.

Table 4. 2. Germplasm reactions to common bean anthracnose and grain yield means in Potchefstroom and Cedara during the 2018/2019 growing season.

Germplasm	Seed type/Colour/Origin	Disease severity		Yield Kg/ha	
		Cedara	Potchefstroom	Cedara	Potchefstroom
A 43	Dark red kidney	2.33	1.00	2668	678
A 55	Black	1.00	1.00	2636	1551
AB 136	Mesoamerican	1.00	0.67	374	180
AMENDION	Calima	1.00	1.00	3164	2191
AURORA	Small white	4.67	1.00	3873	2672
BAT 332	Carioca	3.00	1.00	2092	1182
BELDAKMI-RMR-18	Pinto	7.33	1.00	3567	2147
BELDAKMI-RMR-19	Pinto	7.67	1.00	2892	2611
BELMINEB-RMR-1	Great Northern	5.67	1.00	2881	3049
BELMINEB-RMR-7	Great Northern	5.67	1.00	2771	1107
CAL 143	Calima	1.00	1.00	3410	2671
CANADIAN WONDER	Red kidney	4.67	1.00	1039	676
CAR 2008	Carioca	4.33	1.00	3984	2222
CERILLOS	Alubia	1.33	1.00	1509	657
CNC	Small black	1.00	1.00	3255	2091
CONDOR	Small black	7.33	1.00	1506	1236
CORNELL 49242	Mesoamerican	1.00	1.00	2268	1693
DON TIMOTEO	Chile	1.00	1.00	2569	1487
DOR 710	Red kidney	1.67	1.00	2323	1687
EARLY GALLATIN	Medium white	5.00	2.00	1717	1080
ECUADOR 299	Medium pink	2.67	2.00	2473	2202
FLOR DE MAYO	Pink	1.00	1.00	4542	3336
G 21212	Small Red	6.00	1.00	3252	1789
G 2333	Small Red	1.00	1.00	1274	909
G 2858	Carioca	6.67	1.00	4793	3931
G 5686	Nueva Granada	3.33	1.00	3500	829
GN 1140	Great Northern	1.00	1.00	2017	2584

GOLDEN GATE WAX	Andean/Mesoamerican	4.67	2.67	2489	1898
	Table 4.2 continued				
HURON ELECTION	Small white	5.33	1.00	2443	2884
KABOON	Andean	2.67	0.67	617	220
KW 814	Brown	3.67	1.00	1948	1549
MEXICO 222	Andean	3.00	1.00	1328	764
MEXICO 235	Mesoamerican	2.33	3.33	4072	4373
MEXICO 309	Mesoamerican	6.00	2.67	3345	2713
MICHELITE	Small black	3.67	1.00	371	202
MDRK RED KIDNEY	Mesoamerican	4.00	1.00	1392	681
MKUZI	Carioca	6.33	1.00	3203	2696
MONTCALM	Red dark kidney	1.33	1.00	2563	2198
PAN 72	Small white	6.67	1.00	2770	1389
PC 50	Red	1.00	1.00	2928	1676
PERRY MARROW	Andean	1.67	1.00	1056	516
PI 207262	Mesoamerican	1.00	1.00	2070	770
PUEBLA 152	Small black	1.00	1.00	3017	2413
RED MEXICAN	Medium red	1.00	2.00	4265	3391
TENDERGREEN	Black	3.00	1.00	1488	1334
TO	Mesoamerican	1.00	1.00	3930	2676
TU	Mesoamerican	1.00	1.00	2153	2042
VAX 4	Brown	3.00	1.00	3452	2162
VAX 6	Brown	3.00	1.00	1769	1532
WIDUSA	Andean	5.33	1.00	465	804
ZAMBEZI	Carioca	5.33	3.00	3047	1407
Mean		3.261	1.196	2520,20	1779,18
Resistant (1-3)		29	50	**Grand Mean =** 2149,68	
Moderate (3.1-6)		16	1		
Susceptible (6.1-9)		6	0	**Yield Within location(For both) P-value** <0.05	

Table 4. 3. Cultivar reactions to common bean anthracnose in Potchefstroom and Cedara during the 2018/2019 growing season

Cultivar	Seed type	Cedara	Potchefstroom
BONUS	RSS	5.33	1.00
BWINDI	RSS	1.00	1.00
CALEDON	Small white	1.00	1.00
DBS 360	RSS	5.33	1.00
DBS 830	RSS	5.33	1.00
DBS 840	RSS	3.00	1.00
KAMIESBERG	RSS	7.00	1.00
KRANSKOP	RSS	4.67	1.00
KRANSKOP-HR-1	RSS	5.33	1.00
PAN 116	RSS	5.33	1.00
PAN 123	Small white	6.33	1.00
PAN 148 PLUS	RSS	5.00	1.00
PAN 185	Small white	4.00	1.00
PAN 9216	RSS	2.33	1.00
PAN 9249	RSS	3.00	1.00
PAN 9280	RSS	2.33	1.00
PAN 9292	RSS	4.33	1.00
RS 5	RSS	8.00	1.00
RS 6	RSS	6.00	1.00
RS 7	RSS	4.67	1.00
SEDERBERG	RSS	3.67	1.00
SW1	Small white	7.00	2.33
TEEBUS	Small white	6.00	3.33
TEEBUS-RR-1	Small white	7.67	3.33
TYGERBERG	RSS	3.00	1.00
WERNA	RSS	5.67	1.00
Mean		4.705	1.231
Resistant (1-3)		7.00	24.00
Moderate (3.1-6)		14.00	2.00
Susceptible (6.1-9)		5.00	0.00

Table 4. 4. Chi-square test for variation in disease severity and Welch two sample t-test for yield between locations.

Disease severity Pearson's Chi-squared			
	X^2	DF	p-value
Germplasm	110.63	8	<0.05
Cultivar	103.21	7	<0.05
Common bean yield Welch two sample t-test t		DF	p-value
Germplasm	5.3165	298.72	<0.05
Cultivar	3.8615	153.93	<0.05

Between locations, there was a significant ($P < 0.05$) difference on yield (Table 4.4). Cedara had the highest and widely distributed means of yield compared to Potchefstroom (Fig. 4.2). The variation is not associated with disease severity on germplasm varieties as there's a very weak correlation at r=0.070 between yield and disease.

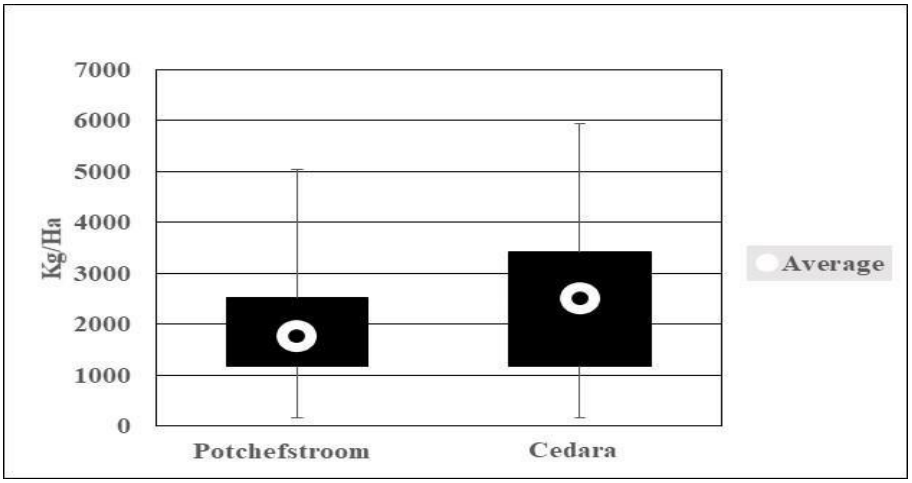


Figure 4. 2 Germplasm yield variation between two locations (Potchefstroom and Cedara) with contrasting environmental conditions.

However, there was a significant ($P < 0.05$) difference within germplasm evaluated in Potchefstroom, the minimum yield was obtained on a highly resistant ABI 136 (1) genotype

and the lowest yield was recorded on a genotype that rated level 3.33 Mexico 235 of resistance (Table 4.2).

The differences in yield are associated with annual rainfall in the regions, however, the yield quality of the susceptible genotypes was compromised with the presence of lesions making low grade because of appeal. During November (planting) and March (harvesting) the average temperature in Potchefstroom and Cedara was 30℃ and 25.26˚C, and total rainfall was 59.4 mm and 90.21 mm, respectively. Potchefstroom had a dry season with high fraction of the rainfall received late in the year. From the 10 resistant cultivars, only 4 were highly resistant, with 3 red speckled types and only one small white canning cultivar. From the total 6 evaluated small white canning beans only 3 were highly susceptible, and this reaction was limited in Cedara. The prevalence of different *C. lindemuthianum* races was common in KwaZulu-Natal where 4 races (7, 81, 83 and 89) were identified and the virulence was high with ratings over 5 obtained. The effect of climatic conditions between locations was a key factor, as it was raised by Edington (1994). Several genotypes that were previously identified by Mohammed (2003) and Muth (2009), as stable landraces for anthracnose breeding programmes were further validated in the current study, this includes G 2333 and Cornell 49242 with *Co-4*^{2}, *Co-5*^{2} and *Co-2* resistance genes and alleles, respectively.

4.5 Conclusion

The occurrence of *C. lindemuthianum* is widespread across selected common bean production locations in South Africa with potentially virulent races identified. The study found significant variation between the two distinct sites and between genotypes making it possible for selection of resistant genotypes for breeding purposes. Cedara had the most races identified and were more virulent compared to Potchefstroom. The damage in yield induced by the different races was mostly cosmetic. The incorporation of superior genotypes such as AB 136, G 2333, Cornell 49242, PI 207262, and region based TO and TU in commercial cultivars is highly

recommended especially to small white cultivars that have shown to succumb to anthracnose in both locations. Furthermore, the widely used landraces such as DOR 710, Don Timoteo, A 55, Montcalm, Red Mexican, Cal 143, CNC, Amendion, Flor De Mayo, GN 1140, Cerillos, PC 50, and Puebla 152 for resistance to other bacterial and fungal diseases is desirable because they were immune to anthracnose pathogenic races.

CHAPTER 5: SCAR MARKERS ASSISTED EVALUATION OF SELECTED ELITE SOUTH AFRICAN GENOTYPES FOR COMMON BEAN ANTHRACNOSE RESISTANCE.

5.1 ABSTRACT

Breeding for resistance is the most effective disease management option, especially for seedborne diseases such as common bean anthracnose caused by *C. lindemuthianum*, and for the poor farmers that use stored seed. The current study sought to phenotypically and genotypically evaluate the different elite genotypes commonly or successfully used in South Africa to breed for resistance for various diseases affecting common bean. A total of five SCAR markers were used. Three previously reported races 7, 81, and 89 of anthracnose were used to evaluate 26 genotypes and two F_2 populations. The selection of these races was based on the field reaction of genotypes, the genotypes were more susceptible to the races identified in KwaZulu-Natal. The F_2 generations were developed with a special interest in the *Co-4* locus and associated alleles. Genotype PI 207262 and TO were used as donor parents for female parents, RS 7 and SW1, respectively. Twenty-three of the genotypes showed complete to partial resistance(R) and only three were susceptible(S) and they were commercial cultivars SW1, RS 7, and Teebus. The population of RS 7 X PI 207262 showed a segregation ratio of 15:7 suggesting the presence of a dominant gene or combination of alleles conferring resistant reaction, as well as a possible dominant complementary factor from the susceptible parent. All five of the markers were able to amplify bands. Makers SAS13, SBB14, SY20, and SCO8 were all loci specific, however, they were not allele specific. SBB12 was loci and allele specific as a single distinct band was detected in PI 207262 for *Co-9*. The current study results showed the presence of resistance genes in the *Co4* loci with alleles to explore for South African breeding programs.

5.2 INTRODUCTION

Common bean anthracnose is a fungal disease caused by *C. lindemuthianum* (Sacc. and Magn.) Lams. -Scrib and is of major importance globally. Anthracnose occurs worldwide where common beans are produced and cause significant losses under favourable conditions, especially in temperate and subtropics climates (Schwartz, 2005). Several control strategies have over the years been proposed and applied to better manage the disease, this includes planting a pathogen-free seed, field sanitation, crop rotation, shifting planting dates, mixing of varieties, and plant resistance (Chaves, 1980; Schwartz, 1989; Mohammed, 2003). As much as these methods are effective collectively, the application is not always viable for small scale and subsistence farmers, because they are sophisticated for the level of their knowledge, technology, and costs involved does not justify their adoption in a short term. Despite the challenges associated with the adoption of the methods, resistance breeding stands out as the main preferred form of control by these farmers. This is because of the durability it provides and complementation of the use of stored seeds from previous seasons (Silbernagel and Zaumeyer, 1973; Zaumeyer and Meiners, 1975; Chaves, 1980), which is a customary practice by subsistence farmers. In the developed world, anthracnose is better managed through the application of various control methods, but resistant varieties are the main practice (PastorCorraleas and Tu, 1989, Tu, 1992, Kelly and Vallejo, 2004).

International Centre for Tropical Agriculture (CIAT) (1987) introduced the 12 differentials for anthracnose races identification. These differentials are made up of *Co* genes (*Co-1* to *Co-17*) (Kelly and Young, 1996). All the *Co* genes offer a significant level of controlling anthracnose. In addition to these genes are allelic variants that are recently explored or discovered more often than before with the aid of molecular markers. The potential of marker assisted selection (MAS) in breeding is fundamentally important as Kelly and Miklas (1999) recognized this. The benefits associated with molecular breeding, particularly with pathogens such as *C.*

lindemuthianum that has a high and complex pathogenic variability, include incorporation of genetic pyramiding to a cultivar race specific resistance without losing the resistance it possesses, making the resistance durable across different environments and different races (Kelly and Miklas, 1999). Some of the known alleles include *Co-1*^{2}, *Co-1*^{3}, *Co-1*^{4}, *Co-1*^{5}, *Co-3*^{2}, *Co-3*^{3}, *Co-3*^{4}, *Co-3*^{5,} *Co-4*^{2}, *Co-4*^{3,} and *Co-5*^{2} (Kelly and Young, 1996; Young *et al*., 1998; Geffrey *et al*., 1999; Alzate-Martin *et al*., 2001). The transition in MAS has evolved to a degree whereby identification of resistance sources can be specific and in multiple alleles of which their value can further be evaluated and correlated, thereby selecting the best resistance genes for a specific pathogen race. Recently, Zuiderveen *et al*. (2016) using Single Nucleotide Polymorphism (SNPs) for GWAS (genome wide association study), demonstrated the existence of multiple races resistance in Co-1 from different cultivars, and new sources of anthracnose resistance in Andean beans were discovered. Another benefit of interest associated with markers is that the uncertainty of masked genes epistasis is eliminated by the technology of markers (Kelly and Miklas, 1999; Zuiderveen et al., 2016). Further additional genes have been identified from other sources, this includes genes such as *Co-12, Co-13, Co-14, Co-15, Co-w, Co-x, Co-y,* and *Co-z* (Coimbra-Gonçalves *et al*., 2016; Gonçalves-Vidigal *et al*., 2016). However, due to the disease pathogen *C. lindemuthianum* variability nature, individually none of these genes is offering full resistance against all known races of anthracnose. Because of the spread of the genes and presence of multiple races of the pathogen, race-specific resistance seems a better mechanism under different conditions and locations, however, this can be short-lived at the presence of a pathogen with mutations taking place in its genetic architecture favouring virulent race development (Burt *et al*., 2015).

As much these genes cannot provide complete resistance against all races of *C. lindemuthianum* their resistance individually varies and genes such *as Co-4, Co-5,* and *Co-6* are regarded as the elite since they harbour the highest resistance spectrum, individually or in combination (Souza

et al., 2014). The gene *Co–4* has been mapped to Pv08 (Meletto *et al.,* 2004) and is known to be multi-allelic (Van Ooijen, 2006). The *Co–4* loci are considered valuable since different alleles at this locus are known to control 97% of the known races of *C. lindemuthianum.* Molecular markers specific to different alleles have been developed (Gept *et al*., 2008). Among them are Sequence Characterized Amplified Region (SCAR) markers. SCAR markers are strategically used as a support tool for the validation of the presence or absence of a resistance gene or allele in a genotype. Therefore, it is the purpose of this study to develop F_2 crosses using differential lines PI 207262 and TO as sources of *Co-4* genes and alleles to evaluate and validate resistance, and screening of selected elite South African common bean sources of disease resistance using six commercial SCAR markers.

5.3. MATERIALS AND METHODS

5.3.1 Glasshouse crosses

A set of 28 genotypes (including 2 recombinant inbred lines (RILs)) was selected for evaluation by phenotypical screenings and validation using commercial SCAR markers. Breeding crosses by using selected well-known anthracnose resistant differential cultivars PI 207262 and TO was done with susceptible cultivars RS7 and SW1, red speckled sugar beans, and small white, respectively, to develop F_2 populations. All two genotypes PI 207262 and TO, were selected because of the *Co-4* gene and alleles, yield performance and resistance to the races identified in KwaZulu-Natal. The study was conducted in a glasshouse with a temperature of 18^{o}C – 26^{o}C regulated by air condition. A set of 3 seeds per bag of 30 cm diameter (heat sterilized soil), were planted weekly for three weeks (3X5X3X4) in five bags for each genotype. Cross-pollination was conducted daily as soon as the flowers started to grow. Transfer of pollen was done using a tweezer and 70% ethanol was used to sterilize the tweezer between crosses. For each cross made, a labelling tag was used to detail the parents and the date the cross was made.

5.3.2 Inoculum and inoculation

Inoculum for anthracnose races 7, 81 and 89 was prepared by extricating spores from single conidial isolates by gently scraping the culture colonies using a sterile spatula. The detached spores were mixed with distilled water and filtered through cheesecloth. The final concentration was adjusted to 1.2 x 10^6 spore/mL with the aid of a haemocytometer. Seeds of all 27 genotypes were planted in plastic pots of 5 cm diameter filled with *Culterra soil mix* and a cover of *Hygrotech* medium-grade vermiculite was added for regulated aeration. The seedlings were inoculated 14 days after planting using an atomizer with an adjusted inoculum solution of 1.2 x 10^6 spore/mL until runoff. The inoculated seedlings were placed in a dew chamber for 3 days at ± 2^{o}C with a relative humidity of greater than 90% and a room temperature of 20^{o}C. The plants were then transferred to the glasshouse for evaluation.

5.3.3 DNA extraction and PCR

Seeds were germinated and the first true leaves were used for DNA extraction. DNA extraction was performed using Cetyl Trimethylammonium Bromide (CTAB) based extraction method (Ausubel *et al*., 1994) with some modifications. The DNA samples were rinsed with 70% ethanol and incubated at 37°C at least 1 h. The DNA was dissolved in distilled sterile water and placed overnight at 4°C. An endogenous control marker was used to assess the suitability of the DNA for PCR reactions. Five SCAR markers (SB12, SBB14, SY20, SC08, SAS13) were used in the analysis. A no-template control (NTC) was included in each run to discriminate or omit contamination on DNA. No specific positive control was run alongside the samples because the twelve differential cultivars were part of the test and validated and they were the main target.

5.3.4 Data collection and analysis

A scale of 1-9 was used to evaluate the disease incident of the F2 generations (Table 4.2). Ratings of 1 to 3 were rated as resistance(R) and 4 to 9 as susceptible(S). Chi-Square Goodness

of fit was conducted using R studio at a P-value of 0.05. PCR products were confirmed on 2% agarose gels visualized with ethidium bromide staining and the band sizes analysed using Genetools (Syngene, Cambridge, UK).

5.4 RESULTS AND DISCUSSION

5.4.1 Race 7, 81, and 89 characterizations to selected genotypes

From the 28 genotypes evaluated only 19 (70%) are completely resistant to all three races (7, 81, and 89). Race 89 continued to show its significance in the South African climate and most importantly to some of the elite evaluated genotypes by affecting all 7 genotypes (Michelite, Michigan Dark red kidney, Perry marrow, Cornell 49242, Teebus, RS 7 and SW1) (Table 5.1). None of the races identified were severe on RS 7, which is the recent updated commercial variety of the red speckled type of common beans available in South Africa. Teebus and SW1 are the white canning beans commercial varieties, but Teebus was the most affected compared to RS 7 and SW1.

Table 5. 1. Genotype reaction to anthracnose races 7, 81, and 89.

	Isolates from separate locations		
Genotype	**7**	**81**	**89**
AB 136	R	R	R
Michelite	S	S	S
Michigan Dark Red Kidney	S	R	R
Perry marrow	S	R	R
Cornell 49242	R	R	S
Widusa	R	S	S
Kaboon	R	R	R
Mexico 222	R	S	S
PI 207262	R	R	R
To	R	R	R
Tu	R	R	R
G 2333	R	R	R
PUEBLA 152	R	R	R
RS 7	S	S	S
SW1	S	S	S
DOR 710	R	R	R

Don Timoteo	R	R	R
A 55	R	R	R
Montcalm,	R	R	R
Red Mexican	R	R	R
CNC	R	R	R
Amendion	R	R	R
GN 1140	R	R	R
PC 50	R	R	R
Teebus	S	S	S

5.4.2 Glasshouse crosses

When PI 207262 was used as a donor(male) parent to RS 7 female parent, a cross was made and a segregation ratio of 15:7 was observed for race 7 (Table 5.2). Such a pattern shows the presence of a dominant gene or combination of alleles conferring resistant reaction, as well as a possible dominant complementary factor from the susceptible parent. PI 207262 consists of *Co-4*^{2} and *Co-9* genes (Alzate-Marin *et al*., 2006) that are resistant to races 7, 81, and 89. A cross of SW1 and TO show a ratio of 3:1 (Table 5.2), indicating that it carries a dominant and independent resistance gene to all three races.

Table 5. 2. Chi-square goodness of fit for the F_2 generation.

			Observed		**Expected**				
	Race	**Total no of plants**	***R***	***S***	***R***	***S***	**Ratios**	X^2	***P-value***
RS7 X PI 207262	7	45	36	9	33.75	11.25	15:7	0.60	0.74
SW 1 X TO	7	41	30	11	30.75	10.25	3:1	0.84	0.65
RS7 X PI 207262	81	29	25	4	21.75	7.25	3:1	1.94	0.37
SW 1 X TO	81	35	21	14	26.25	8.75	3:1	4.20	0.12
RS7 X PI 207262	89	40	35	5	30	10	3:1	3.33	0.18
SW 1 X TO	89	38	24	14	28.5	9.5	3:1	2.84	0.24

5.4.3 Molecular marker analysis

SCAR makers SAS13, SBB14, SY20, and SCO8 were all loci specific, however, they are not allele specific. SBB12 was loci and allele specific as a single distinct band was detected in PI 207262 for *Co-9*. SAS13 amplified bands for all genotypes including the crosses, however, this was expected for genotypes with the *Co-4* gene as it has been reported that SAS13 is not specific for *Co-4*^{2} and does amplify *Co-4* regardless of the allele present (Park and Yu, 2004). The presence of SAS13 in RS 7 justifies the presence of the complementary resistance observed in phenotypic screening which is linked to the *Co-4* gene. The presence of SAS13 in SW1 is regarded as a false positive because there is no link of *Co* genes to the variety only *Ur* and bacterial diseases resistance genes. SBB14 was present in PI 207262 but not in the F_2 cross of RS7 X PI 207262 nor parent in RS7 (Fig. 5.1). SBB14 produced a distinct band for G 2333(*Co-4*^{2}) in agreement with Geetha (2013). However, SBB14 was absent in SW1 but present in F_2 of SW1 X TO(*Co-4*). The marker SBB14 did not discriminate against the different alleles in the locus in the case of the F_2 generations. Marker SY20 linked with *Co-4* amplified a distinct band in G 2333(*Co-4*^{2}, *Co-5, Co-7*) and three more bands on TO(*Co-4*), Widusa (*Co-9*), and PI 207262(*Co-4*^{3}) (Fig. 5.1). The presence of marker SY20 on Widusa is a false positive as there is no link associated with the genotype target locus.

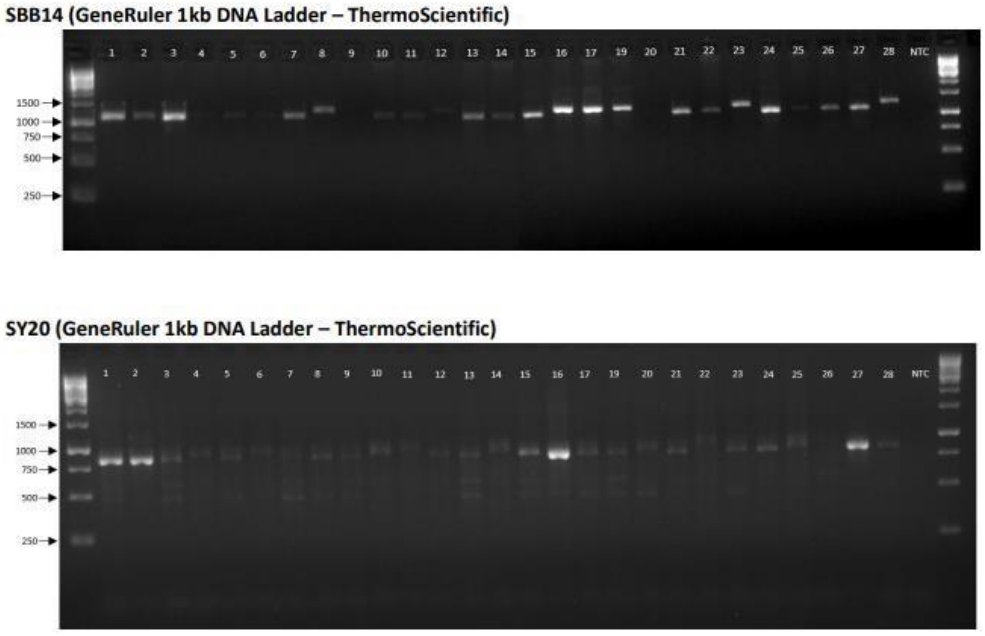


Figure 5. 1 Amplification of molecular SCAR maker SBB14 and SY20 for anthracnose resistance gene *Co-4*[2], and *Co-4*, respectively.

Marker SCO8 is linked with *Co-4* which is absent in PI 207262 was detected by the marker, however, SCO8 was also detected on RS 7 and RS7 X PI 207262. The presence of the gene *Co-4* in PI 207262 can be attributed to the marker being unable to differentiate between alleles in the target locus. Also, the *Co-4* gene presence was detected by the SCO8 in TO as is the case in RS 7 (Fig. 5.2) (Table 5.3).

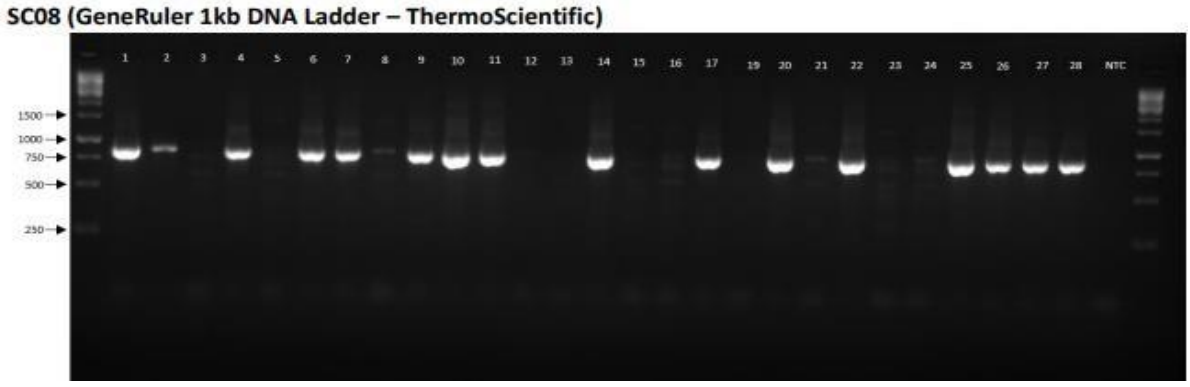


Figure 5. 2 Amplification of molecular SCAR marker SCO8 for anthracnose resistance gene *Co-4*[3]..

Table 5. 3. Genotype SCAR maker response.

	Genotype	Resistance gene	SB12 *Co-3³*	SBB14 *Co-4²*	SY20 *Co-4*	SCO8 *Co-4*	SAS13 *Co-4; Co-4²*
1	**PI 207262**	*Co-43, Co-3³*	+	+	+	+	+
2	**TO**	*Co-4*	-	+	+	+	+
3	**PUEBLA 152**		-	+	-	-	+
4	**RS 7**		-	-	-	+	+
5	**SW1**		-	-	-	-	+
6	**RS7 X PI 207262**		-	-	-	+	+
7	**RS7 X PUEBLA 152**		-	+	-	+	+
8	**SW 1 X TO**		-	+	-	-	+
9	**DOR 710**		-	-	-	+	+
10	**Perry Marrow**	*Co-1³*	-	-	-	+	+
11	**Don Timoteo**		-	-	-	+	+
12	**Tu**	*Co-5*	-	-	-	-	+
13	**A 55**		-	+	-	-	+
14	**Montcalm,**		-	+	-	+	+
15	**Red Mexican**		-	+	-	-	+
16	**G 2333**	Co-4², Co-5, Co-7	-	+	+	-	+
17	**Cornell 49242**	*Co-2*	-	+	-	+	+
19	**CNC**		-	+	-	-	+
20	**Amendion**		-	-	-	+	+
21	**GN 1140**		-	+	-	-	+
22	**PC 50**		-	+	-	+	+
23	**Teebus**		-	+	-	-	+
24	**Michelite**	*Co-11*	-	+	-	-	+
25	**MDRK**	*Co-1*	-	+	-	+	+
26	**Mexico 222**	*Co-3*	-	+	-	+	+
27	**Widusa**	*Co-1⁵, Co-9³*	-	+	+	+	+
28	**AB 136**	*Co-6, Co-8*	-	+	-	+	+

The findings of SAS13 agreed with the findings of the cross RS 7 X PI 207262 and the reaction of the genotypes by showing that RS 7 carries complementary genes or alleles that give them complementary resistance and these genes or alleles are from the *Co-4* locus. Because it is known to amplify consensus sequences common to all alleles at the *Co*-4 locus (Awale and Kelly, 2001). SW1 is a new variety that is still undergoing trials in the field and canning (Fourie, 2019); therefore, the false-positive conclusions are supported by field characterization whereby the variety showed symptoms that are severe compared to RS 7 (which has a *Co-4* gene or allele). Also, the lack of consistency in detecting the presence of the different *Co* genes alleles as it has been the case with the other genotypes further validates this observation.

5.5. Conclusion

Although no allelism investigation was conducted, the use of the different *Co-4* genes and alleles in South African varieties through the breeding program is of immense importance as it provides more options of alleles from the different sources thereby assuring sustainable or durable resistance. The most important sources being the Mesoamerican genotype; PI 207262, TO, and G 2333, however, it is also equally important to incorporate the Andean genotypes in the program to prevent the circumvention of the Mesoamerican resistance.

The current study results showed the presence of resistance genes in the *Co4* loci with alleles to explore for South African breeding programs. All markers linked to the alleles of the *Co-4 gene* were positive thereby validating the existence of the known target genes in the different varieties, especially on the differential genotypes. All five markers are recommended for MAS breeding for anthracnose. Of interest, the positive results of SCAR marker SBB14 on SWXTO agreed with the phenotypic data that showed resistance from most of the plants that were inoculated with races 7,81, and 89. It is therefore recommended that marker SBB14 be a marker of choice for the advancement of SWXTO crosses.

CHAPTER 6: SUMMARY AND CONCLUSION

6.1 SUMMARY

Common bean is an important grain crop and food legume especially for the poor population of South Africa because it is rich in protein making it an affordable alternative of red meat. However, South Africa's annual production is below annual consumption. Nevertheless, the susceptibility of the crop to diseases such as anthracnose caused by *C. lindemuthianum*, makes it difficult to produce quality and sufficient common bean in South Africa. Therefore, the purpose of this study was to investigate the status of common bean anthracnose as a potential destructive epidemic in South Africa under different production regions, by phenotypically and genetically evaluate selected germplasm for resistance and develop resistant-inbred lines with the aid of SCAR markers, using locally sourced genetic materials as a long-term solution for ascertaining increased yields, thus increasing profits for small-scale famers.

Disease field surveys were conducted in selected common bean production regions in South Africa, specifically in KwaZulu-Natal, Mpumalanga, and Free State and North West provinces. Selected genotypes were carefully chosen for screening in Cedara and Potchefstroom. Finally, inbred lines were developed using resistant germplasm and susceptible cultivars. The inbred lines were subjected to molecular evaluation using commercial SCAR markers.

A total of eight races (3, 6, 7, 81, 83, 89, 263 and 323) were identified from 32 isolates. Only AB 136, G 2333, Kaboon, TU and PI 207262 exhibited complete resistance. Cornell 49242 was only mildly susceptible to race 89 in both Cedara and Greytown. The 2018/2019 common bean production season was characterised by drought in Potchefstroom; however, disease presence was recorded. The inoculated trial in Potchefstroom was evaluated on race 6 that was previously collected from local affected crop and fields. A total of 70 (92%) of the evaluated genotypes were resistant to anthracnose race 6 in Potchefstroom. On the contrary, in Cedara where the evaluation was on naturally occurring inoculum only 38 (49%) of genotypes were

immune to anthracnose. Pathogenic races were identified at a later stage in this study as 7, 83, 87 and 89 for the Cedara trial. Cedara had the highest level of disease severity. Between locations, there was a significant ($P<0.05$) difference on yield. Cedara had the highest and widely distributed means of yield compared to Potchefstroom.

Successful crosses of SW1 X TO and RS 7 X PI 207262 were made with segregation ratios of 3:1 and 15:7, respectively. Makers SAS13, SBB14, SY20, and SCO8 were all loci specific, however, they were not allele specific. SBB12 was loci and allele specific as a single distinct band was detected in PI 207262 for *Co-9.*

6.2 CONCLUSION

The current study findings have demonstrated the variability of *C. lindemuthianum* in South Africa through its widespread distribution using the pathogenicity test. Both the Andean and Mesoamerican differential cultivars were affected by the races identified. The most severe *C. lindemuthianum* races were 7, 81, 83 and 89 and were identified in KwaZulu-Natal.

The damage caused by anthracnose was mostly cosmetic to selected commercial cultivars in KwaZulu-Natal with a potential to significantly lower yield quality. The commercial cultivars resistance is attributed to some of the evaluated resilient germplasms used to breed for bacterial and other devastating fungal diseases of common bean. The red sparkled type had high resistance to anthracnose, and high yields compared to the small white type making them more attractive for the farmers because of reduced risk. The SCAR makers were useful for marker-assisted breeding targeting the different important *Co* gene resisting anthracnose

Overall, the study has shown the presence of a wide geographic distribution of *C. lindemuthianum* races, diverse valuable genetic resources, and effectiveness of the applied SCAR markers for selection breeding. Therefore, it is recommended that the valuable

germplasms are incorporated to the susceptible cultivars to improve resistance and ensure stability in South African cultivars.

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