EVALUATION OF THE CIAT PHASEOULUS CORE COLLECTION FOR RESISTANCE TO ANTHRACNOSE

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Dissertation Submitted to the University of Zambia in partial fulfillment of the Requirements of the Degree of Master of Science in Plant Breeding and Seed Systems

SCHOOL OF AGRICULTURAL SCIENCES

DEPARTMENT OF PLANT SCIENCE

THE UNIVERSITY OF ZAMBIA

LUSAKA

2021

DECLARATION

I, **Zombe Kapata Nalupya** hereby declare that the work presented in this dissertation was my own and has never been submitted for a degree at this or any other University.

Signature ________________________________

Date ________________________________

APPROVAL

This dissertation of Zombe Nalupya Kapata was approved as fulfilling part of the Requirement of the award of degree of Master of Science in Plant Breeding and Seed Systems by the University of Zambia

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ABSTRACT

Colletotrichum lindemuthianum, the causative pathogen of common bean (*Phaseolus vulgaris*) anthracnose, is highly variable. Therefore, understanding its race structure and identification of new sources of resistance is necessary for the development of varieties with durable resistance. The objectives of this study were (i) to characterize three isolates of *C. lindemuthianum* collected from three major bean-growing regions in Zambia, and (ii) evaluate the CIAT *Phaseolus* core collection for resistance to *C. lindemuthianum* races 37, 73, and 566, and a blend of 20 races. The three isolates collected from three major bean-growing districts in Zambia, namely Mporokoso, Mpika, and Mbala, were characterized as race 37, 73, and 566, respectively. A subset of the CIAT core collection comprised of 885 accessions of common bean (*Phaseolus vulgaris*), 13 accessions of scarlet runner bean (*Phaseolus coccineus*), and 11 accessions of year bean (*Phaseolus dumosus*) were evaluated for resistance to races 37, 73 and 566, and a blend of 20 races in a greenhouse at University of Zambia, Lusaka, Zambia. About 72%, 66%, 48% and 9% of *P. vulgaris* accessions evaluated were highly resistant to races 37, 73, 566 and a blend of 20 races, respectively. Also, accessions of *P. coccineus* and *P. dumosus,* highly resistant to races 37, 73 and 566, were identified. These have a potential to be used as a resource for development of resistance varieties especially in countries where the breeding programs include secondary gene pools such as these. Eight out of 331 *P. vulgaris* accessions were highly resistant to all three individual races (37, 73, and 566) and to a blend of 20 races. These eight accessions constitute a valuable breeding resource for developing varieties with durable resistance to *C. lindemuthianum*.

DEDICATION

I dedicate this work to my children, **Wane, Natemwa, Joshua** and **Lumba** for all the long hours of being away from home and from you, for the love of gaining enlightenment so that I can not only inspire you to greater heights but pass on the baton to you.

ACKNOWLEDGEMENT

Firstly, I would like to thank the Almighty God, my creator and sustainer of life, for the sufficient grace he supplies on a daily basis in all my endeavors.

I would like to sincerely thank my supervisor Dr. Kelvin Kamfwa for his guidance, advice and the practical approach to this work. To My co – supervisor Dr. Davies Lungu, whose experience in this field remains unmatched.

My heartfelt appreciation is extended to the Agricultural Productivity Program for Southern Africa (APPSA) under Zambia Agricultural Research Institute (ZARI) for the sponsorship without which the work would not have been possible. Thanks also in this regard goes to Kirk house Trust for providing materials and equipment's partly used in this study. My gratitude goes to CIAT for providing the germplasm

My appreciation goes to Tience Company limited based in Chipata for the partial sponsorship rendered to me

I remain grateful to Mr. Mukuma Chikoti for undeserved guidance and encouragement throughout the experiment and analysis. The input of my fellow students by providing the necessary competitions, the arguments, discussions, disagreements and agreements cannot go without mention. These resulted in my academic character formation and I will always hold them dearly in my memory.

I acknowledge the mentorship of Dr. Tamala Kambikambi for continuous mentorship, Dr. Richard Chanda and all the members of staff at the University of Zambia, School of agricultural science. My husband and the entire family for the emotional, financial and spiritual support rendered to me during the study, your support enabled me to go through this work with ease.

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CHAPTER 1

1.0 INTRODUCTION

The genus *Phaseolus* has over 80 domesticated and wild species (Freytag and Debouck, 2002), of which the species *Phaseolus vulgaris* (common bean) is the most widely distributed. In addition to *P. vulgaris,* the other domesticated species of the genus *Phaseolus* include scarlet runner bean (*Phaseolus coccineus*)*,* year bean (*Phaseolus dumosus*), tepary bean (*Phaseolus acutifolius*), and lima bean (*Phaseolus lunatus*). *P. coccineus* and *P. dumosus* are the two most closely related species to *P. vulgaris* among the domesticated species (Broughton et al., 2003).

Common bean is a major source of protein, essential micronutrients (iron and zinc), dietary fibers, and income for many households in Africa and Latin America (Wortmann, 1998). Diseases are a major production constraint of common bean and are partly responsible for low yield worldwide (Beebe, 2012). Anthracnose) caused by the fungus *Colletotrichum lindemuthianum* is a serious disease of common bean; in susceptible varieties, it can cause yield losses of up to 100% (Pastor-Corrales and Tu, 1989). Anthracnose is a seed-borne disease that infects beans at any growth stage, and symptoms can appear on leaves, stems and pods. On leaves, symptoms may appear on the veins of the underside of the leaf and on the petiole as brick-red spots that turn dark-brown to black. On the pods, anthracnose symptoms appears first as small, tan to rust-colored spots that enlarge to dark sunken areas surrounded by a slightly raised black ring (Schwartz and Pastor-Corrales, 2005).

Planting anthracnose infected seed is one of the major transmission methods for anthracnose (Ferreira et al., 2013), therefore, use of clean anthracnose-free planting seed is an effective control method. This method, however, may not be effective in Africa because the majority of farmers use farm-saved recycled seed. The recycled seed may carry the pathogen and transmit it to the next generation. Fungicides can effectively control anthracnose but most farmers in Africa cannot afford them and there are health concerns associated with the use of fungicides. Development and use of anthracnose resistant varieties is the most cost-effective strategy for controlling this disease in beans

The *C. lindemuthianum* has co-evolved with the Andean and Middle American gene pools of common bean resulting in some races being more virulent on Andean beans than Middle-American and vice-versa (Melotto et al., 2000; Mahuku and Riascos, 2004). *C. lindemuthianum* is highly variable and exists in many races (Sicard et al., 1997; Balardin et al., 1997; Balardin and Kelly 1998; Sharma et al., 1999; Rodríguez-Guerra, et al., 2003; Rodríguez-Guerra et al., 2008). To date, over 182 races have been identified worldwide using a set of 12 differential cultivars of common bean (Padder et al., 2019). The high genetic variability of the *C. lindemuthianum* makes it difficult to develop varieties with durable resistance, and requires continuous identification of new sources of resistance. Genotypes that may be resistant to anthracnose in a particular growing region may be susceptible in other regions due to differences in the races among regions. Furthermore, multiple races might occur at the same time in a field, hence, the need for varieties to carry resistance against multiple races.

Resistance to anthracnose is controlled mainly by major genes; to date, over 11 major genes have been reported on eight chromosomes (Ferreira et al., 2016). Resistance genes are classified as Andean or Middle American, based on the gene pool of origin of the bean host (Kwak and Gepts 2009). Middle American genes include *Co-2, Co-3, Co-4, Co-5, Co-6, Co-11, Co-16, Co-17, Co-u,* and *Co-v* on chromosomes Pv02, Pv03, Pv04, Pv07, Pv08, and Pv11 (Mastenbroek 1960; Bannerot et al., 1971; Fouilloux 1976; Young and Kelly 1996; Geffroy 1997; Gonçalves-Vidigal et al., 1997; Young et al., 1998; Gonçalves-Vidigal et al., 2007; Geffroy et al., 2008; Coelho et al., 2013; Trabanco et al., 2015). Andean genes include *Co-1, Co-12, Co-13, Co-14, Co-15, Co-x, Co-w, Co-y, Co-z, Co-Pa* and *Co-AC* on chromosomes Pv01, Pv03, and Pv04 (Cardenas et al., 1964; Geffroy et al., 1999; Melotto and Kelly 2000; Gonçalves-Vidigal and Kelly 2006, 2008, 2009, 2012; Geffroy et al., 2008; Sousa et al., 2015; Lacanallo and Gonçalves-Vidigal 2015; de Lima Castro et al., 2017; Gilio et al., 2017). Resistance to ANT provided by these major genes is race-specific with some genes being effective against specific races but less effective against other races (Mungalu et al., 2020). However, *Co-4* confers broad-spectrum resistance to a wide-range of races and has been widely deployed in breeding programs (Pastor-Corrales et al. 1994). No single gene or genotype confer resistance to all races of *C. lindemuthianum* but a combination of major genes does confer broad resistance to diverse races. For example, the genotype G2333, which possesses the *Co-4*, *Co-5* and *Co-7* genes, is resistant to a wide range of races (Pastor-Corrales et al., 1994; Young et al. 1998; Vallejo and Kelly, 2009). Even G2333, which is widely acknowledged to have high levels of resistance to many races is susceptible to race 3481 (Mahuku et al., 2002). Because of the high genetic variability of *C. lindemuthianum*, there is a need to continuously be searching for new sources of resistance.

Gene banks hold collections of plant genetic resources for long-term conservation and use by plant breeders, researchers, and others. The International Center for Tropical Agriculture (CIAT) gene bank in Cali, Colombia, holds 37,987 *Phaseolus* accessions (as of 22/01/2021) , including the largest collection of common bean genetic resources in the world. While this large number is useful for preserving the genetic diversity of the crop but it can impede the end use of these materials in breeding or research. To circumvent the logistical challenges of working with a large number of accessions in gene banks, the concept of core collection was introduced by Frankel (1984) and is used by almost all gene banks. A core collection is a limited set of accessions (about 10% of all accessions in the gene bank), with minimum repetitiveness and representing the genetic diversity of a crop species and its wild relatives (Brown, 1989). CIAT gene bank has a core collection comprised of 1,500 *Phaseolus vulgaris* accessions. This collection serves as a representative sample for the 37,987 *Phaseolus* accessions found in its entire collection (https://ciat.cgiar.org/what-we-do/crop-conservation-and-use/bean-diversity/, (information accessed 22nd January 2021). The *Phaseolus* core collection is comprised of *Phaseolus* species including *P. vulgaris* (common bean), *P. coccineus* (scarlet runner bean) and *P. dumosus* (year bean). The *Phaseolus* CIAT core collection is an important genetic resource and a reservoir for traits such as ANT resistance. Evaluation and characterization of core collection enhances the value of accessions for potential use in breeding and research. Current knowledge is void of the evidence of the core collection having been evaluated for resistance to races 37, 73, and 566. Characterizing the reaction of the core collection for resistance to ANT could enhance its value and potential use as an important resource to support development of ANT resistant varieties.

1.1 Objectives

1. To characterize three isolates of C. lindemuthianum collected from three major bean-growing regions in Zambia.
2. Evaluate a subset of the CIAT *Phaseolus* core collection for resistance to races 37, 73, 566 and a blend of 20 races of *C. lindemuthianum*

1.2 Hypotheses

1. There exists phenotypic variation of *Colletotrichum lindemuthianum* in Zambia
2. There exists new sources of resistance to anthracnose in the CIAT *Phaseolus Core collection*

1.3 Justification

Anthracnose caused by *C. lindemuthianum* pathogen is an important bean disease which causes economic losses to the crop. It has been listed among the major production constraints in both tropical and temperate environments worldwide. (Graham and Ranalli, 1997). It can cause total crop failure if it attacks a susceptible variety. The major challenge in breeding for anthracnose resistance is that the pathogen is highly genetically variable and it has co – evolved with the host. The co- evolution is fast and ongoing. Barrus in 1911 first noticed differences between the virulence of two races of anthracnose against 139 bean cultivars. Therefore, his report confirmed the variability of the pathogen.).

The high level of pathogenic variability leads to frequent formation of new races which results in increasing potential for losing resistance in originally resistant varieties, therefore, there is need to phenotype the core collection to identify various sources of resistance to the pathogen. The core collection represents the global germplasm collected and characterized. The establishment of the ex situ germplasm collection has been the result of global effort and represents a rich resource in terms of genetic and uniqueness of cultivars.

The study will result in identification of the sources of resistance within the CIAT core collection. Anthracnose resistance is genetically controlled therefore selection is possible when developing varieties that are resistant to different races of anthracnose. Gene pyramiding would be exploited which can lead to simultaneous expression of more than one gene in a variety to develop resistance. This is a cheaper and feasible way of resolving the challenge especially for the resource poor farmers. The results will assist in identifying those genotypes that can be further used in breeding programs with an objective of breeding for resistance against various races of the pathogen. (Genchev *et al* 2014) confirmed that a complex resistance to all physiological races of *C. lindemuthianum* can be achieved by pyramiding of the respective specific genes in a single plant.

CHAPTER 2.

2.0 LITERATURE REVIEW

2.1 The Genus Phaseolus

The genus *Phaseolus* has over 80 cultivated and wild species (Freytag and Debouck, 2002) and the specie *Phaseolus vulgaris* (common bean) is the most widely distributed. In addition to *P. vulgaris*, the other cultivated species of the genus *Phaseolus* include scarlet runner bean (*Phaseolus coccineus*), year bean (*Phaseolus dumosus*), tepary bean (*Phaseolus acutifolius*) and lima bean (*Phaseolus lunatus*). *P. coccineus* and *P. dumosus* are the two most closely related species to *P. vulgaris* among the cultivated species (Broughton et al. 2003).

The specie P. *coccineus* commonly known as runner bean has a twining perennial vine. It is a native to the mountains of Central America, grown for both as a vegetable as well as for its edible pods and/or as an ornamental plant usually for appreciation for its showy flowers. It grows at higher elevations than the common beans. It forms tuberous roots from which new shoots sprout annually in areas with frost where it is not evergreen. In Mesoamerican, the thick starchy roots are used as food. It is most attractive to humming birds and bees and most productive in cooler summers (Susan, 2014).

The specie *P. dumosus* is the lowest of any specie in the *Phaseolus genus* Secondary gene pool. Currently it has a small geographical range restricted to central and southern Guatemala. It also grows under challenging environments such as high altitude and cool wet climatic conditions. CIAT bean breeding programme has succeeded in transferring valuable resistance genes from *P. dumosus* to *P. Vulgaris* (Tohme, pers. Com – cited by Angela *et al.,* 2016). The specie *P dumosus* is closely related to *P. vulgaris* and *P. coccineus*. It has been found to be less genetically diverse which suggests a more recent domestication (Debouck, 2002). Resistance to leaf blight, white mould and anthracnose has been identified

The specie *P. vulgaris,* the most widely cultivated specie are grown and consumed mostly in developing countries of Africa, Latin America and Asia. It is the most important legume for direct consumption.

2.2 Origin and Classification

The common bean *P. vulgaris* L. is a domesticated plant which belongs to the class *Magnoliopsida* in the *Leguminoceae* family originated in Mexico, Central America (Purseglove, 1988). Recent studies based on the molecular analysis of wild relatives of the crop have also suggested central Mexico as the Centre of origin (Bitocchi *et al.,* 2013, Belluci *et al.,* 2014 – Cited by Pader *et al.,* 2017). The crop was independently domesticated in both the Andean and Mesoamerican and was transported to Africa in the 1600s (Deron *et al.,* 2016), therefore all the bean genotypes generally originate from two gene pools which are the Andean (Large seeded) and the Mesoamerican (Small seeded).

Common beans are further classified into the bush type which is the determinate and the climbing type which is the indeterminate type (Vining or Trailing) (Sigh, 1992 and waters *et al,* 1997). In the determinate type stem elongation stops when the terminal flower of the main or lateral branches have developed. On indeterminate types flowering and pod formation continues as long as moisture and temperatures are favorable for growth to occur.

In addition to the determinate and the indeterminate classification four more plant growth types have been identified and these are: Type I, type II, Type III and Type IV. Type I is a determinate bush, Type II is an upright short vine with a narrow plant profile and three to four stems, Type III is indeterminate but with a prostrate vine while Type IV is indeterminate with strong climbing tendencies (Sigh 1992 – cited by Stanley, 2006). The growth habits have become helpful in identification and classification of new upright beans, a recent innovation with high yielding potential and suitable in areas where land is a limiting factor, but all the growth types are available in different regions of Zambia where beans are grown.

2.3 Importance of Common Bean

Common bean is one of the most important legume crops. (Larisa *et al* 2020), a major source of food and nutritional security, and revenue for many households in African and Latin American countries (Akidobe and Maredia, 2012). It is rich in dietary protein which complements carbohydrate sources such as rice, maize, cassava and wheat. Many bean plant parts are used for food: fresh and dry beans, leaves and green pods, however beans are now increasingly produced for income generation. Grain is the most important, of which fresh pods and dry grains are the most marketed products (*http://www.africancrops.net/rockefeller /crops/beans/index.htm*). Beans form a major part of diets in most boarding schools, hospitals and prisons in Zambia because it is a cheap source of proteins which makes it competitive for the Zambian government to feed the masses found in these institutions.

The crop is increasingly being cultivated for income generation for both small scale and large-scale farmers especially in the northern part of Zambia. The crop haulms are suitable for use as animal feed, as part of the components for compost manure making, as mulch in orchards and gardens and as a substrate for growing mushrooms under controlled environments.

In Zambia, The Golden Valley Research Trust (GART) which promotes and disseminates information about use of cover crops for conservation farming has listed beans as one of the legumes recommended to be used as a cover crop. Cover crops provide benefits such as weed suppression, moisture conservation, nutrient recycling, soil protection, animal fodder and management of pests and diseases. (Imelda 2010). The crop is suitable for intercropping and crop rotation regimes as it fixes nitrogen in the soil and helps to restore fertility to the soil as well as provide soil cover in minimum tillage farming systems. (Piha & Munns, 1987).

It is also rich in minerals, certain vitamins and dietary fibers. The crop has a wide range of ecological preferences in that it survives better in a wide range of temperatures ranging from 14 to 26^0C. It requires an annual precipitation between 400 and 1600 mm per year depending on the maturity period of the cultivar. The crop prefers temperatures ranging from 14 to 26°C, a slightly acid soil pH (average 5-6), show a wide range for days to maturity (70-200 days) and seed yield potential depending on the variety can 400-5000 kg/ha (Wortmann *et al* 1998; Debouck, 1999). These attributes allow beans adapt to a wide range of environments making it an easy crop to grow by most rural farmers.

2.4 Bean Production Statistics

Global production statistics currently indicate 31.4 Million metric tons (Shalibande, 2019). The major producers being USA, Brazil Myanmar and China, but Brazil and Mexico are leading producers and consumers contributing about 24% to world production .The major African producers are Kenya, Tanzania, Uganda and Rwanda (faostat.@www.fao.org). East Africa contributes about 25% with consumption exceeding 50kg per capital per annum Chavez-Servia *et al*. 2010). The average production statistics in Zambia is about 24,000 metric tons annually (Sichilima *et al* 2016). This creates an annual deficit of 500 metric tons which is met through importation.

2.5 Challenges of Bean Production

Commercially cultivated bean varieties are low in productivity due to so many biotic and abiotic factors. Among the biotic factors numerous pests and pathogens often greatly reduce common bean production (Kelly and Bornosk *et al* 2018). There are many pathogens that have been identified to attack beans. These include bacteria, fungi and viruses. About 200 of these are known to be able to attack the crop but among the 200, less than 10 are able to attack and cause economic losses to the crop. In bean production biotic factors affecting bean production and causing economic losses have been cited to be: Anthracnose, Angular Leaf Spot (ALS) Bean Stem Margot, Bean Mosaic Virus (BMV), Root roots and Bruchids among many others Anthracnose is among the most important diseases of beans which can causes serious economic losses (Padder *et al* 2017). Anthracnose is a serious seed bone disease known world over. The disease can cause total crop failure if it attacks a susceptible variety. It has been reported that infection of bean anthracnose on susceptible cultivars result in up to 100% losses when the environmental conditions are cool and humid. (Sharma *et al*., 1994; Fernandez *et al*., 2000; Padder *et al.,* 2007; Sharma *et al.,* 2008).

2.6 Bean Anthracnose

Anthracnose caused by the seed- borne hemibiotrophic fungus *Colletotrichum lindemuthianum* has been cited as one of the most important disease of beans (Padder *et al* 2017). The scientific community has been studying the disease causal agent *C. lindemuthianum* and their interactions with its host for over 100 years. This information has helped the community to understand the pathosystem and have come up with ways and means of managing the disease. The fungus was discovered by Lindemuth in 1875 (Thomazella 2002). It was *first* described by Saccado in 1978 and was reported not only on *P. vulgaris* but also on other *Phaseolus* genuses in different parts of the world. Taxonomically it belongs to Fungi *Assomycotina, Pezizomycotyna Sordarmycetes*, *Hypocreomycetide* and *Glomerellale*s. The infectious nature of the pathogen was established by Frank in 1883 (Day, 1919) It overwinters in seed and crop residue (Paddler, 2017) and affects all aerial parts of the bean plant. Anthracnose caused by a pathogen known as *C. lindemuthianum* is the most destructive disease of beans in the tropical and the sub- tropical regions especially under cool and humid climates (Tu 1988, Shamar et al 1994).

The pathogen is known to attack the crop at all the stages of growth from seedling to maturity depending on the prevalence and the favorable conditions essential for initiation of infection and further development of the disease. The pathogen has a wider host range among the cultivated legumes and it has been isolated from lima bean (*P. lunatus* L.), scarlet runner beans (*P. coccineus*), tepany beans *(P. acutitolius var.latifolius* L.), Mung bean (*Vigna radiate*), cow pea (*Vigna unguiculata*), kudzu beans (*Dolichos bitloris* L.), and broad beans (*Vicia faba* L.), soybean (*Glycine max*), Pea *(Pisum sativum*) and black gram (*Vigna mungo*) (Yuesuf, 2005).

2.6.1 Taxonomy

C. lindemuthianum is considered as hemibiotrophic fungus that is it is parasitic in living tissue for some time and later it continues to live in dead tissue. Its taxonomical classification was a difficult, confused task. This fungus had been named with different synonymous throughout the years. Then, it could be hardly identified through classical taxonomy, because it produces acervuli with or without fruiting body depending on the quality and amount of substrate (Sicard *et al.,* 1997) Now, the fungal names are given according to principles and rules of the International Code of Botanical Nomenclature, although there is still some controversy in the designated names to some fungus. The *C. lindemuthianum* classification was made by the (Alexopoulos 1979) In this case, most authors agreed that *C. lindemuthianum* belongs to: Family, *Melanconiaceae*; Order, *Melanconiales*; Sub Class, *Coelomycetidae*; Class, *Deuteromycetes;* Sub Division, *Deuteromycotina*; Division *Amastigomycota*; Kingdom *Myceteae*; Super Kingdom, *Eucariota* .

2.6.2 Biology

C. Lindemuthinum is an ascomycete characterized by bearing spores in a sac (Asci) and also a deutromycetes whose multiplication is ensured by asexual reproduction and uses conidia for further infection and spread (Alexopoulos 1962, bailey et al., 1992). Although the continuity is ensured by asexual reproduction, sexual forms of the pathogen have been observed in laboratory trials but they are not reproducible due to low viability of the spores (Bryson et al 1992). After this experiment, it was therefore concluded that sexual reproduction occurs in natural populations leading to recombination between different loci (Sicard et al 1997). The fungus is found in nature in an imperfect stage but can overwinter as mycelia or conidia, the perfect stage *Glomerella cingulata* is rarely found in nature. Its conidia are oval shaped and dark brown in color (Agrios 1997). On the host they form pink masses of conidia packed into acervuli. *C. lindemuthianum* is distinguished from other species of the same genus by its growth characteristics and a dark pigmentation on cultures (Tesfaye, 2003).

The pathogen has been found to withstand unfavorable conditions in nature by its mechanism of overwintering in plant residue, however, the most common survival mechanism is through infected seed, which ensures its passing on to the next season. (Tu, 1981, Young and Kelly, 1997). The development of the disease requires high humidity

(87 – 93%) and a temperature range of 15 – 28^0C and rainy conditions with windy weather are necessary for epidemic spread (Tu, 1981).

The pathogen can survive up to 5 years and does overwinter on crop debris. Seed infection is the primary means and ways by which the pathogen spreads. As long as the seed remains viable, the fungus also remains viable therefore, the production and use of improved varieties of certified seed against anthracnose is one control measure known to be effective in controlling the disease (Mohammed, 2013).

The survival of the anthracnose fungus is influenced by the environment, type of infected tissue and depth of burial in the soil. On average it persists in the soil between 9 to 18 months which is long enough to save as source of primary infection to subsequent crops of beans. The pathogen overwinters longer on infected stems and pods than it does on infected seed (Robert, 2018).

The fungus can survive over the winter in dry straw, but not in wet or burned straw. The disease is spread by spores carried by raindrops, machines, wind and people. Therefore, the burial of infected tissue which is achieved by cultivation reduces the viability of the pathogen in all tissue types. The study indicated that the pathogen could affect the subsequent bean crop after a 3-year period of fallow or zero tillage, therefore crop rotation should not be used in isolation to control bean anthracnose but should be used in an integrated control system. (Dulard, 1990)

2.6.3 Morphology and Etiology

The conidia are born on acervuli, the acervuli are mostly in groups, coalescing and covering lesions on infected plants. Stae are few and longer than conidial mass. The conidial masses are Orange to bright Orange. Mycelia are scanty and white. Conidia are oblong to dumble shape, one celled, round of about 9 – 15 x 3 –4 µm (Muthur and Kongsdad, 2000) Conidia are uninucleate and usually have a clear vacuole – like body near the center. *C. lindemuthianum* has a unicellular conidium which in an aqueous environment produces a single germ tube. The conidium germinates within six to nine hours and produces one to four germ tubes (Zaumeyer and Thomas, 1957) upon contact with the host the germ tube tip swells and differentiates into a thick walled heavily melanized appresorium. A penetration hypha arises from below the appresorium and penetrates cuticle and host cell wall during pathogenesis. Inside the lumen, a globose infection vesicle develops which in turn gives rise to a primary hypha (Zaumeyer and Thomas, 1957). Following infection, the symptoms begin to show after three to seven days depending on the prevailing environmental conditions.

2.6.4 Bean Anthracnose distribution

Anthracnose was first described from plant specimens obtained in Germany in 1875 (walker, 1957) since then, the disease has become one of the most important and widely distributed throughout the world. It has been reported in USA (zaumeyer 1957) European countries (Hubelling, 1977) Canada (Tu, 1980) Latin America [Tu, 1983, CIAT' 1988). In Africa, it is particularly important in Uganda, Kenya, Tanzania, Rwanda, Burundi, Ethiopia and D.R. Congo (Batureine, 2009).

In Zambia, the disease is highly prevalent and severe in wetter agroecology of Kasama, Northern Zambia where seventeen pathotypes where found (Kachapulula *et al.,* 2010) plant residues contribute to pathogen survival and dissemination (Chaves 1980) infected seed is cited to play an important role in the international distribution of pathogen. This is especially true for African countries where farmers continuously exchange and use infected seed contributing the distribution of the pathogen. In Brazil more than 25 different *C. lindemuthianum* races have been identified (Thomazella et al 2002). In Tanzania yield losses remain very high (40-80%) and are estimated to be worth $304 million per annum. In Uganda, anthracnose is the most important disease in the high altitude, low temperature areas (Opio et *al.,* 2006). In Sudan, field losses in these regions, due to seedling, leaf, stem and pod infections are up to 90% under climatic condition favorable to the disease. The infected seeds are the most important means of dissemination of this pathogen, which explains its worldwide distribution (Mudawi, 2009).

Economic importance of anthracnose in recent years has declined in developed countries through the effective use of clean seed and development of resistant varieties [(Yuesuf

2005, Awigchew 1982, Allen 1983). However, in developing countries, it remains serious and it is regarded as one of the principal diseases of beans throughout tropical regions including Latin America and Eastern Africa (Allen 1983).

2.6.5 Host Range of Colletotrichum

Colletotrichum species have been one of the major plant pathogens in the world with its host range varying from cereals, legumes, fruits, vegetables and ornamental plants (Bailey and Jeger1992). They cause huge crop losses in tropical, subtropical and temperate regions (Freeman, 2000). The pathogen is reported to cause anthracnose in about 121 host genera belonging to 45 different plant families (Farr et al.2009). *C. lindemuthianum* specie which affects legumes has been isolated from lima bean (P. lunatus L.), scarlet runner beans (*P. coccineus*), tepany beans (P. *acutitolius* var.latifolius L.), Mung bean (*V. radiate*), cow pea (*V. unguiculata*), kudzu beans (*Dolichos bitloris* L.), and broad beans (*Vicia faba* L.), soybean (*G. max*), Pea (*P. sativum*) and black gram (Vigna 2005).

2.6.6 Symptoms and Losses associated with Bean Anthracnose

Generally, the typical symptoms are deep, shrunken lesions containing flesh-colored spores on bean pods that are the most distinctive symptoms of anthracnose. Lesions also commonly appear on stems, hypocotyls and leaf veins of seedling plants, with more advanced disease resulting in wilting and flagging of chlorotic leaves similar to that of other foliar pathogens. (Pastor-Corrales and Tu, 1989). Further advancement of the disease leads to complete girdling and eventually death of the plant.

Symptoms of Anthracnose disease has been observed to appear on health plants between three and seven days after major rains and high temperatures which are conducive for its development. Pathogenesis is considered to be successful when attachment of disbursed fungi propagules is achieved (Harmar *et al*., 1988). However, there are suggestions that adhesion of ungerminated conidia is largely a passive process because in experiments where respiration and transcription inhibitors were used showed no effects on the adhesion. It is also observed that anthracnose conidia will adhere rapidly to a wide range of plant and artificial surfaces suggesting that adhesion is none specific (Mercure *et al.,* 1994, 1995).Leaf infections and symptoms appear as lesions on leaf petioles and veins. Early infection signs usually appear on the lower leaf surface and veins which show brick red to purplish discoloration. Brown lesions of different sizes develop around small veins. As the disease progresses vein necrosis occurs first then wilting and bleaching often appears at the tip of the leaflet before spreading over the margin and finally over the center of the blade (Godoy *et al.,* 1987). During this stage hyphae proliferate throughout host tissue and cells. It extends to also to intracellular spaces where it produces cell wall degrading enzymes and low molecular weight phytotoxins. These phytotoxins contribute to necrotrophic growth of the pathogen (Bailey *et al.,* 1992). As the diseases progresses, conidiophores rapture through the host cuticle and form acervuli on the plant surface.

When it comes to pod infections and symptoms, the stems are infected first by anthracnose fungus before the pods are attacked. Infection of the stem is identified by dark brown eyelike spots which develop longitudinal along the stem. (Tu, 1983). If eye spots enlarge in young seedlings the stems may break off but in order stems eye spots become limited. After stem infection, it is passed to the pods. Infection of the bean pods results in rust-colored lesions that develop into sunken cankers with black ring borders. The most striking symptoms are small brown specks or rust brown spots. Each of the tiny black specks contain a mass of pinkish spores (Godoy *et al.*,1997). These spores are visible as viscous droplet in humid conditions. The lesions on the pod usually reach a diameter of 5-8 mm and they are slightly sunken on the center and have a dark brown or purplish brown margin (Tu, 1983)

Severely infected premature pods abort and fall early, while pods that mature produce infected seed with dark cankers that make the seed unattractive and unmarketable to consumers. Water splashes disperse spores and result in the secondary spread of disease.

2.7 Control Measures of bean Anthracnose

There are several methods that have been identified as control measures for bean anthracnose, but no single method is effective enough for the control of the pathogen. Therefore, integration of several methods is recommended in order to eliminate the anthracnose disease. The methods include cultural, physical, chemical, and biological and host plant resistance. (Mohammed, 2013)

Culturally, anthracnose disease is best controlled by using disease free seed. Seed produced under wet and humid conditions should be avoided to be used as seed for the next season. Crop rotation is recommended at least three years interval which helps in reduction of the inoculum in the field. Stations of cleaning and bagging where anthracnose has been a source of contamination dust should be cleaned of debris between shipments and the shipments be isolated (http://www.ipmcentres.org/cropprofiles/docs/TNsnapbeans2012.pdf). Seed storage facilities and commonly used agricultural materials such as leather, rubbers, denim and painted metal should be disinfected with a 10% bleach solution of 0.525% of sodium hypochlorite (Jik), followed by chlorine dioxide (aquacare) and chloroxylenol (Dettol) (Buruchara *et al.*, 2010)

Crop rotation should include none host plants like cereal and s*olanaceous* crops, this strategy reduces bean anthracnose due to reduction of initial infection from the initial inoculum. Additionally, fields should not be worked when plants are wet because this facilitates rapid fungal spore movement from diseased plants to healthy plants under these conditions (Tu, 1986). Weekly scouting for symptoms of the disease helps to avoid using seed from diseased plants for the next season. Cultivar mixtures containing at least 60% of a resistant cultivar have been reported to offer a good control of anthracnose (Tesfaye, 2003).

The most commonly used physical methods include hot water seed treatment and solarization. The hot water seed treatment involves soaking the seed in hot water at 64 to 72^0 F for 15 hours, followed by another soaking at 117^0 F for 25 minutes has been reported to kill the fungus in infected seed without compromising the germination percentage of the seed (Bush, 2009). Solarization method involves covering the soil with a transparent plastic sheet for a month before sowing. This has been proved not only to reduce disease severity but also disease incidence (Mohammed *et al* 2013)

Chemical control through use of fungicides is recommended. Fungicides containing Chlorothalonil, Zineb, Maneb, Benomil, Folfet or Captafol have been recommended to be used at first sight of the symptoms of the disease **(**Agrios, 1997). Chemicals like Maneb and Zineb at 3.5g/l, Benomyl at 0.55g/l (Beshir, 1997), Captafol at 3.5kg/ha, Carbendazim at 0.5kg/ha and Fentinhydroxide at 1.2g/l (Pastor-Corrales and Tu, 1989) have been reported to be effective in controlling bean anthracnose. Benomyl seed dressing followed by a foliar difenoconazole spray, or difenoconazole application alone, showed a promising anthracnose control strategy (Tesfaye, 2003).

Host plant resistance is the most effective method of anthracnose management as it permits use of cultivars that have been tested for resistance against various forms of *C. lindemuthianum,* however, integrating resistance with other control measures gives a good control of the disease.

2.7.1 Host Plant Resistance

Plant resistance is the relative amount of its inheritable qualities that influence the ultimate degree of damage done by any pest or pathogen. It is expected that even common beans should express a similar phenomenon against all pathogens (Panter, 1951). Two kinds of resistance to crop pathogens are recognized which are vertical and horizontal resistance. Vertical resistance is conditioned by single genes which are part of gene – for gene relationship. This resistance is less durable as compared to horizontal resistance which is known to be conditioned by multiple genes (Venderpark, 1975, Robinson 1997). Horizontal resistance invariably occurs in the absence of vertical resistance. Unfortunately, it tends to breakdown when crops are bred for vertical resistance or when crops are bred under the protection of chemicals.

Resistance is the most effective and efficient approach of managing anthracnose but the challenge has been presented by different forms in which the pathogen exists. There are no single genes that are effective against all known races of the pathogen, the protection offered by single genes is short lived. This simply means that plants that are known to be resistant to one race may be susceptible to another race. The broken resistance is as a result of adaptation of the pathogen to the local environment. It is therefore, recommended to test the cultivars in these environment in order to assess their tolerance to the locally adapted races (http//www:ipmcenters.org/cropprofiles/docs/TNSpanbeans2012.pdf).

Bean breeders should seek various sources of genetic resistance. Cultivars such as AB 136 and G2333 could be used as sources of resistance in breeding programs since they have been identified to be highly resistant to different races of *C. lindemuthianum* found in Africa, North and South America (Mohammed, 2013). Alternatively, durable resistance should be acquired through exploitation of horizontal resistance breeding (Pastor-Corrales *et al.,* 1995). Although the genetic basis of durable resistance in plants has not been fully understood, it is frequently presumed that quantitative resistance conditioned by minor genes and supposed to act in a race – none specific manner would provide durable resistance (Johnson 1981 – cited by Perseguin *et al.,* 2016). Cultivar mixtures which contains at least 60% of a resistant cultivar have been reported to offer a good control of anthracnose. This could be another added approach in reducing effects of anthracnose on seed quality and quantity in beans.

2.7.2 Mechanisms of Resistance

Plants possess a wider array of physical and chemical strategies for defense against invasion by pathogens. Host resistance can be based on physical factors and structural barriers. These represent the first line of defense against fungal pathogens. Physical barriers which include topography, waxes and leaf hairs impede formation of infection structures. Physical barriers such as the mechanical strength of the cuticle and epidermal wall and the resistance of their structural polymers to enzymatic degradation may contribute to grater resistance in species of mature leaves and stems as compared to young plant organs (Health 1981 – cited by Stanley 2006).The second line of defense is that of biochemical which is considered more important as it determines the success of an infection.

A common feature of a host resistance is the browning and death of host cells once infection has occurred. This is referred to as Hypersensitive Reaction (HR) (Bailey, 1991) and (O' Connell and Bailey 1986) Researchers distinguished two kinds of resistance in the bean anthracnose pathosystem. One form involves early death of epidermal cells and the other one involves delayed death of infected cells. In many resistant cultivars single epidermal cells die as soon as they are in contact with the pathogen. This type of reaction is dependent on localized accumulation of phytoalexins, an example of HR based on the host cell incompatibility (Stanley, 2006).

Another group of metabolites associated with host resistance are known as Hydroxyproline – Rich Glycoprotein ($HRGP_s$). These accumulate in bean seedlings infected with *C. lindemuthianum* and it has been reported that a greater amount is produced in resistant varieties as compared to susceptible ones. HRGPs act by increasing the structural resistance of cell walls to pathogenic penetration. In addition to HRGPs other plant defense proteins are thought to be secreted at the host – pathogen interface. These include protein inhibitors of fungal hydrolases which exert their hydrolytic activity towards bacterial and fungal cell walls causing release of elicitor – active fragments (Esqurre' – Tugaye' *et al*., 1992).

2.8 Pathogen Variability

Despite availability of management tools for the bean anthracnose disease, which include folia spray with fungicides, use of certified seed, genetic resistance and crop rotation, the crop is still under threat to the disease because of high pathogenic variability. The variability has been researched upon by a number of scientists in Mesoamerican, North and South Andes which are the three centre of origin of common beans. There is a suggestion that co- evolution between the pathogen and common bean the host has led to parallel gene pools. (Gepts, 1988, Sicards *et al.*, 1997) based his subdivision of *C. lindemuthianum* isolates on molecular and virulence markers into three groups corresponding to the gene pool. Polymorphism for resistance was further established in both between and within the three centres of origin (Gonzales *et al.*, 1998). A better resolution of the structure of the variability in *C. lindemuthianum* is obtained by combining virulence and molecular analyses.

The race structure of *C. lindemuthianum* is highly variable and new ones keep emerging time after time (Tu 1987, Nkalubo 2006). The fungus has races that vary from country, region, location and variety (CIAT, 1997). The origin of pathogenic variability is of sexual reproduction mechanisms such as recombination of nuclear genes. Pathogens that reproduce sexually like *C. lindemuthianum* species do produce variants more readily than those mostly asexual (Ogallo, 1991). Numerous anthracnose pathogen variants or physiological races are identified by their reaction on a set of host varieties commonly referred to as host differentials. Different races differ from each other primarily on the basis of pathogenicity (Agrios1997). Research has demonstrated high variability of the pathogen by using the international set of bean differentials for classification of *C. lindemuthianum.* For example (Alzate *et al.,* 2004 identified 50 pathotypes in Brazil between 1994 and 2002, while Mahuku and Riascos, 2004 identified 90 races from 200 *C. lindemuthianum* isolates collected from Andean and Mesoamerican bean varieties and regions. To date about 1590 isolates of *C. lindemuthianum* inoculated on 12 bean differential cultivars have resulted in the identification of 182 races worldwide (Appendix 1).

Race 0 does not cause infection on any of the 12 differential cultivars but is present in Mexico, France and India (González-Chavira *et al.,* 2004; Sharma *et al*., 2007). However, race 0 infects other bean cultivars, so there is a need to include a susceptible cultivar in the differential set that does not possess any major anthracnose resistance. The identified races are assigned a value based on the binary nomenclature system. Each differential cultivar has an assigned number (*2n*), where *n* corresponded to the place occupied by the cultivar within the differential series. The designation of a race number is obtained by summing the *2n* values of all cultivars exhibiting susceptible reactions to the isolate used for inoculation. (Balardin *et al*., 1997). For example if a race is virulent on Widusa and Kaboon, then the race number will be;Wedusa = 2^n where n = 4, 2^4 =16
Kaboon = 2^n where n = 5, 2^5= 32

$(2^4 = 16) + (2^5 = 32)$

$16 + 32 = 48$.

Therefore a race virulent on Widusa and Kaboon only will be race 48 (Balardin et al., 1997, Kelly and Vallejo, 2004)

2.9 The Core Collection Concept

When materials grown in centers of diversity face extinction, they cause genetic erosion in crops. Establishment of germplasm containing as many genetic components as possible such as varieties, lines and clones with the highest variability possible becomes necessary in resolving the challenge (Buelnal *et al*., 2001). The management, evaluation and use of large germplasm is expensive and inefficient due to redundancies, duplication and the difficulties in analyzing all the conserved accessions in detail (Xu *et al.,* 2016). Constitution of the core collection makes it easier because accessions can be represented by low redundancy thus using only a lower percentage of the size of the entire germplasm (Gomes *et al.,* 2019)

The core set is constituted from a set of lines/landraces based on morphological diversity of qualitative traits. The core collection is the representative of sub – samples of germplasm collection that have been developed to improve the efficiency of evaluating the accessions while increasing the probability of finding the genes of interest. These accessions are selected to represent climatic conditions and geographical availability of the species especially in the primary centers of origin. (Hormann *et al*., 2006) Genetic diversity conservation is an important prerequisite for developing new varieties with desirable agronomic characteristics. A large number of germplasm collections have been established worldwide, However, many of them face major challenges due to large size and lack of adequate information about population structure and genetic diversity. Very little is known about the genetic diversity and structure of such collections at the interspecific and intraspecific level. (Lee *et al*., 2016). In order to realize efficient use of large germplasm collections, the core collection concept has been proposed.

A core collection is a subset of germplasm collection of a representative species that represent the genetic diversity of the entire core collection. (Frankel, 1984). The good attribute of the core collection is one that has no redundant accessions, it should be small enough and should represent the total genetic diversity. The major reasons for establishing a core set is to reduce the number of representations to about 10% while ensuring that the genetic diversity is maintained (Thies and Ferry 2002, Hanson 2004). Ideally the diversity of a core collection should be at least 70% of that found in the entire collection (Brown and Spillane 1999)

Different types of data which may include molecular markers agronomic traits and geographic traits and geographic origin can be used (Quenouille *et al*., 2006). Different methods are used for selecting a core set depend on the type of research to be conducted. The data generated out of the core set becomes valuable for breeding purposes. (Lee, 2016). In Capsicum core collection establishment, five different methods were used. The core set were selected based on, 1) Genotypic analysis of entire collection, 2) Genotype analysis of each cluster after grouping based on genotype dissimilarity,3) Phenotype analysis of the entire collection, 4) A combination of phenotype and genotype analysis of entire collection, 5) A combination of genotype analysis of each cluster after grouping based on genotype dissimilarity (Lee, 2016). The accessions selected for the core collection may be used in future studies of genome association as well as in genetic crosses in breeding programs aimed at developing improved cultivars with high genetic diversity which can meet current and future market needs.

CHAPTER 3

3. MATERIALS AND METHODS

Objective 1

3.1 Plant Material for Differential Cultivars

A set of 12 differential standard cultivars of *P. vulgaris* was used to characterize three isolates of *C. lindemuthianum* (Pastor – Corrales, 1991*)*. Of these 12 differential cultivars, four are Andean while the remaining eight are Middle American (Appendix 2). The four Andean genotypes contain the *Co-1* locus, which is multi-allelic ($Co\text{-}1^2$, $Co\text{-}1^5$). Small quantities of seeds of the 12 differentials were obtained from CIAT, Cali, Colombia and multiplied in the greenhouse at the University of Zambia, Lusaka, campus for use in characterization.

3.2 Disease Sample collection and *C. lindemuthianum* isolation

Naturally infected bean pods and leaves showing typical anthracnose symptoms were collected from farmer fields located in agricultural camps found in the Mbala, Mporokoso, and Mpika districts in the Northern and Muchinga Provinces of Zambia. Only one farmer field was sampled by random selection in each of these three districts. This was because the sampling was restricted to targeted farmers where the disease pressure higher. Four samples were collected in each farmer field. Disease samples were packed in envelopes and transported to the laboratory for storage and isolation.

Isolation and characterization were conducted following a protocol described in Mungalu et al. (2020). To isolate the fungus, the interface of the infected and healthy tissue on the lesions, was cut into small pieces (0.5 to 1cm ^{2}). These small pieces were surface-sterilized and incubated on petri dishes containing either Potato Dextrose Agar (PDA) (39g/l) (races 37 and 566) or Modified Mathur's media (dextrose 8g/l, MgSO4·7H2O 2.5 g/l, KH2PO4 2.7g/l, neopeptone 2.4g/l, yeast extract 2.0g/l, agar 16g/l) (race 73) in the dark for 10-12 days until sporulation. Some isolates sporulate better on PDA while others on Modified Mathur’s media. The margin of the growing fungus from the tissue on the petri dish was excised using a cork borer and transferred to a new plate, which was then incubated in the dark until sporulation. Single spore/monosporic cultures were made on PDA and Modified Mathur’s agar and incubated in the dark for 10-15 days.

When isolates had sporulated, a spore suspension for seedling inoculation was prepared by flooding plates with distilled water. The culture surface was scraped with a glass rod to dislodge spores into spore suspension. The spore suspension on the plate was then filtered through a double-layered cheesecloth and the concentration adjusted to 1.2×10^6 spores per ml using a haemocytometer. Tween 20 was then added to the inoculum to a final concentration of ~ 0.01% (1 drop of Tween 20 from a pasteur pipet for every 100 ml of inoculum).

3.3 Inoculation of the Differential Cultivars

Six seeds of each of the 12 differential cultivars were surface sterilized and pre-germinated on petri dishes in an incubator at 27^oC to ensure uniformity. Two pre-germinated seeds of each differential were transferred to 250 ml plastic pots containing clay loam soil. Each pot was considered as a replication; therefore, each differential was grown in three replications, with two seedlings per replication (total of six seedlings for each differential). Pre-germinated seeds in the pots were grown in the greenhouse for 7 to 10 days to primary leaf stage (fully expanded). After 10 days, seedlings were inoculated with individual isolates by spraying the leaves on top und underside and on the stem until run off. A standardized concentration of $1.2 \text{ x}10^6$ spores per ml was used for each isolate. Inoculated plants were left on the bench top to dry and later were incubated in a humidity chamber with >90% humidity for 72hrs at 23- 25^0C. Seedlings where then removed from the humidity chamber and transferred to the greenhouse ($25\text{-}28^0C$) where they were kept for 5-7 days for anthracnose to develop.

3.4 Race Characterization

After the disease had developed, seedlings were rated for anthracnose reaction based on a 1-9 severity scale (Balardin et al., 1997). Plants with no visible symptoms or with few very small lesions mostly on primary leaf veins were considered resistant (1-3), seedlings with small lesions on leaves and seedling stem were considered moderate resistant (4-6) and seedlings with numerous small or enlarged lesions, with sunken cankers on leaves and seedling stem, or dead plants were considered as susceptible (7-9). The races of *C. lindemuthianum* were identified (Annex 2) using a set of 12 differential cultivars, which are associated with a binary system for the determination of each race (Pastor-Corrales 1991)

Objective 2: Evaluation of a subset of the CIAT Phaseolus core collection for resistance to races 37, 73, 566 and a blend of 20 races of *C. lindemuthianum*

3.5 Plant Material for the Core Collection

An entire core collection comprised of 1,500 *Phaseolus* accessions were sourced from the CIAT gene bank (Palmira, Colombia). However, only 907 *Phaseolus* accessions produced seed when planted in Zambia for seed increase. Therefore, only these 907 accessions were used in the current study. Because of insufficient seed quantities for some of the 907 accessions, the number of accessions screened for each race was variable, i.e., 907, 883, 900, and 331 for races 37, 73, 566, and a blend of 20 races, respectively. Of the 907 accessions that were screened for resistance to race 37, 885 were *P. vulgaris*, 11 were *P. coccineus* and 11 were *P. dumosus*. Of the 883 accessions that were screened for resistance to race 73, 869 were *P. vulgaris*, 10 were *P. coccineus* and four were *P. dumosus*. Of the 900 accessions that were screened for resistance to race 566, 881 were *P. vulgaris,* 13 were P. *coccineus* and 6 were *P. dumosus*. All the 331 accessions screened with a blend of 20 races belonged to *P. vulgaris.*

3.6 Evaluation of the core collection for resistance to races 37, 73, 566 and a blend of 20 races

The core collection was inoculated with *C. lindemuthianum* races 37, 73, and 566, which were characterized in Objective 1. Races 37 and 566 were classified as mixed races of both the Andean and Middle American gene pools, while race 73 is Middle American. Classification of races into either Andean or Middle American was based on the reaction of the Andean and Middle American race differential cultivars. A race that was virulent only on Andean host genotypes was classified as Andean while a race virulent only on Middle American hot genotypes was classified as Middle American. A race that was virulent on both Andean and Middle American differentials was classified as mixed race. Additionally, the core collection was inoculated with inoculum that had a blend of 20 characterized races. The 20 included the characterized races 37. 73 and 566 The characterization followed a procedure described by Kelly and Vallejo, 2004) After constituting and adjusting the concentration of the inoculum, equal measurements of 5mls were obtained and mixed. (Races 17, 19, 23, 38, 39, 45, 49, 55, 63, 95, 178, 499, 1091, 1141, 1153, 1199, 1331, 1344, 1407, and 1631). Of these 20 races, 2 are Andean (38 and 566), 2 are Middle American (race 1153 and 1344) and the remaining races were classified as mixed races of Andean and Middle American gene pool. The core collection seed was planted on Styrofoam trays with 200 wells. Each well was 3cm long, 3cm wide and 5cm deep. The genotypes G2333 and Kabulangeti (local landrace) were included in the evaluation as resistant and susceptible checks, respectively. Evaluation was conducted using a completely randomized design (CRD) with 3 replications. Each replication had two seedlings (a total of six seedlings per accession). Inoculum preparation and inoculation for the core collection followed the same procedure described under race characterization (Mungalu *et al* 2020)

Seedlings on Styrofoam trays were grown to the unifoliate (fully expanded primary leaf) stage and then inoculated with races 37, 73, 566, and a blend of 20 races on four separate days. Inoculated seedlings were transferred to the high humidity (>90%) growth chamber where they were kept for 72 hours at between 23 – 25^0C. After this period they were removed from the growth chamber and transferred to the greenhouse (25 – 30^0C were they were kept for 5 to 7 days to allow for ANT development. ANT severity was scored based on a 1-9 severity scale (Balardin et al., 1997), As indicated in objective 1.

3.7 Statistical Analysis

Analysis of variance (ANOVA) on ANT severity scores was conducted using PROC MIXED in SAS 9.3 (SAS Institute, 2011) based on the following statistical model: $Y_{ik}=\mu+\alpha_i+\gamma_k+E_{ik}$, where: Y_{ik} was anthracnose severity score, for accession *i*, in replication *k*; αi was the fixed variable effect of the accession *i*; γ was the random variable effect of a replication; E was the residual associated with replication *k* for accession *i*. The severity score data of accessions for all three races and a blend were not normally distributed, therefore, they were transformed (logarithmic transformation) before use in ANOVA. Means are reported in their original values.

CHAPTER 4

4 .0 RESULTS

4.1 Race characterization

The isolate named MPOSK1 from Shibwalya Kapila camp in Mporokoso district was virulent on differentials Michelite (binary number 1), Perry Marrow (4) and Kaboon (32) (Table 1). The other nine differentials were resistant to this isolate. Based on the reaction of the differentials, isolate MPOSK1 was characterized as race 37. Race 37 was classified as a mixed race of Andean and Middle American gene pool because it was virulent on both Andean differentials (Perry Marrow and Kaboon) and a Middle American differential Michelite.

The isolate named MPIK1 from Malashi camp in Mpika district was virulent on differentials Michelite (1), Cornell 49242 (8) and Mexico 222 (64) (Table 1). The other nine differentials were resistant. Based on the reaction of the differentials, isolate MPIK1 was characterized as race 73. Race 73 was classified as a Middle American race because it was virulent only on Middle American differentials.

The isolate named MBMW1 from Mwamba camp in Mbala district was virulent on MDRK (2), Perry Marrow (4), Widusa (16), Kaboon (32), and TU (512) (Table 1). The other seven differentials were resistant. Based on the reaction of the differentials, isolate MBMW1 was characterized as race 566. Race 566 was classified as a mixed race of Andean and Middle American gene pool because it was virulent on both Andean and Middle American differentials.

Table 1. Reaction of differential cultivars to three isolates of *Colletotrichum lindemuthianum*

Differential	Host Gene pool	Host genes	Binary number	Isolates		
				MPOSK1 Race 37[a]	MPIK1 Race 73[b]	MBMW1 Race 566[a]
Michelite	MA	*Co-11*	1	S	S	R
MDRK	A	*Co-1*	2	R	R	S
Perry Marrow	A	*Co-1*^{3}	4	S	R	S
Cornell 49242	MA	*Co-2*	8	R	S	R
Widusa	A	*Co-1*^{5}	16	R	R	S
Kaboon	A	*Co-1*^{2}	32	S	R	S
Mexico 222	MA	*Co-3/Co-9*	64	R	S	R
PI 207262	MA	*Co-3*^{3}, *Co-4*^{3}	12	R	R	R
TO	MA	*Co-4*	256	R	R	R
TU	MA	*Co-5*	512	R-	R	S
AB 136	MA	*Co-6*, *Co-8*	1024	R	R	R
G 2333	MA	*Co-4*^{2}, *Co-5*^{2} *Co-7*	2048	R	R	R

MA= Middle American; A=Andean; S=Susceptible; R=Resistant

[a] Mixed race, [b] Middle American race

4.2. Resistance Reaction to race 37

Significant (P<0.01) differences among accessions of *P. vulgaris* and *P. dumosus* were observed for resistance to race 37. No significant differences were observed among *P. coccineus* accessions for reaction to race 37. Of the 885 *P. vulgaris* accessions screened for resistance to race 37, 635 (72%) were highly resistant (score of 1-3), 84 (10%) were moderately resistant (score of 4-6), and 166 (19%) were highly susceptible (score of 7-9) (Table 2). The population mean for *P. vulgaris* accessions was 3. The frequency distribution of the severity scores for *P. vulgaris* accessions screened with race 37 was skewed to resistance (Figure 1). Of the 11 *P. dumosus* accessions screened for resistance to race 37, 10 (91%) were highly resistant (score of 1-3), 1 (9%) as moderately resistant, and no accessions were highly resistance. The population mean of *P. dumosus* accessions was 1.1. All the 13 (100%) *P. coccineus* accessions screened with race 37 were highly resistant (score of 1-3). The population mean for *P. coccineus* accessions was 1.1 (Table 1)

4.3 Resistance Reaction to race 73

Significant (P<0.01) differences among accessions of both *P. vulgaris* and *P. dumosus* were observed for resistance to race 73; no significant differences were observed for the *P. coccineus* accessions. The population mean of *P. dumosus accessions* was 1.6. All the 10 (100%) *P. coccineus* accessions screened with race 73 were highly resistant (score of 1-3). The population mean for *P. coccineus* accessions was 1.1 (Table 1).

Of the 869 *P. vulgaris* accessions screened for resistance to race 73, 575 (66%) were highly resistant (score of 1-3), 90 (10%) were moderately resistant (score of 4-6) and 204 (23%) were highly susceptible (score of 7-9) (Table 2). The population mean for *P. vulgaris accessions* was 3.3. The frequency distribution of the severity scores for *P. vulgaris* accessions screened with race 73 was skewed to resistance (Figure 2). Of the four *P. dumosus* accessions screened for resistance to race 73, three (75%) were highly resistant (score of 1-3), 1 (25%) was moderately resistant and no accessions were highly resistance.

4.4 Resistance Reaction to race 566

Significant (P<0.01) differences between accessions of *P. vulgaris* and *P. dumosus* were observed for resistance to race 566 and no significant differences were observed for the *P. cocineus accessions.* No *P. coccineus* accessions were highly susceptible. The population mean for *P. coccineus* accessions was 1.3 (Table 1). Of the 881 *P. vulgaris* accessions screened for resistance to race 566, 423 (48%) were highly resistant (score of 1-3), 80 (9.1%) were moderately resistant (score of 4-6) and 378 (42.9%) were highly susceptible (score of 7-9) (Table 2). The population mean for *P. vulgaris accessions* was 4.7. The distribution for the reaction of *P. vulgaris* to race 566 followed a bimodal distribution (Figure 1). Of the 13 *P. coccineus* accessions screened with race 566, 12

(92%) were highly resistant (score of 1-3) and one (8%) was moderately resistant. Of the 6 *P. dumosus* accessions screened for resistance to race 566, 5 (83%) were highly resistant (score of 1-3). There were no accessions moderately resistance. Only 1(17%) was highly susceptible (score of 7-9). The population mean of *P. dumosus accessions* was 2.3.

4.5 Resistance reaction to a blend of 20 races of *C. lindemuthianum*

A total of 331 *P. vulgaris* accessions were evaluated for resistance to a blend of 20 races. The *P. coccineus* and *P. dumosus* accessions were not screened for resistance to the blend due to lack of seed. Significant differences were observed between accessions in their reaction to a blend of races. Of the 331 *P. vulgaris* accessions screened, 30 (9%) were highly resistant (score 1-3 on a 1 to 9 scale), 20 (6%) were moderately resistant (Score 4-6) and 281 (85%) were susceptible (score 7-9) (Table 3). The average severity score for the 331 accessions was 8.0. The frequency distribution of the severity scores was skewed to susceptibility. A total of eight accessions, which were highly resistant to races 37, 73 and 566 were also resistant to the blend of 20 races (Figure 2).

4.6 Intersections of Reactions accessions of *Phaseolus vulgaris* highly resistant to Race 37, 73,566 and a blend of 20

The specie *Phaseolus vulga*ris being the most widely distributed (Freytag and Debouck,2002) requires detailed understanding with regards to its reactions to different races in Zambia. It was therefore necessary to isolate the accessions of the primary gene pool and observe their reactions to race 37, 73, 566 and a blend of 20 as indicated in figure 2. A total of 635 were resistant to race 37, 575 were highly resistant to race 73, 425 were highly resistant to race 566 and 30 were highly resistant to the blend of 20 races. 8 accessions were found to be resistant to race 37,73, 566 and a blend of 20.These genotypes identified as supper resistant to *Colletotrichum lindemuthianum* form a breeding resource as far as breeding for durable resistance is concerned. It is evident that the single race and multiple race inoculation exhibited different degrees of pathogenicity with multiple race being the most virulent. Results of this study are in tandem with what Chilipa et al, 2016 reported. Similarly Aliyu et al 2012 reported a synergistic interactions among the multiple races used to screen for resistance to Colletotrichum lindemuthianum, therefore, the blend approach could be a better option when screening for anthracnose resistance.

Breeding for race specific bean genotypes to *C. lindemuthianum*. is less effective and a non-durable control strategy. This is because new races of *C. lindemuthianum* continually emerge, rendering single gene deployment non-durable (Kiryowa et al., 2021). Therefore the deployment of varieties carrying genes resistant to all local races (gene pyramids) has been viewed as the most durable and most sustainable way (Kiryowa et al., 2021) combating *C. lindemuthianum*. Figure 2 shows potential number of bean genotypes that can be used in breeding for broad-spectrum resistance genotypes. In the study, 8 bean genotypes where found to have multiple resistance to 20 local races that were identified.

Table 2. Means and ranges for anthracnose severity measured in the *Phaseolus* core collection accessions inoculated with races 37, 73, 566 and a blend of 20 races of *Colletotrichum lindemuthianum*.

Race	Species	Checks		Core Collection accessions		
		G2333	Kab	Mean	Range	Anova
37	*P. vulgaris*	1	9	2.9 ± 0.09	1.0 - 9.0	**
	P. coccineus	1	9	1.1 ± 0.10	1.0 - 2.0	ns
	P. dumosus	1	9	1.1 ± 0.41	1.0 - 5.5	**
73	*P. vulgaris*	1	9	3.3 ± 0.10	1.0 - 9.0	**
	P. coccineus	1	9	1.1 ± 0.10	1.0 - 2.0	ns
	P. dumosus	1	9	1.6 ± 0.63	1.0 - 3.5	**
566	*P. vulgaris*	1	9	4.7 ± 0.11	1.0 - 9.0	**
	P. coccineus	1	9	1.3 ± 0.23	1.0 - 4.0	**
	P. dumosus	1	9	2.3 ± 0.09	1.0 - 7.0	**
Blend (20 Races)	*P. vulgaris*	1	9	8.0 ± 0.12	1.0 - 9.0	**

****Significant at α=0.01; ns=non-significant; Kab =Kabulangeti (susceptible check); G2333 = resistant check**

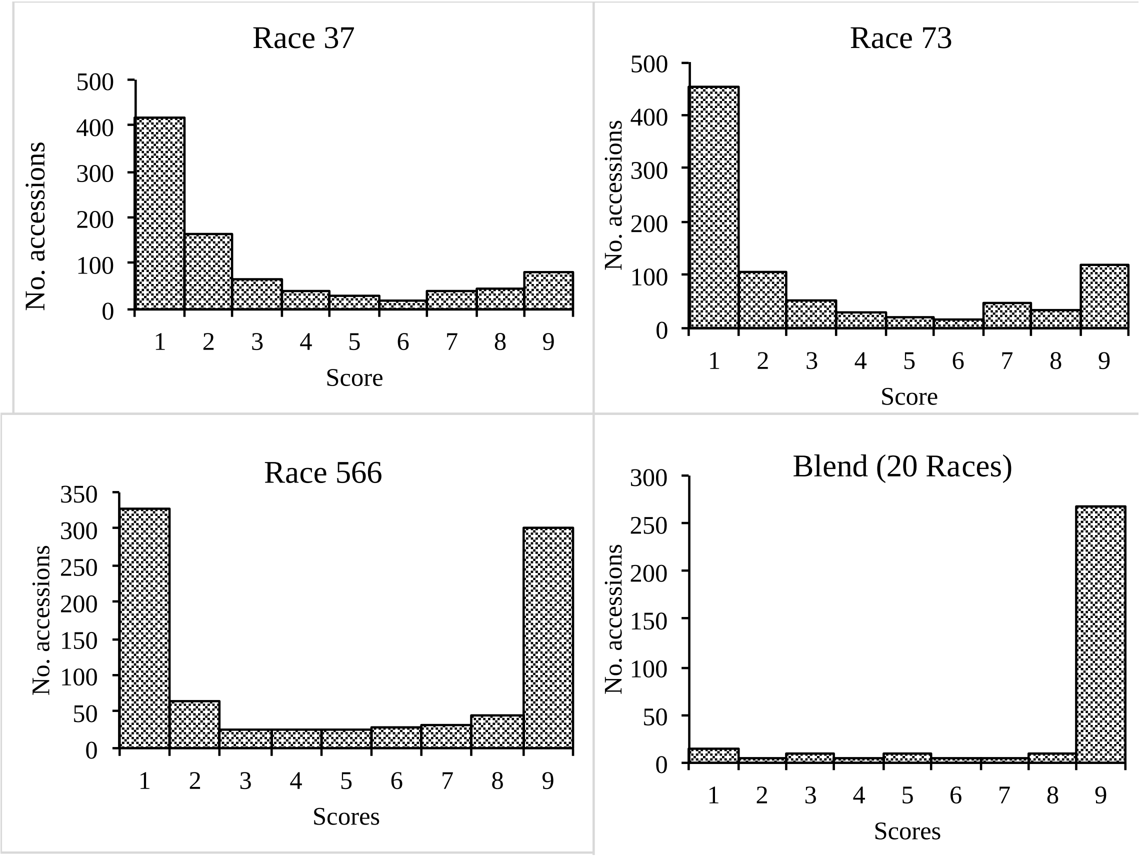

Figure 1. Frequency distributions of *P. vulgaris* accessions in the core collection for severity scores for races 37, 73, 566 and a blend of 20 Races

Table 3. Number and percentage of *Phaseolus* accessions that were resistant (R), moderately resistant(MR) and susceptible(R) to races 37, 73, 566 and a blend of 20 races

Races	Species	Total # Genotypes	R* (1-3)		MR (4-6)		S (7-9)	
			Number	%	Number	%	Number	%
Race 37	*P. vulgaris*	885	635	71.7	84	9.6	166	18.7
	P. coccineus	11	11	100	0	0	0	0
	P. dumosus	11	10	90.9	1	9.1	0	0
Race 73	*P. vulgaris*	869	575	66.2	90	10.4	204	23.4
	P. coccineus	10	10	100	0	0	0	0
	P. dumosus	4	3	75	1	25	0	0
Race 566	*P. vulgaris*	881	423	48.0	80	9.1	378	42.9
	P. coccineus	13	12	92.3	1	7.7	0	0
	P. dumosus	6	5	83.3	0	0	1	16.7
Blend (20 Races)	*P. vulgaris*	331	30	9.0	20	6	281	85

***R=Resistant (score of 1-3 on a scale of 1-9); MR=moderately resistant (score of 4-6 on a scale of 1-9); S=Susceptible (score of 7-9 on a scale of 1-9)**

Table 4. The *Phaseolus vulgaris* accessions highly resistant (score of 1-3 on a scale of 1-9) to race 37, 73, 566 and a blend of 20 races

Accession number	Common names	100 seed weight (g)	Country	Scores for all 3 races and blend (20 Races)
G10843	Guatemala 1334	19	Guatemala	1
G2775	Ojo De Cabra	36	Mexico	1
G3514	De Vara	24	Mexico	1
G4327	Frijola	45	Mexico	1
G8776	Genuine Cornfield	26	United States	1
G19021	Mezclado	20	Mexico	2
G686	-	25	Guatemala	2
G21134	Kipin, Piquin, Sahuin	44	Mexico	3

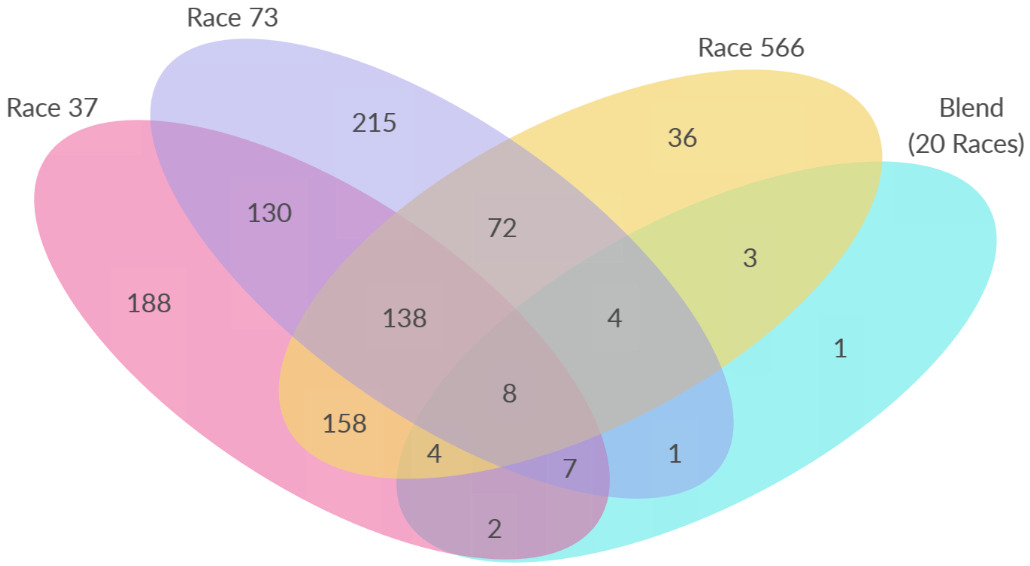

Figure 2. Venn diagram of the number of *Phaseolus vulgaris* accessions that were highly resistant (score of 1-3 on a scale of 1-9) to individual races (37, 73, 566) and a blend (20 races)

CHAPTER 5

5. 0 DISCUSSION

The fungus *Colletotrichum lindemuthianum* is a highly variable pathogen, which includes multiple races. Characterization of races found in a given growing environment is important for deciding which resistance genes to deploy in that environment. In this study, three isolates MPOSK1, MPIK1 and MBMW1 from three major bean-growing regions of Zambia were characterized into races 37, 73 and 566. Races 37 and 566 are mixed (Andean and Middle American) while race 73 is Middle American. This is the first report of race 37 in Zambia. Middle American race 73 has a wider geographic spread and has been reported in several countries including the US. It was, however, interesting to discover the presence of race 73 in Zambia where nearly all beans grown are Andean. The confirmation of the presence of race 73 in Zambia implies that the race has adapted to the Andean gene pool

The presence of three different races in three growing regions suggests that *C. lindemuthianum* is variable in Zambia. Presence of mixed races and Middle American race underscores the need for pyramiding both Andean and Middle American anthracnose resistance genes in Zambian varieties. The Andean locus *Co-1*, found in the Andean race differential Kaboon, which in the current study provided resistance to race 73, could be considered for inclusion in a gene pyramid program together with the Middle American locus *Co-4* found in G2333 which provided resistance to races 37 and 566. A gene pyramid of the Andean locus *Co-1* and the Middle American locus *Co-4* in the same genetic background could result in varieties with durable resistance to multiple ANT races.

Continuous identification of new sources of resistance is necessary when breeding for a highly variable pathogen such as *C. lindemuthianum*. The CIAT core collection is a valuable source of resistance to diseases such as anthracnose. A total of eight *P. vulgaris* accessions were identified in this study as highly resistant to races 37, 73, 566, and a blend of 20 races. These highly resistant accessions constitute a valuable genetic resource to breeders to develop common bean varieties with durable and broad-spectrum resistance to *C. lindemuthianum*. The current study has provided important information on the status of the core collection for resistance to anthracnose, which has enhanced its value as a source of germplasm in breeding for resistance to anthracnose. Accessions that were resistant to the three races came from the primary gene pool of common bean (*P. vulgaris*) and from its secondary gene pool (*P. coccineus* and *P. dumosus*). The resistant *P. coccineus* accessions could be used as sources of resistance in countries that have *P. coccineus* breeding program and where it is a food crop, e.g., in some European countries bordering the Mediterranean sea.

Resistance to race 566 among *P. vulgaris* accessions showed a bimodal distribution pattern suggesting qualitative inheritance (major gene) for resistance to race 566 in the core collection. Mungalu et al. (2020) also reported a bimodal distribution of resistance to race 566 among recombinant inbred lines and two major effects QTL on Pv02 and Pv04. It is plausible that one of these two genes could be the one responsible for the observed resistance to race 566 in the core collection. Frequency distributions of resistance to races 37 and 73 also suggested qualitative inheritance involving major genes. Zuiderveen et al. (2016) reported a major-effect QTL for resistance to race 73 using the Andean diversity panel, which is consistent with the observed frequency distribution for race 73 in the current study.

It is evident in this study that the single race inoculation and a blend exhibited different degrees of pathogenicity, with the multiple race of the blend being the most virulent. Results of this study are consistent with those reported by Chilipa et al, 2016.Similarly, Aliyu et al reported a synergistic interactions among the multiple races used to screen for resistance to C. lindemuthianum .Although a majority of the *P. vulgaris* accessions were highly susceptible to the blend of 20 races, the race blend approach was useful for identifying high level and broad-spectrum resistance, which is useful in breeding for durable resistance, however, where a particular race is prominent in a region, breeding for a single race can be an option for short term purposes.

CHAPTER 6

6.0 CONCLUSION

In this study, three isolates from three major bean-growing regions of Zambia were characterized as *C. lindemuthianum* races 37, 73 and 566. Races 37 and 566 are a blend of Andean and Middle American while race 73 is Middle American confirming the presence of both Andean and Middle American races in Zambia. Additionally, accessions of *P. vulgaris*, *P. coccineus*, and *P. dumosus* were highly resistant (a score of 1-3) to races 37, 73 and 566. A total of eight common bean genotypes were identified as highly resistant to races 37, 73, 566 and a blend of 20 races. These eight accessions constitute a valuable breeding resource for developing common bean varieties with durable and broad-spectrum resistance to *C. lindemuthianum*. More studies should be undertaken which should include other bean growing regions in Zambia with increased sampling intensities for the farms and fields.

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LIST OF APPENDICES

Appendix 1. List of Variables for Symptom Evaluation Description According to Barladin et al., (1990)

Score	**Description**
1	Leaves with no visible symptoms
2	Few isolated small lesions on mid - veins in the lower leaf surface
3	A higher frequency of small lesions on mid – veins in the lower leaf surface
4	Lesions in the mid vein occasionally in secondary leaf vein
5	Many small lesions scattered on mid and secondary veins
6	Many small lesions in the lower and upper leaf surface
7	Large lesions scattered over the leaf blade
8	Many large coalesced lesions accompanied by tissue breakdown and chlorotic or abscised leaf
9	Severely diseased or dead leaf

Appendix 2. Anthracnose differential series, resistance genes, host gene pool, and the binary number of each cultivar used to characterize races of anthracnose in common bean

Differential Cultivar	Host Genes	Place of cultivar	Gene pool	Binary Number	Growth Habit
Mitchelite	Co - 11	0	MA	1	II
MDRK	Co – 1	1	A	2	I
Perry Marrow	Co – 1^{3}	2	A	4	II
Cornel 49242	Co – 2	3	MA	8	II
Widusa	Co – 1^{5}	4	A	16	I
Kaboon	Co – 1^{2}	5	A	32	II
Mexico 222	Co – 3	6	MA	64	I
PI 207262	Co – 4^{3}, Co – 9	7	MA	128	III
TO	Co – 4	8	MA	256	I
TU	Co – 5	9	MA	512	III
AB 136	Co – 6, Co – 8	10	MA	1024	IV
G 2333	Co – 3^{5}, Co – 4^{5}, Co – 5^{2}	11	MA	2048	IV

Appendix 3. Analysis of variance for Race 37, Race 73, Race 566 and blend of 20 Races

(a) Analysis of Variance for Race_37

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Gtyp	900	(353)	14098.4542	15.6649	34.69	<.001
Rep	1		12.9532	12.9532	28.68	<.001
Residual	527	(870)	238.0053	0.4516		
Total	1428	(1223)				

(b) Analysis of Variance for Race_73

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Gtyp	882	(371)	17398.0550	19.7257	42.37	<.001
Rep	1		6.1966	6.1966	13.31	<.001
Residual	588	(809)	273.7422	0.4655		
Total 1471 (1180)						

(c) Analysis of Variance for Race_566

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Gtyp	895	(358)	23949.826	26.760	20.17	<.001
Rep	1		10.657	10.657	8.03	0.005
Residual	641	(756)	850.607	1.327		
Total 1537 (1114)						

(d) Analysis of Variance for the blend of 20 races

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Gtyp	328	(925)	3686.2354	11.2385	20.11	<.001
Rep	1		0.8008	0.8008	1.43	0.233
Residual	126	(1271)	70.4025	0.5588		
Total	455	(2196)				