

**RESISTANCE SPECTRUM TO ANTHRACNOSE DISEASE AND THE
GENETIC EFFECT OF PYRAMIDED GENES ON YIELD IN COMMON
BEAN**

BY

**MOSES KIRYOWA
BSc. AGRIC (MAK)
MSc. CROP SCIENCE (MAK)
REG. NO: 2008/HD02/14611U**

**A THESIS SUBMITTED TO THE DIRECTORATE OF RESEARCH AND
GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN PLANT BREEDING AND
BIOTECHNOLOGY OF MAKERERE UNIVERSITY**

OCTOBER, 2019

DECLARATION

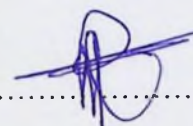
This thesis is entirely my own work and has not been presented for a degree award in any University

Signed: 

Date. 27th/09/2019

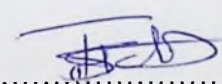
MOSES KIRYOWA, Makerere University

This thesis has been submitted for examination with our approval as the University Supervisors:

Signed 

Date. 27/9/19

PROF. PHINEHAS TUKAMUHABWA, Makerere University

Signed 

Date. 27/9/2019

DR. STANLEY NKALUBO, National Agricultural Research Organization

DEDICATION

To my children Josiah, Elisha and Karen; wife Rachael; and Jajja Anette

For your patience, love and prayers

ACKNOWLEDGEMENT

I express my sincere gratefulness to the following people and organizations who contributed in various ways to make this work a success;

My graduate advisors and supervisors; Prof. Phinehas Tukamuhabwa and Dr. Herbert Talwana of the School of Agricultural Sciences, Makerere University; Dr. Stanley Nkalubo and Dr. Pamela Paparu of the Legumes Program, National Crops Resources Research Institute (NaCRRI); and Dr. Clare Mukankusi of Centro Internacional de Agricultura Tropical (CIAT)-Uganda, for advising and guiding me until this work became a success. I specially thank Professor Patrick Rubaihayo for the critical supervision and guidance during write-up of this thesis.

Field research and laboratory technical staff, Vincent Kyaligonza and Tony Mukwaya at Biosciences lab facility, NaCRRI; Allan Male, Samuel Kiviiri and Cathy Acam at the CIAT lab facility, National Agricultural Research Laboratories (NARL), for their technical support in laboratory based research work; Sheikh Ssebuliba, Naluwooza, Arafat, Steven Musoke and Khasifa of CIAT-Uganda, NARL and Juliet Nakimera and Lydia of NaCRRI for all the field and screen house support.

Fellow students undertaking different aspects of beans research namely Aston Ebinu, Patrick Ongom, Esther Arengo, Dennis Ocii, and Nicholas Olango for their continual encouragement and sharing of experiences. Fellow students in the first cohort of the PhD program in Plant Breeding and Biotechnology such as Geoffrey Kawube, Jushua Mudingoto, Gabriel Ddamulira, Anthony Pariyo, Sadiq Kassim, Arthur Wasukira, Laban Turyagyenda, Alexander Bombom, Henry Wagaba, Abel Sefasi, Maphosa Mcbesi, Langa Tembo to mention but a few

The Uganda National Council of Science and Technology (UNCST) Millennium Science Initiative (MSI) project, the National Agricultural Research Organization (NARO) Agricultural Technology and Agricultural Advisory Services (ATAAS) project and the Pan-African Bean Research Alliance (PABRA) for funding my tuition and this research work.

Prof. Adipala Ekwamu, Dr. Patrick Okori, Dr. Richard Edema, Prof. and Mrs. Gibson, for conceiving, delivering the vision of a PhD in Plant Breeding and Biotechnology at Makerere University and for their constant parental encouragement and guidance.

The National Agricultural Research Organization (NARO) training committee, Directorate of Human Resource for supervisory and financial support; and particularly Dr. Julius Mukalazi Director of Research at Abi Zonal Agricultural Research and Development Institute (AbiZARDI) where I was serving as a Research Officer during my PhD Research.

My family, Jajja Anette Kabanda, Uncle John Mukwaya, Aunties Hellen and Anita Kabanda for shaping my life and giving me a firm educational background. Rachael my wife and the children Karen, Elisha and Josiah for their love, patience and understanding during this study.

The good and Almighty LORD who gave me patience and endurance through it all (Psalm 8).

TABLE OF CONTENTS

Cover page.....	i
Declaration.....	ii
Dedication.....	iii
Acknowledgement.....	iv
Table of contents	vi
List of Tables.....	xi
List of Figures.....	xii
List of Plates	xii
General Abstract.....	xiii
 CHAPTER ONE	 1
General introduction.....	1
1.1 Background.....	1
1.2 Bean production.....	2
1.3 production constraints	2
1.3.1 Importance of the bean anthracnose disease.....	3
1.3.2 Distribution, epidemiology and symptoms of <i>C. lindemuthianum</i>	4
1.3.3 Anthracnose symptoms and epidemiology.....	4
1.3.4 Pathogenicity characterization of <i>C. lindemuthianum</i>	6
1.3.5 Molecular characterization of <i>C. lindemuthianum</i>	6
1.3.6 Host-pathogen co-evolution	7
1.4 Host plant resistance to bean anthracnose	7
1.4.1 Host resistance genes.....	7
1.4.2 Marker assisted selection (MAS) in resistance breeding in common beans	8
1.5 Problem statement	9
1.6 Justification.....	10
1.7 Overall objective.....	11

1.8 Specific objectives	12
1.9 Hypotheses	12
CHAPTER TWO	13
Literature review.....	13
2.1 Pathogenic variation of <i>Colletotrichum lindemuthianum</i>	13
2.2 Resistance gene pyramiding and its effectiveness.....	17
2.3 Assessment for yield penalty and possible yield penalty among pyramided populations ..	22
CHAPTER THREE	26
Pathogenic variation of <i>Colletotrichum lindemuthianum</i> in Uganda	26
Abstract.....	26
3.1 Introduction	27
3.2 Materials and methods.....	27
3.2.1 Collection of <i>C. lindemuthianum</i> samples.....	27
3.2.2 Isolation of <i>C. lindemuthianum</i>	29
3.2.3 Characterization of <i>C. lindemuthianum</i>	29
3.2.4 Race determination	30
3.3 Results	31
3.3.1 <i>C. lindemuthianum</i> samples and isolates collected	31
3.3.2 Isolate and cultivar variability	34
3.3.3 Race determination and distribution.....	36
3.4 Discussion.....	39
3.5 Conclusion.....	43
3.6 Recommendation	43
CHAPTER FOUR.....	44

Gene pyramiding and assessing effectiveness of single and pyramided genes in conferring broad-spectrum resistance to diverse races of <i>C. lindemuthianum</i>	44
Abstract.....	44
4.1 Introduction	45
4.2 Materials and methods.....	45
4.2.1 Plant genetic materials.....	45
4.2.2 Molecular markers used in Marker Assisted Selection	46
4.2.2.1 DNA extraction and storage	48
4.2.2.2 DNA amplification, gel electrophoresis and imaging	48
4.2.3 Development of populations.....	50
4.2.3.1 Pedigree steps of the pyramiding scheme.....	50
4.2.3.2 Fixation steps of the pyramiding scheme	52
4.2.4 Raising bean populations for phenotypic screening	52
4.2.5 Phenotyping for anthracnose resistance among F ₆ lines	53
4.2.5.1 F ₆ lines used for phenotyping	53
4.2.5.2 <i>C. lindemuthianum</i> races and inoculum preparation	53
4.2.5.3 Inoculum and disease scoring.....	53
4.2.6 Data analysis.....	54
4.3 Results	55
4.3.1 Inheritance profile of resistance genes among F ₄ lines.....	55
4.3.2 Specific molecular marker analysis and polymorphism.....	58
4.3.3 Phenotypic evaluation of F ₆ lines for resistance to bean anthracnose disease	59
4.3.3.1 <i>C. lindemuthianum</i> isolates used	59
4.3.3.2 Evaluation of families with pyramided genes	59
4.3.4 Effectiveness of single and pyramided genes in conferring broad spectrum resistance in diverse <i>C. lindemuthianum</i> races.....	64
4.3.5 Pairwise comparison of severity of <i>C. lindemuthianum</i> between gene groups	65

4.4 Discussion.....	67
4.4.1 Gene pyramiding and development of advanced populations.....	67
4.4.2 Marker analysis and polymorphism during gene pyramiding.....	68
4.4.3 Effectiveness of single and pyramided genes in conferring broad resistance to bean anthracnose	68
4.5 Conclusion.....	73
4.6 Recommendation	74
CHAPTER FIVE	75
Genetic variability and relationship between pyramided resistance genes and yield traits among advanced common bean populations	75
Abstract.....	75
5.1 Introduction	76
5.2 Materials and methods.....	77
5.2.1 Field experimental set up and evaluation of advanced populations	77
5.2.2 Data analysis.....	77
5.2.2.1 Genetic variability and parameter estimation among populations	77
5.2.2.2 Correlation analysis of pyramided genes and yield traits.....	78
5.2.2.3 Path coefficient analysis of pyramided genes and yield traits.....	78
5.3 Results	79
5.3.1.1 Genetic variability among advanced common bean populations	79
5.3.1.2 Phenotypic and Genotypic coefficient of variability among populations	80
5.3.1.3 Heritability and genetic advance among advanced bean populations	81
5.3.2 Yield traits performance among advanced bean populations.....	82
5.3.3 Correlation between number of pyramided genes and yield traits	86
5.3.4 Path coefficient analysis of number of pyramided genes and yield traits	86
5.4 Discussion.....	87
5.4.1 Genetic variability among the advanced populations.....	87

5.4.2 Relationship between number of pyramided genes and yield	89
5.5 Conclusion	90
5.6 Recommendation	91
CHAPTER SIX	92
General overview.....	92
6.1 General conclusion	93
6.2 General recommendation.....	94
References	95

LIST OF TABLES

Table 1.1: Bean anthracnose resistance genes, their sources and linked markers.....	8
Table 3.1 Differential cultivars used to characterize <i>C. lindemuthianum</i> , their binary codes, resistance genes and gene pool.....	30
Table 3.2: <i>C. lindemuthianum</i> samples and isolates collected	31
Table 3.3 Analysis of variance for bean anthracnose severity on 12 differential cultivars.....	34
Table 3.4 Reactions of <i>Colletotrichum lindemuthianum</i> isolates on the differential cultivars	35
Table 3.5 Characterization of <i>C. lindemuthianum</i> pathotypes using differential cultivars	37
Table 3.6 Incidence, Severity and races of <i>C. lindemuthianum</i> by district	38
Table 3.7 Pathogenicity of 27 races on 12 bean differential cultivars and a susceptible check	39
Table 4.1 Parents used in the gene pyramiding scheme	46
Table 4.2 PCR-based markers used in Marker Assisted Selection.....	47
Table 4.3 Ingredients of a PCR master mix using PCR reagents and a Bioneer PCR premix..	49
Table 4.4 Grouping of the F ₆ advanced lines based on number and type of genes inherited	55
Table 4.5: Sixty nine (69) F ₆ families with their profile of inherited resistance genes	56
Table 4.6 Reaction of pyramided lines and parents to four races of <i>C. lindemuthianum</i> and four SCAR markers	61
Table 4.7 Analysis of variance for severity of four races on different gene-groups	64
Table 4.8 Mean severity scores of cultivars in the different gene groups inoculated with diverse <i>C. lindemuthianum</i> races.....	64
Table 4.9 Pair-wise comparison of mean severity scores of the different gene groups	66
Table 5.1 Analysis of variance Mean Squares for the different yield traits	80
Table 5.2 Parameter estimation among advanced bean populations	81
Table 5.3 F ₅ Advanced bean populations and their yield performance	82
Table 5.4 Comparison of performance between parents and their derived advanced populations	84
Table 5.5 Correlation matrix for seed weight per plant and different plant growth and yield characters	86

Table 5.6 Direct and indirect path coefficients for seed weight per plant.....	87
--	----

LIST OF FIGURES

Figure 3.1: Map of Uganda indicating sampling areas for bean anthracnose	28
Figure 4.1 Cascading gene pyramiding scheme used to develop advanced populations	51
Figure 5.1 Trend of Seed weight per plant (SWP) with increasing number of pyramided genes	85
Figure 5.2 Trend of Number of pods per plant (NPP) with increasing number of pyramided genes	85

LIST OF PLATES

Plate 1.1 Bean anthracnose disease symptoms.....	5
Plate 3.1 <i>Colletotrichum lindemuthianum</i> in culture.....	33
Plate 3.2 Conidia of <i>C. lindemuthianum</i>	33
Plate 3.3 Bean anthracnose disease symptoms on inoculated seedlings	34
Plate 4.2 Gel photos showing banding patterns of DNA fragments amplified with the different SCAR markers.....	58
Plate 4.3A Conidia of <i>C. lindemuthianum</i> in culture; races 352 and 713	60
Plate 4.3B Conidia of <i>C. lindemuthianum</i> in culture; races 767 and 2047.....	60
Plate 4.4A Leaf trifoliates of three F ₆ cultivars with single resistance genes in comparison with a known susceptible cultivar seven days post inoculation	62
Plate 4.4B Leaf trifoliates of three F ₆ cultivars with pyramided resistance genes in comparison a known resistant cultivar, seven days post inoculation	63

GENERAL ABSTRACT

Bean anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. et. Magn) Lams. Scrib., is one of the most widespread and economically important fungal diseases of the common bean. The pathogen possesses a high genetic and pathogenic variability, which causes it to overcome resistance in bean cultivars. Pyramiding of resistance genes in commercial varieties is a strategy that would ensure more effective resistance and thus a reduction in yield losses. This study was conducted with the overall objective to enhance broad-spectrum resistance to anthracnose disease among common bean varieties for increased productivity in Uganda. The specific objectives were; a) To determine the pathogenic variability of *Colletotrichum lindemuthianum* in Uganda; b) To assess effectiveness of single and pyramided resistance genes against bean anthracnose disease; and c) To determine genetic variability and relationship between pyramided genes and yield associated traits among advanced common bean populations

To determine pathogenic variation of *C. lindemuthianum*, samples of common bean tissues with anthracnose symptoms were collected from Kabarole, Sironko, Mbale, Oyam, Lira, Kapchorwa, Maracha and Kisoro. 51 isolates were used to inoculate 12 standard differential cultivars under controlled conditions. Five plants per cultivar were inoculated with each isolate and evaluated for their reaction using a 1 – 5 severity scale (Inglis *et al.*, 1988). Races were classified using the binary nomenclature system proposed by Pastor Corrales (1991). Sequence Characterized Amplified Region (SCAR) markers were used to facilitate the process of pyramiding and tracking three anthracnose resistance genes/ alleles (*Co-4*²/*Co-4*³, *Co-5* and *Co-9*) using a cascading pedigree pyramiding scheme. Detached leaf trifoliates of F₄₆ plants were screened under controlled conditions with four *C. lindemuthianum* races and severity scored on a 1 – 9 scale (Balardin *et al.*, 1997). 53 F₄₅ and 69 F₄₆ families were evaluated in the field for yield and agronomic performance; and correlation and path analysis done to establish relationship between pyramided resistance genes and yield. Disease severity and yield traits data were subjected to ANOVA to reveal extent of variability.

Twenty seven (27) pathogenic races were identified. Sironko district had the highest number of races followed by Mbale and Kabarole. Races 2047 and 4095 were the most frequent. Race 4095 was the most aggressive and followed by races 2479, 2047 and 2045. Races, 4094 and 2479 caused a susceptible reaction on the differential cultivar G2333, known to possess a high

degree of resistance. Differential cultivars G2333, Cornell 49-242, TU and AB136 were the most resistant.

The five pyramid group means were significantly different from each other ($P < 0.01$) of which, $Co-4^2 + Co-5 + Co-9$ and $Co-4^2 + Co-5$ exhibited the lowest mean disease score to all the four races indicating a high degree and spectrum of resistance. The group $Co-4^3 + Co-9$ had the highest mean disease severity. The single-genes were significantly different from each other ($P < 0.01$). The $Co-4^2$ and $Co-5$ genes both conferred resistance to all the four races 352, 713, 767 and 2047, while the group with none of the o resistance gene inherited (5.9 ± 0.21) was overcome by all the four races. $Co-4^2$ group had the least mean severity across races followed by $Co-5$, $Co-4^3$, $Co-9$ and *No-gene*. The single gene $Co-4^2$ was not significantly different from the best pyramids $Co-4^2 + Co-5 + Co-9$ and $Co-4^2 + Co-5$ ($P < 0.01$) and was better than the other three pyramids. Similarly the $Co-5$ single gene was not significantly different from $Co-4^2 + Co-5$, $Co-4^2 + Co-9$ and $Co-5 + Co-9$ pyramid groups ($P < 0.01$). The single gene $Co-9$ was found to be antagonistic in combination with other resistance genes and should be avoided in resistance gene pyramiding programs. The two single genes $Co-4^2$ and $Co-5$ are recommended for use in resistance gene pyramiding programs.

ANOVA revealed significant variability ($P < 0.01$ and $P < 0.05$) for all yield traits among advanced bean lines, and further revealed that Phenotypic Coefficients of Variability (PCV) estimates were higher than the Genotypic Coefficients of Variability (GCV) estimates indicating importance of environmental effects in the expression and improvement of these traits. Broad sense heritability (h_b^2) and genetic advance among populations was low for number of pods per plant, number of seeds per plant and seed weight per plant implying that selection would not be effective in improving these traits but other breeding strategies such as heterosis breeding would be appropriate for improving the traits. Three $F_{4,6}$ lines with pyramided genes namely D16.3.3.11.160.5.6, C44.1.4.5.142.4 and C16.1.3.8.136.2 were among the best 10 yielders. However, number of pyramided genes had a significant negative correlation with seed weight per plant (-0.17), number of pods per plant (-0.24, $p < 0.05$) and number of seeds per plant (-0.19, $p < 0.1$); and path coefficient analysis revealed a significant ($P < 0.05$) negative indirect effect of number of pyramided genes on seed weight per plant via number of seeds per plant (-0.25). These findings suggest a yield penalty arising from pyramiding resistance genes against the *C. lindemuthianum* pathogen.

CHAPTER ONE

General introduction

1.1 Background

The common or dry bean, *Phaseolus vulgaris* (L.), is a diploid ($2n = 22$) with a genome size ranging from 450 to 650 mbp for haploid genome (Broughton *et al.*, 2003). It was domesticated about 7,000 years ago (Kaplan, 1965) in two centers of origin; Mesoamerica (Mexico and Central America) and the Andean region. Common bean was introduced in sub-Saharan Africa in the 16th century (Greenway, 1945) by Portuguese traders (CIAT, 2001) and in the highlands of Eastern Africa, now a secondary center of genetic diversity, about 400 years ago (Schwartz and Pastor-Corrales, 1989). Beans are the most important legume for human consumption in the world and they are considered to be the second most important source of dietary protein and the third most important source of calories (FAOSTAT, 2012) for nearly 500 million people in Africa, Latin America and the Caribbean (Cortés *et al.*, 2013). Common bean is characterized as a “nearly perfect” food due to its high protein content and generous amounts of fiber, complex carbohydrates, and other dietary necessities (CIAT, 2001). A single serving of beans provides at least half the United States Department of Agriculture’s recommended daily dietary allowance of folic acid (CIAT, 2001) one of the vitamins in the B complex that is especially important for pregnant women (Peake, 2013). It also supplies 25 to 30% of the recommended levels of Iron (Fe) and meets 25% of the daily requirement of Magnesium (Mg) and Copper (Cu) as well as 15% of the Potassium (K) and Zinc (Zn) (CIAT, 2001). The nutritional balance of beans, therefore, makes it important in preventing malnutrition especially among the low income class.

In Uganda, beans are the most important source of proteins providing up to 45% of the protein intake for the population (Kilimo Trust, 2012). The National Development Plan (2010), under Section 5.1 on Agricultural development, identified beans among the 10 selected national strategic commodities and called for their accelerated production. Although beans are largely produced for subsistence mainly by women farmers in sub-Saharan Africa (Wortmann *et al.*, 1999), their short maturity period, ease of handling and storability make them a coveted cash crop for small-scale farmers (David, 1999). Therefore, beans play an essential role in the sustainable livelihoods of smallholder farmers, providing both food and income security. Beans

are also highly valued by the resource poor because all parts of the plant can be used; the grain is eaten fresh or dried, the leaves are used as vegetables and the stalk is used to make soda ash (David, 1999).

1.2 Bean production

Global bean production in 2013 was approximately 23.4 million metric tons, with 23.2% and 25.8% of the world production in Latin America and Africa, respectively (Murrel, 2016), with an annual market value of about US \$10 billion (FAOSTAT, 2012). Brazil is the main producer of common bean followed by India, China, United States of America and Mexico (FAOSTAT, 2008). Tanzania, Uganda and Kenya lead in production on the African continent with volumes estimated at 950,000MT, 455,000MT and 390,598MT respectively in 2010 (Kilimo Trust, 2012). Other important producers in Africa include D.R Congo, Burundi, Rwanda and Ethiopia (Pachico, 1993). Beans are the most important legume crop in Uganda occupying an important niche in the agricultural sector and farm household economy (Opio *et al.*, 2001) and accounting for 6.1% of national agricultural GDP, ranking fifth behind bananas, cassava, beef and milk (FAOSTATS, 2008). By 2010, Ntungamo district was the leading producer with a total of 138,000 MT, followed by Mubende with 78,000 MT and Amuru district with 75,000 MT (UBOS and MAAIF, 2010).

1.3 Production constraints

The average global yield of common beans has been observed to be generally declining since the early 2000s (FAOSTAT, 2008). Recorded yields of bush beans frequently reach an average of 3 MTha⁻¹ in experimental conditions while national averages throughout the tropics averages 0.65 MTha⁻¹ implying a wide yield gap in relation to yield potential (Kalyebara, 2008). Uganda's common bean productivity per hectare has also been on the decline since 2001 with recorded yield average of 0.4 – 0.8 MTha⁻¹ depicting a major shortfall from the potential yield of 1.5 – 2.5 MTha⁻¹ realized with improved varieties (Kalyebara, 2008). This is attributable to several production constraints such as pests, diseases, declining soil fertility, drought, poor agronomic practices (Beebe, 2012), low adoption and low replacement rates of improved seed (ISSD, 2012).

Abiotic stresses like drought and low soil fertility are estimated to affect 60% of bean growing areas worldwide especially tropical lowlands of South America and Africa (Beebe *et al.*, 2012). These lower bean yields to 1.0 MTha⁻¹ in the tropics (Wortmann *et al.*, 1999). Soil constraints including low levels of phosphorous (P) and nitrogen (N); aluminum (Al) and manganese (Mn) toxicities associated with acid soils; and low calcium (Ca) availability, are the most widespread limitations (Thung and Rao, 1999) especially in degraded soils with low levels of organic matter (Beebe *et al.*, 2012).

Diseases and pests are the two most important biotic constraints of common bean production worldwide (Rodriguez and Barnado, 2014). More than 45 diseases are reported to affect the common bean incited by fungi, bacteria, viruses and nematodes (Vieira, 1983). At lower altitudes in the Great Lakes Region and elsewhere in eastern and southern Africa, insect pests such as the bean fly (*Ophiomyia spp*) are a significant limiting factor to production of beans and cause substantial damage especially on less fertile land (Shwartz and Pastor-Corrales, 1989).

The major diseases of beans in Africa are anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., angular leaf spot (*Pseudocercospora griseola* (Sacc.) Crous & Braun), rust (*Uromyces appendiculatus* F. Strauss), common bacterial blight (*Xanthomonas phaseoli* (Erw. Smith) Dowson), several common bean viruses transmitted by beetles and white flies, and bean root rots caused by a complex of pathogens (Schwartz and Pastor-Corrales, 1989). It is estimated that 10% of the total yield loss (Schwartz *et al.*, 2005) estimated at 2,288,000 tons annually in Africa (Wortmann *et al.*, 1999) is caused by bean diseases.

1.3.1 Importance of the bean anthracnose disease

Bean anthracnose is a fungal disease of the common bean caused by *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scrib. The disease is severe and can cause 100% yield losses on susceptible cultivars or when infected seed is planted and favorable conditions prevail (Benard-Capelle *et al.*, 2006). It was once considered the most important disease in bean producing areas of Eastern USA and losses amounting to \$1.5 million dollars were reported in Michigan State (Tu, 1983). The disease has been responsible for significant economic losses in

North, Central and South America, Europe, Africa, Australia and Asia (Tu, 1983). Yield losses of up to 95% were recorded in Columbia and over 92% in Malawi (Allen, 1983). In Tanzania yield losses of 40 – 80% were reported and estimated to be worth \$304 million US dollars per annum (Mohammed, 2013). In Uganda yield losses of up to 30 - 70% were reported (Nkalubo *et al.*, 2007).

1.3.2 Distribution, epidemiology and symptoms of *C. lindemuthianum*

Bean anthracnose occurs worldwide and is reported wherever the common bean is grown. It was reported in the USA (Zaumeyer and Thomas, 1957), European countries (Hubbeling, 1977), Canada (Tu and Aylesworth, 1980), Latin America (CIAT, 1988) and in Africa particularly in Uganda, Kenya, Tanzania, Rwanda, Burundi, Ethiopia and D.R Congo. Infected seeds are the commonest means of dissemination of the pathogen (del Rio and Bradley, 2004), which in part explains its worldwide distribution.

Symptoms of appear as dark, sunken, lenticular necrotic lesions containing the acervuli of the pathogen. The initial symptoms of anthracnose appear as a dark brown to black lesion along the veins on the underside of the leaves (Buruchara *et al.*, 2010) and susceptible genotypes may exhibit symptoms on all aerial parts of the plant (CAB International, 2004). Seed borne infection usually induces dark brown to black eye-shaped lesions longitudinally on the hypocotyls and cotyledons. On the hypocotyls the lesions enlarge and may cause the stem to break. On older stems, lesions are sunken and may reach 5 – 7 mm. Earlier signs of leaf infections occur on the petiole and on the lower leaf surface (Plate 1.1a and c), where small lesions extend along the veins developing a brick red to purple red coloration becoming black with age. Later similar symptoms appear on the upper leaf surface (CAB International, 2004).

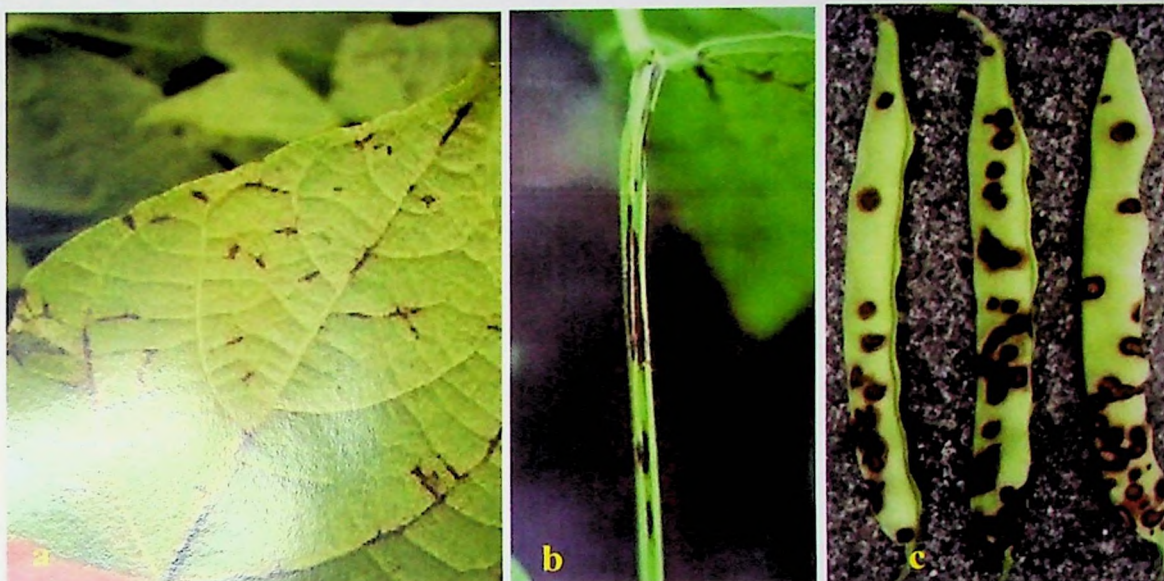


Plate 1.1: Bean anthracnose disease symptoms; a = Leaf symptoms; b = stem and petiole symptoms; c = pod lesions; Photo credit: E. Bush, Virginia State University

On the pods lesions are typically sunken and are encircled by a slightly raised black ring surrounded by a reddish-brown boarder (Plate 1.1b). Under severe infection, young pods may shrivel and dry prematurely (Buruchara *et al.*, 2010). The fungus may also penetrate the seed coat and become firmly established within the seed which, when planted serve as the source of infection in the succeeding crop (Buruchara *et al.*, 2010).

Bean anthracnose is favored by cool and wet weather with temperatures of 17 – 24°C (Tu and Aylesworth, 1980). After artificial inoculation, good infection is obtained when plants are incubated at or near 100% relative humidity at 22°C for 5 – 7 days (Pastor-Corrales *et al.*, 1985). The spread of the disease from a focus to other susceptible plants is influenced by environmental factors responsible for inoculum dispersal, such as rain-splash or wind-blown rain and through movement of insects, animals and man, especially when the foliage is moist (Buruchara *et al.*, 2010). The number of foci of the initial inoculum has been shown to be linearly related to disease incidence on leaves but not on pods. Subsequent pod and seed infections can bring on epidemics (Kumar *et al.*, 1999).

1.3.4 Pathogenic characterization of *C. lindemuthianum*

There are many *C. lindemuthianum* variants or physiological races (CIAT, 1997) whose identification is based on their reaction on a set of differential cultivars (Agrios, 1997) differing in their genes for resistance or susceptibility to one or more races of the pathogen. The occurrence of pathogenic variability in plant pathogens has direct implications on breeding for resistance and therefore the methodologies employed in race determination (Buruchara, 1991). This is important for identification of different races of *C. lindemuthianum*. The differential cultivars used are internationally standardized, genetically pure and able to give clear resistant or susceptible reaction. A set of 12 differential cultivars and a system of nomenclature were proposed and adopted for use in identification of races of *C. lindemuthianum* during the first Latin American workshop on anthracnose held at Cali, Colombia in 1988 (Buruchara, 1991). The system of nomenclature that was adopted is called the binary system, which identifies a race by a number that is a result of the sum of all binary values associated with a cultivar when it gives a susceptible reaction to the race.

1.3.5 Molecular characterization of *C. lindemuthianum*

The use of molecular techniques offers a complementary and alternative solution for determining genetic variability in pathogen populations. Molecular tools have been used to study variability of *C. lindemuthianum* at genetic level including Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Microsatellites (RAMs) and primers derived from the DNA sequence of repetitive-elements (Rep)-PCR which uses primers derived from the 'enterobacterial repetitive intergenic consensus' (ERIC) sequence and the conserved repeated bacterial DNA element 'BOX' for analysis (Miklas *et al.*, 2001; Melotto and Kelly, 2001; Kelly and Vallejo, 2004; Mahuku and Riascos, 2004). Using these molecular tools, great genetic variability of *C. lindemuthianum* has been observed and reported (Mahuku and Riascos, 2004). There is still a challenge of lack of association between pathogenicity data derived from screening from differentials and molecular-derived data has been observed in many cases (Balardin *et al.*, 1997; Sicard *et al.*, 1997; Mahuku and Riascos, 2004).

1.3.6 Host-pathogen co-evolution

Host-pathogen gene-for-gene (GFG) coevolution between common bean, *Phaseolus vulgaris*, and its pathogen *C. lindemuthianum* was reported to be responsible for the differentiation of host resistance in the centres of diversity of common beans (Gepts, 1988; Geffroy *et al.*, 1999; Alzate-Marin *et al.*, 1999; and Chiorato *et al.*, 2006) and therefore affects the deployment strategy of resistance genes. It was observed that physiological races of *C. lindemuthianum* of Mesoamerican origin were more virulent in both Mesoamerican and Andean bean genotypes while Andean races were virulent only in Andean genotypes (Balardin and Kelly, 1998). It was also observed that resistance genes of Mesoamerican origin were highly effective when transferred to beans of Andean background and deployed in regions where Andean isolates prevailed such as East Africa, Colombia, and Ecuador (Miklas *et al.*, 2006), while genes of Andean origin were very effective when transferred to beans of Middle American background and deployed in regions where isolates of Mesoamerican origin prevailed such as Central America, Mexico and USA (Miklas *et al.*, 2006).

1.4 Host plant resistance to bean anthracnose disease

1.4.1 Host resistance genes

The use of host plant resistance is reported as the most effective, safe and inexpensive strategy for the control of bean diseases (CAB International, 2004; Otsyula *et al.*, 2005) through the development and use of cultivars with specific genes. This requires continuous identification and characterization of new disease resistance genes and their introduction into commercial bean varieties (Vidigal Filho *et al.*, 2007).

Resistance to bean anthracnose is conditioned by thirteen (13) major genes *Co-1* to *Co-13* (Lacanallo *et al.*, 2010) with only *co-8* being recessive. Loci *Co-9/Co-3³* and *Co-7/Co-3* are allelic (Méndez-Vigo *et al.*, 2005) and multiple alleles exist at the *Co-1*, *Co-3*, *Co-4*, *Co-5* loci. Genes *Co-1*, *Co-12* and *Co-13* are Andean in origin while the rest are Mesoamerican genes (Kelly and Vallejo, 2004) representing the two centers of origin of common beans (Table 1.1).

Table 1.1: Bean anthracnose resistance genes, their sources and linked markers

Gene symbols		Pedigree	Gene pool	Linked markers	Map location
Original	New				
<i>A</i>	<i>Co-1</i>	MDRK	A	OF10 ₅₃₀	B1
	<i>Co-1</i> ²	Kaboon		SE _{ACT/MCCA}	
	<i>Co-1</i> ³	Perry Marrow		-	
	<i>Co-1</i> ⁴	AND 277		-	
	<i>Co-1</i> ⁵	Widusa		OA18 ₁₅₀₀	
<i>Are</i>	<i>Co-2</i>	Cornell 49242	MA	OQ4 ₁₄₄₀ , OH20 ₄₅₀ , B355 ₁₀₀₀	B11
<i>Mexique 1</i>	<i>Co-3</i>	Mexico 222	MA	-	B4
	<i>Co-3</i> ²	Mexico 227	MA	-	
<i>Co-9</i>	<i>Co-3</i> ³	BAT 93	MA	-	B8
<i>Mexique 2</i>	<i>Co-4</i>	TO	MA	SAS13, SH18	
	<i>Co-4</i> ²	SEL 1308	MA	SBB14, OC8	
	<i>Co-4</i> ³	PI 207262	MA	OY20	B7
<i>Mexique 3</i>	<i>Co-5</i>	TU	MA	OAB3 ₄₅₀	
	<i>Co-5</i> ²	SEL 1360	MA	SAB3	
<i>Q</i>	<i>Co-6</i>	AB 136	MA	OAH1 ₇₈₀ , OAK20 ₈₉₀	B7
-	<i>Co-7</i>	HI, MSY 7-1, G2333	MA	-	-
-	<i>co-8</i>	AB 136	MA	OPAZ20	-
-	<i>Co-9</i>	BAT 93	MA	SB12	B4
-	<i>Co-10</i>	Ouro Negro	MA	F10	B4
-	<i>Co-11</i>	Michelite	MA	-	-
-	<i>Co-12</i>	Jalo Vermelho	A	-	-
-	<i>Co-13</i>	Jalo Listras Pretas	A	OPV20 700	B3

A = Andean; MA = Meso American; Source: Vallejo and Kelly (2009)

The independent resistance genes *Co-1*, *Co-2*, *Co-3*, *Co-4*, *Co-5*, *Co-6*, *Co-7*, *co-8*, *Co-9*, and *Co-10* are present in the cultivars Michigan Dark Red Kidney (MDRK), Cornell 49-242, Mexico 222, TO, TU, AB 136, G2333, BAT 93, and Ouro Negro, respectively (Alzate-Marin *et al.*, 2003). Alleles *Co-1*, *Co-1*², *Co-1*⁵, *Co-3*² and *Co-4*² are reported in the cultivars Perry Marrow and Kaboon, Widusa, Mexico 222, and SEL 1308, respectively (Kelly and Vallejo, 2004).

1.4.2 Marker assisted selection (MAS) in resistance breeding of common beans

DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding through marker-assisted selection (MAS), which is proven to speed up breeding through laboratory based selection of individual plants with the desired trait(s). MAS has been

used to improve germplasm and develop new common bean varieties that are better adapted to biotic and abiotic constraints (Miklas *et al.*, 2006). Sequence Characterized Amplification Region (SCAR) markers were reported among the most useful of molecular markers in plant breeding programs (Young *et al.*, 1998) and have been used widely in MAS (Garzon *et al.*, 2008). The SCARs *SAS13*, *SH18*, *SBB14*, *SAB3*, *SB12* tag the loci/alleles *Co-4*, *Co-4*², *Co-4*², *Co-5* and *Co-9* respectively for anthracnose resistance. The challenge however is that there are no reported markers tagging some specific resistance genes such as *Co-4*³ allele which occupies the same locus at the *Co-4*² allele and *Co-7* gene.

1.5 Problem statement

Global yields of common beans are reported to have declined since the early 2000s (FAOSTAT, 2015). In Uganda yields reported on farmers' fields were significantly lower than research experimental yields by about 2.0 MTha⁻¹ (Kalyebara, 2008). Schwartz *et al.* (2005) attributed to bean diseases 10% of observed yield decline. Anthracnose caused by *Colletotrichum lindemuthianum* is an important disease of common bean resulting in major economic losses worldwide. It is one of the most important diseases of common beans in Uganda causing yield losses of –up to 70% among susceptible varieties (Nkalubo *et al.*, 2007). Control of the disease using host-plant resistance is recommended as the most cost-effective and environmentally safe strategy (Otsyula *et al.*, 2005).

Pursuing host resistance as a strategy to control anthracnose disease is, however, complicated by the fact that *C. lindemuthianum*, the causative pathogen, possesses a high degree of genetic and physiologic variability (Sharma *et al.*, 1999; Mahuku and Riascos, 2004; De Silva *et al.*, 2017). The pathogen is reported to have a reproductive/ Telemorphic phase, *Glomerella lindemuthianum* which is partly responsible for its genetic and physiologic variability through genetic recombination (De Silva *et al.*, 2017). This enables it to adapt to new sources of host resistance and easily breakdown single gene resistance (Melotto *et al.*, 2000). Effective control through breeding requires periodic surveillance of *C. lindemuthianum* population structure to understand level of variability and distribution and emergence of new races. There is need,

therefore, to update *C. lindemuthianum* variability in Uganda and identify new sources of effective resistance to the current pathogen structure for use in breeding programs in Uganda. More than 13 major anthracnose resistance genes have been reported from diverse common bean cultivars (Lacanallo *et al.*, 2010), yet none of the known genes confers resistance to all known races of the *C. lindemuthianum*. Moreover, breakdown of single gene resistance due to emergence of new races is well documented such as in the case of the Andean *Co-1* and Mesoamerican *Co-2* genes, which were reported to breakdown, due to occurrence of new races 31 and 89, after being incorporated into susceptible cultivars (Kruger *et al.*, 1977).

Resistance gene pyramiding, the combination of two or more resistance genes in a cultivar, is recommended as a strategy to increase broad-spectrum resistance against diverse *C. lindemuthianum* pathogen. But conventional gene pyramiding is tedious, time consuming and costly (Joshi and Nayak, 2010). Though parents G2333 and PI207262 possess natural gene pyramids *Co-4²+Co-5+Co-7* and *Co-4³+Co-9* respectively (Kelly and Vallejo, 2004), and could be grown in disease prone environments, their small (Mesoamerican) seed type is not popular among Uganda farmers, traders and consumers, who prefer large kidney (Andean) seed type. Moreover, it is not known which gene combinations would give the most effective resistance against *C. lindemuthianum* race diversity in Uganda. Therefore, there is need to generate information on the effectiveness of single resistance genes *Co-4²*, *Co-5*, *Co-9* and *Co-4³* and pyramided gene combinations “*Co-4²+Co-5+Co-9*”, “*Co-4³+Co-5+Co-9*”, “*Co-4²+Co-9*” and “*Co-5+Co-9*” against *C. lindemuthianum* in Uganda.

Disease resistance is reported to cost the plant in terms of yield through associated traits (McGrann *et al.*, 2014) but the extent of the cost/ penalty depends on the resistance gene(s) in question. For anthracnose resistance genes in common bean, this yield cost is not well elucidated and therefore warrants an investigation

1.6 Justification

Understanding the current *C. lindemuthianum* variability in Uganda is crucial in reveal the existing pathological races and their distribution. The use of standard differential cultivars to pathogenically characterize *C. lindemuthianum* races would also reveal new sources of broad-

spectrum resistance genes for use in Uganda bean breeding programs. Current breeding efforts against bean anthracnose disease would not yield much in absence of key information on race occurrence and distribution and sources of effective resistance to bean anthracnose disease.

Resistance gene pyramiding is the most plausible means for achieving broad-spectrum and durable resistance against diverse *C. lindemuthianum* races. Assessing effectiveness of different single and pyramid gene combinations is crucial as this would reveal the most effective single genes pyramid gene combinations against *C. lindemuthianum* in Uganda. Achieving broad-spectrum resistance among commercial varieties would minimize on farm losses, save time and funds required in breeding replacement varieties due to resistance breakdown.

The use of molecular techniques would be more practical in resistance gene pyramiding as the identification of plants carrying two or more resistance alleles of different genes would be made easy. This would ease the constraints of classical breeding which is limited by the length of screening procedures and reliance on the environmental factors. Molecular markers, therefore, would be crucial in facilitating precision selection of individual plants and speeding up the process of gene pyramiding.

Understanding relationship between pyramided resistance genes and yield would provide insight in whether pyramided resistance genes impose a yield cost/ penalty through negative association with yield related traits. This information would help breeders design gene pyramiding strategies that would maximize yield gain among progenies and limit the potential yield cost. This would address the risk of achieving highly effective resistance at the cost of the economic.

1.7 Overall objective

The overall objective of the study was to enhance broad-spectrum resistance to anthracnose disease among common bean varieties for increased productivity in Uganda

1.8 Specific objectives

1. To determine the pathogenic variability of *Colletotrichum lindemuthianum* in Uganda
2. To assess effectiveness of pyramided resistance genes against bean anthracnose disease
3. To determine genetic effects of pyramided genes on yield traits among populations of common bean

1.9 Hypotheses

1. *Colletotrichum lindemuthianum* has a high pathogenic variability, is location specific and new races have emerged in bean growing areas in Uganda
2. Resistance gene pyramids are effective in conferring broad-spectrum resistance to bean anthracnose disease and resistance spectrum increases with number of genes pyramided
3. Common bean populations possess high variability and number of pyramided resistance genes has no negative correlation with yield traits.

CHAPTER TWO

Literature Review

2.1 Pathogenic variation of *Colletotrichum lindemuthianum*

There are many anthracnose pathotypes or physiological races identified by their pathogenicity reactions on a set of host differentials (CIAT, 1997). The highest number of *C. lindemuthianum* races was reported in the two centers of diversity of common beans, the Andean and Mesoamerican regions (Gepts, 1988; Pastor-Corrales *et al.*, 1995).

Barrus (1911) made the first report of α (alpha) and β (beta) races; Burkholder (1923) described the γ (gamma) race, while Andrus and Wade (1942) reported the δ (delta) race; Hubbeling (1957) also reported the δ (delta) race, Schnock (1975) indicated κ (kappa) race (Leaky and Simbwa-Bunya, 1972) and Tu (1992) suggested existence of clearly different physiological races of *C. lindemuthianum*, including α , β , δ , ϵ , γ , κ and λ (lambda), while Kelly *et al.* (1994) reported two races, 7 and 73, in Michigan and North Dakota. Since race 73 overcomes the *Are* gene for anthracnose resistance and race 7 overcomes the *A* gene, both of which were extensively used in the breeding program, to confer resistance to *C. lindemuthianum*, use of gene pyramiding as a disease resistance strategy was suggested.

Using the binary nomenclature system, 38 races were reported in Mexico (Rodriguez, 1991), seven races were identified in a group of 10 isolates from Nicaragua (Rava *et al.*, 1993), 33 were characterized from a group of 178 isolates from Colombia (Pastor-Corrales *et al.*, 1995) and three races were described in the United States (Balardin and Kelly, 1996). Sicard *et al.* (1997) studied population subdivision of *C. lindemuthianum* in the Mexican, Ecuadorian and Argentinean centers of diversity of common beans. They used Random Amplified Polymorphic DNA (RAPD) markers and virulence on 12 differential cultivars to assess diversity of the pathogen. Findings revealed significant differentiation of the three regions with Mexico having the highest polymorphism. Fifteen physiological races were identified out of 45 isolates collected from five wild common bean populations in the South Andean center of origin.

Balardin *et al.* (1997) used the 12 standard differential bean cultivars to characterize 138 *C. lindemuthianum* isolates collected from Argentina, Brazil, the Dominican Republic, Honduras,

Mexico, and the United States and forty one (41) races were identified and categorized races found over a wide geographic area and races restricted to a single country. Races 7, 65, and 73 were widespread with race 73 being the most common while race 7 was found once in Argentina and Mexico but at a higher frequency in the United States. Race 65 was found repeatedly in Brazil and the United States. Although 39% of the races were detected repeatedly and three races were widespread, no race was isolated from both *P. vulgaris* gene pools. Phenetic analyses showed no obvious patterns correlated with virulence clusters and no geographic pattern was evident. Molecular polymorphism generated by RAPD markers confirmed the extensive variability in virulence of *C. lindemuthianum*. Virulence phenotypes were grouped into 15 clusters with the two largest clusters containing isolates from all the geographic regions sampled. Molecular polymorphism was observed among isolates from races 65 and 73 within and among countries, except among Brazilian isolates of race 65. The genetic diversity of *C. lindemuthianum* was greatest in the Mexican and Honduras regions

Sharma *et al.* (1999) collected 85 isolates of *C. lindemuthianum* from Himachal Pradesh state of India and characterized them based on their reaction on the standard differential cultivars and CIAT differentials. Using the standard differential cultivars, 12 races were characterized and the races designated IndI and IndIX were different from those identified in Europe and USA forming a new race group from the Indian Subcontinent. Using the CIAT differential set 19 races were identified, of these only races 65 and 73 were similar to the races reported in North American. Padder *et al.* (2007) analyzed five *C. lindemuthianum* populations in Himachal Pradesh state, India for genetic diversity on the basis of allele frequencies of 12 RAPD markers and reported diversity within each population to be high with values ranging from 0.26 – 0.31 Nei's genetic diversity

Mahuku and Riascos (2004) assessed genetic variability of 200 *C. lindemuthianum* isolates collected from the Andean and Mesoamerican bean varieties and regions and observed high levels of *C. lindemuthianum* variability and genetic diversity (0.97) of 90 races. Ansari *et al.* (2004) characterized pathogenic and genetic diversity of *C. lindemuthianum* isolates collected from a total of 10 countries of Africa, Europe, Central and South American. Using differential cultivars and Amplified Fragment Length Polymorphism (AFLP) they found 30 pathogenic races with race 9 9 as the most widespread. AFLP cluster analysis grouped 86 isolates into three

(3) clusters. This analysis demonstrated that most South and Central American isolates fell among two clusters, and the European and African isolates were genetically closer to Central American isolates.

Alzate-Marin *et al.* (2004) using differential cultivars, reported a total of 50 *C. lindemuthianum* races in Brazil between the period 1996 and 2002. Races 65, 73, 81 and 87 were the most frequent and widely distributed in the country, and were commonly found in the Paraná, Santa Catarina, Goiás and Distrito Federal. Races 1, 5, 17, 67, 79, 85, 86, 93, 96, 102, 105, 111, 117, 121, 123, 125, 137, 193, 217, 320, 321, 339, 343 and 585 were identified in only one State. Although the number of collected isolates was different from each location, data showed that the Paraná presented the highest *C. lindemuthianum* variability with 29 races followed by Goiás with 17 races, Santa Catarina with 16 races and Rio Grande do Sul with 14 races.

Talamini *et al.* (2006) assessed genetic divergence of *C. lindemuthianum* using RAPD markers. Thirty one (31) isolates were collected from three regions of Minas Gerais state in Brazil. Three isolates of the sexual phase of *C. lindemuthianum* (*Glomerella cingulate* f.sp *phaseoli*) were included in the study. Band patterns generated using 11 primers produced 133 polymorphic bands, which were used to determine genetic divergence among and within the pathogen races. The isolates analyzed were divided into six groups with 0.75 relative similarity. Group IV formed by the three isolates of the sexual phase was the most divergent and races previously determined using differential cultivars did not correlate with the results obtained using RAPD markers.

Munda *et al.* (2009) used AFLP markers characterize *C. lindemuthianum* isolates from Slovenia that had been characterized as races 23, 55, 103 and 131 and found two main groups that did not clearly correspond to earlier race classification.

Ribeiro *et al.* (2016) collected 51 isolates from the states of Sao Paulo and Santa Catarina, Brazil and tested for physiological races and for variability among isolates of the race 65 using the 12 standard differential cultivars. They identified 10 physiological races 4, 38, 55, 65, 73, 81, 83, 85, 321, and 351. Races 65 and 81 were predominant, with frequencies of 37.25 and

35.29%, respectively. Regarding the isolates of race 65, wide physiological variability was evident.

Mota *et al.* (2016) studied the variability of *C. lindemuthianum* and *Glomerella spp* using morphological traits such as index of mycelial growth, colony diameter and sporulation capacity; pathogenicity tests and Interretroelement Amplified Polymorphism (IRAP) markers. Although a high level of variability of *C. lindemuthianum* and *Glomerella spp* was observed, they revealed that *Glomerella spp* is not a telemorph (sexual phase) of *C. lindemuthianum* like earlier reported (Souza *et al.*, 2010).

Bigirimana *et al.* (2000) using 12 standard differential cultivars to characterize 12 isolates from Burundi found a high diversity of *C. lindemuthianum* with nine races identified as 9, 69, 87, 384, 385, 401, 448, 449 and 485. Seven of these races (9, 69, 87, 384, 401, 448, and 485) were reported for the first time in Africa while races 401 and 485 had never been reported before.

In Uganda, Leaky and Simbwa-Bunnya (1972) used differential cultivars used by Schriber (1932) and Hubberling (1961) namely Emerson 847, Perry Marrow, Michelite, Dark Red Kidney; and those used by Blondet (1963) and Bannerot (1965) namely Imuna, Kaboon, Coco de la crème, Widusa and Cornell 49-242. They identified races 17, 19, 23, 102, 130, and 453 with isolates collected from Central, Western and South Western regions of Uganda. Nkalubo (2006) reported eight races 23, 55, 102, 130, 227, 375, 511 and 767 using collections from Kabale, Kisoro, Bushenyi and Mpigi districts with race 767 being the most widespread and virulent. Mwesigwa (2008) using RAPD markers, rep-PCR markers and differential cultivars to assess the genetic and physiological diversity of *C. lindemuthianum* isolates collected from Kabale, Mbale, Apac, Mpigi and Wakiso districts Found that 74 isolates formed 3 major clusters and physiological characterization revealed 21 races 0, 2, 3, 4, 6, 14, 128, 262, 264, 268, 320, 452, 481, 1024, 1536, 1538, 1856, 1857, 1989, 3086 and 4033. Comparison of results from the race differentials with those from the molecular techniques did not show a strong relationship. None of these races were similar to those reported by Leaky and Simbwa-Bunnya (1972) and Nkalubo (2006). Nine of them infected Mesoamerican cultivars, three infected Andean cultivars and seven infected cultivars from both gene pools while race 0 did not infect any of the differential cultivars. Races 1024, 1536, 1538, 1856, 1857, 1989, 3086 and 4033

were the most virulent as they infected the highly resistant differential cultivars AB136 and G2333. Races 0 and 6 were the most wide spread.

2.2 Resistance gene pyramiding and its effectiveness

REX Consortium (2016) investigated the deployment strategy that results in the greatest durability of resistance genes. They reported that although theoretical and empirical studies comparing deployment strategies of more than one resistance gene are very scarce, overall durability of disease resistance genes can be increased by combining their presence in the same plant through pyramiding. Their retrospective analyses of field monitoring data suggested that the pyramiding of disease resistance genes within a plant was the most durable strategy and by extension, suggested the combination of disease resistance genes with other practices for pathogen control such as use of pesticides and best cultural practices as a relevant management strategy to slow down the evolution of virulent pathotypes.

Caporalino *et al.* (2014) evaluated three strategies to control root-knot nematodes in lettuce using cultivar mixtures, alternating resistance genes and pyramiding resistance genes, under controlled and field conditions. Results showed that the choice of the single resistance gene and the genetic background in which it was introgressed affected the frequency of resistance breakdown and that pyramiding of two resistance genes in one genotype suppressed the emergence of virulent isolates. Results further revealed that alternating different resistance genes in rotation was efficient in decreasing virulent populations of nematodes in fields due to the specificity of the virulence and the trapping effect of resistant plants; however, mixing resistant cultivars appeared as a less efficient strategy to control nematodes. These findings help demonstrate the general applicability of such strategies for breeding and sustainable management of resistant cultivars against pathogens.

Kelly *et al.* (1994) suggested that pyramiding the *Co-1* Andean and the *Co-2* Mesoamerican genes would afford protection against all known *C. lindemuthianum* races in North America. Young and Kelly (1996) suggested that deployment of major genes such as *Co-6* and *Co-5* in different combinations with *Co-1* gene could contribute to more durable anthracnose resistance in common beans.

Kelly *et al.* (1995) reported on the use of gene pyramiding in common beans for the development of more effective resistance to the temperature-insensitive-necrosis-inducing (TINI) strains of Bean Common Mosaic Virus (BCMV) present in the USA. They indicated that the *bc-3* gene was effective against these strains in the absence of the strain unspecific *bc-u* gene in genotypes possessing the *I* gene. The epistatic *bc-3* gene was observed to interfere with traditional efforts to pyramid the other recessive resistance genes by masking their activity.

Scully *et al.* (1995) pyramided a series of resistance genes to multiple viruses into genetically diverse common bean breeding lines. The specific viruses included bean common mosaic (BCMV), bean yellow mosaic (BYMV), broad bean wilt (BBWV), blackeye cowpea mosaic (BiCMV), clover yellow vein (CYVV), pea mosaic (PeMV), soybean mosaic (SMV), tobacco mosaic (TMV), and watermelon mosaic (WMV) viruses. Five (5) common bean lines [SP 6C, SP 17B, SP 61, SP 180D and SP 377C] resistant to all the nine viral diseases were successfully developed and released.

Liebenberg *et al.* (2005) reported the successful transfer of common bean rust resistance genes from 16 donor lines to adapted germplasm in South Africa since the 1970s. The pyramided resistance genes included *Ur-3+*, *Ur-5*, *Ur-11* and other uncharacterized genes. However, to protect the durability of the resistance sources used, emphasis was placed on concurrent research to combine several genes in a single well-adapted background.

Asensio *et al.* (2005) bred for higher yielding multiple disease resistant Andean cultivars using inter-gene pool populations and studied the feasibility of introgression of resistance to bean common mosaic virus (BCMV), common bacterial blight (*Xanthomonas campestris*) and halo blight (*Pseudomonas syringae*) using gamete selection. It was observed that none of the selected breeding lines had seed sizes as large as the largest Andean parent and that an elite breeding line rather than a landrace cultivar was the most suitable as the last parent in the crossing scheme making maximum genetic contribution to the multiple-parent inter-gene pool crosses and simultaneous selection for plant type, seed traits as well as resistance to diseases.

Pastor-Corrales *et al.* (2007) reported six great northern bean germplasm lines pyramided with rust and mosaic resistance genes, including lines BMN-RMR-8, BMN-RMR-9, BMN-RMR-10,

BMNRMR-11, BMN-RMR-12 and BMN-RMR-13, which were developed by the United States Department for Agriculture (USDA) – ARS Beltsville Agricultural Research Centre. These were reported as the only great northern bean lines in the world to combine in pyramid four genes *Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11* known to confer resistance to all known races of the highly variable bean rust pathogen with the *bc-3* resistance gene that conditions an immunity to all strains of the seed-borne bean common mosaic (BCMV) and bean common mosaic necrosis (BCMNV) viruses.

In order to combat white mold of common beans in the USA, Tera'n and Singh (2010) successfully introgressed high levels of white mold resistance from the secondary gene pool through backcrossing. They pyramided these resistance genes and developed common bean cultivars with high levels of white mold resistance combined with common bacterial blight (CBB), bean common mosaic virus (BCMV) and bean curly top virus (BCTV) resistances.

Ragagnin *et al.* (2009) used markers to pyramid *Co-4*, *Co-6*, *Co-10* anthracnose, *Phg-1* angular leaf spot and *Ur-ON* rust resistance genes in to a susceptible 'carioca' market class cultivar in Brazil. They used TO, AB 136, AND 277 and Ouro Negro as donor parents in a backcross scheme and molecular fingerprinting to select the lines genetically closer to the recurrent parent. Intercrossed BC lines showed that four F_{4.7} pyramid lines had inherited all the target resistance from their respective donor parents against the respective pathogens of the three diseases.

Genchev *et al.* (2010) combined various genes for resistance to anthracnose in Bulgaria using alternating crossing which resulted in recombinant inbred lines (RILs) based on the cross DG 2-36 [Red Hawk/TO//AB136/3/Red Hawk/TO//TU/4/DG 98-53]. Several races of *C. lindemuthianum* identified in Bulgaria were used for phenotypic screening from F₁ to F₅ generation. Seven RILs of F₆ progeny, possessing more than one specific gene for resistance were selected by RAPD and SCAR markers linked to *Co-1* and *Co-4* genes. The line DG 2-36-58-3 was identified as the most promising with resistance to anthracnose provided by *Co-1* and *Co-4* pyramided genes.

Sofkova *et al.* (2010) in Bulgaria used biotechnological and *in vitro* techniques to successfully pyramid resistance genes obtained from *P. coccineus* and *P. acutifolius* mutant lines into

susceptible common bean cultivars for bacteriosis, white mold, anthracnose, rust, and viruses like BCMV, CMV, BYMV and CIYVV. Several cultivars were successfully developed as a result of combining selection for multiple disease resistance.

Rocha *et al.* (2012) assessed SCAR markers and found them to be efficient in selecting plants resistant to both anthracnose and angular leaf spot. Twenty six superior families possessing the highest number of markers were identified using SCAR markers and selected in the F₄ generation. Eighteen of these families were resistant to the races 65 and 453 of *C. lindemuthianum* and five were resistant to the race 63.23 of *Pseudocercospora griseola*.

Ferreira *et al.* (2012) used SCAR, CAPs and RAPD markers to successfully pyramid *Co-2*, *Co-3/9* anthracnose, *I* and *bc-3* common mosaic virus resistance genes into a market class bean genotype.

Singh *et al.* (2014) successfully pyramided high levels of white mold resistance into pinto beans in the USA using multiple-parent populations derived from four sets of crosses [SE154 (VA 19/MO 162//A 195/G 122); SE152 (CORN501/G122//A 195/VCW 55); SE153 (USPT-WM-1/CORN 601//USPT-CBB-1/ 92BG-7); and SE155 (A195/4/NY6020-4/92BG-7//MO 162/19365-25//ICA Bunsu/G 122)] between and within gene pools, involving seven Andean and six Mesoamerican parents. Four large-seeded, < 40g/100 seeds, Andean (SE152-6, SE154-1, SE154-9, and SE155-9) and four medium-seeded, 25-40 g/100 seeds, Mesoamerican (SE153-1, SE153-3, SE153-6, and SE153-7) pyramided lines with white mould resistance were successfully developed. Phenotypic screening under controlled conditions of the pyramided breeding lines and seven resistant parents using four pathogen isolates ARS12D, ND710, CO467 and NY133 revealed that the percentage of resistant plants for the pyramided breeding lines ranged from 63 to 82%.

Wahome *et al.* (2011) evaluated seven groups of snap bean populations of different generations, and 45 bush snap bean lines for resistance to angular leaf spot, anthracnose, and rust diseases in Kenya. Field evaluation showed that two bush lines (KSB 10 W and KSB 10 BR), and one climbing line (HAV 130) had consistent multiple resistance to angular leaf spot, anthracnose and rust diseases. Nine lines and 674 single plants were selected from populations showing

multiple disease resistance. Resistance in selected lines reduced angular leaf spot, anthracnose and rust severity by 17, 16 and 36%, respectively.

Oballa *et al.* (2012) studied the effectiveness of pyramided resistance genes in improving resistance to *Fusarium* root rot in susceptible common bean cultivars in Uganda. Results revealed that pyramiding of resistance genes from different *Fusarium* root rot resistance sources provided a stable source of resistance than using single sources of resistance.

Kivuru (2013) successfully introgressed anthracnose resistance genes *Co-4*² and *Co-6* into common bean lines with resistance to angular leafspot, common bacterial blight, bean common mosaic virus and bean common necrotic virus. Donor parents C4-1308B-3E-8-B and AB136 were used for *Co-4*² and *Co-6* resistance genes respectively. Highly effective resistance to anthracnose disease was observed among the segregating populations.

Njuguna *et al.* (2014) used SCAR marker gamete selection to successfully pyramid multiple resistance genes to angular leaf spot, anthracnose, root rots and bean common mosaic virus into susceptible commercial cultivars in Kenya. Sixteen populations were generated from crosses among six sources of resistance (MEX 54, G10909, G2333, RWR 719, AND 1062 and BRB 191) and four susceptible popular varieties (KAT B1, KAT B9, GLP 585 and GLP 92).

Ddamulira *et al.* (2015) conducted a study to determine the effectiveness of pyramided genes in improving angular leaf spot resistance in susceptible common bean cultivars in Uganda by making crosses among five inbred lines, three resistant donor parents AND277, Mexico 54, G5686 and two susceptible cultivars K132 and Kanyebywa in a cascading gene pyramiding scheme to develop triple crosses (TC). They found that four pyramided genes in the four-parent-crosses (FPC) conferred a more effective resistance against isolate 61:63 than two or three genes.

Mukankusi *et al.* (2016) reported that efforts in Uganda of developing multiple disease resistant common bean varieties by first developing multiple disease resistance parents through gene pyramiding. SCAR and SSR molecular markers tagging resistance genes to anthracnose, *Pythium* root rot, angular leaf spot and Bean Common Mosaic Virus (BCMV) diseases were

used. The target genes and sources of resistance used included the *I* and *bc-3* genes for Bean Common Mosaic Virus (BCMV) and its necrotic strain Bean Common Mosaic Necrotic Virus (BCMNV) from genotype MCM5001; *Co-4*², *Co-5* and *Co-7* genes for anthracnose resistance from genotype G2333; *Prr* gene for *Pythium* root rot resistance from genotype MLB-49-89A, and the *phg* gene for angular leaf spot resistance from genotype MEX54. Findings indicated that lines with pyramided genes were more effective as parents in transferring combined resistance to susceptible cultivars than the individual sources.

2.3 Assessment for yield performance and yield penalty among populations with pyramided resistance genes

According to Smedergard-Peterson, (1985), Bergelsen and Purrington, (1996), Heil and Baldwin, (2002) and Brown, (2002), disease resistance is costly on the plant and may affect crop performance and therefore, has commercial significance.

In spring barley, Kjaer *et al.* (1990) used doubled-haploid progeny of crosses between three mildew resistant (*mlo*) lines and susceptible (*Mlo*) cultivars in trials in which foliar diseases were controlled with fungicides. Mildew resistant lines had an average yield that was 4.2% lower than that of susceptible lines. The yield loss that was associated with *mlo* was caused by the spotting which was a pleiotropic effect of the *mlo* gene.

Thomas *et al.* (1998) run different analyses of the same data and found that grain number per stem was the main yield component affected. McGrann *et al.* (2014) also confirmed that agronomic costs were associated with the use of the mildew resistance, *mlo* in spring barley cultivars as a result of spontaneous necrotic spotting. They further observed that using *mlo* resistance gene enhanced susceptibility to several other facultative pathogens such as *Magnaporthe oryzae*, spot blotch caused by *Cochliobolus sativus* and 40 head blight caused by *Fusarium graminearum*.

In winter barley, le Gouis *et al.* (2001) reported that the *Ym4* gene conferring resistance to barley mild mosaic virus and strain 1 of barley yellow mosaic virus was associated with a 2% reduction in grain yield.

In wheat, Singh and Espino (1997) reported a 6% yield reduction associated with *Lr34* gene pyramided with other *Lr* genes which provided durable resistance to brown rust of wheat. Latter *et al.* (1998) reported a 9% yield penalty associated with three stem rust resistance genes wheat. Sharp *et al.* (2002) reported a 21% yield reduction associated with the resistance gene *Wsm1* for wheat streak mosaic virus.

In maize, Santa-Cruz *et al.* (2014) examined the effects of two quantitative trait loci (QTL) for southern leaf blight resistance on several agronomic traits using two near-isogenic lines (NILs) B73-3B and B73-6A in the background of the maize inbred B73, each carrying one introgression (called 3B and 6A respectively) encompassing a QTL for southern leaf blight (SLB) resistance. Results revealed that both introgressions confer a yield cost associated with the resistance phenotype in the absence of SLB, but only introgression 6A was associated with a statistically significant reduction.

In rice, Suh *et al.* (2013) used marker-assisted backcrossing strategy to pyramid three resistance genes *Xa4*, *xa5* and *Xa21* against bacterial leaf blight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* into susceptible elite japonica rice cultivar Mangeumbyeo. The background genome recovery expressed 92.1% using genome-wide SSR marker analysis. The pathogenicity assays conducted with 18 isolates among advanced backcross lines revealed that pyramiding resistance genes *Xa4*, *xa5* and *Xa21* provided a higher resistance than the individual resistance genes with agronomic and grain quality traits similar to those of the recurrent parent.

Pradhan *et al.* (2015) pyramided three resistance genes *xa5* + *xa13* + *Xa21* against bacterial blight disease into highly susceptible but popular cultivar Jalmagna. Results revealed high levels of resistance against bacterial blight, high morphological and agronomic similarity to Jalmagna and absence of antagonistic effects for yield among pyramided lines.

Suh *et al.* (2015) pyramided five resistance genes *Pi40*, *Xa4*, *xa5*, *Xa21* and *Bph18* for biotic stress into the japonica rice cultivar using marker-assisted selection (MAS). Pyramided lines

with multiple resistances for blast, bacterial blight and brown plant hopper were successfully developed but no agronomic penalty was associated with the pyramided resistance genes.

Tanweer *et al.* (2015) pyramided two rice blast resistance genes, *Pi-b* and *Pi-kh*, into a popular Malaysian rice variety MR219. Background selection revealed more than 95% genome recovery of MR219 in advanced blast resistant lines, while phenotypic screening for blast resistance revealed strongly resistant advanced lines against pathotype P7.2 with significantly similar morphological, yield and grain quality characteristics to those of recurrent cultivar MR219.

In common beans, Coyne *et al.* (2000) reported 'Wei hing' in the USA which was bred with multiple resistances to rust, common bacterial blight and white mold using pedigree selection. Field evaluation results revealed no significant differences between the mean yield of the pyramided cultivar and two other standard cultivar checks.

Liebenberg *et al.* (2005) reported the successful gene pyramiding of three characterized rust resistance genes namely *Ur-3+*, *Ur-5*, *Ur-11* and other uncharacterized genes in to advanced common bean lines. Field testing for agronomic performance of the lines showed that resistance was considerably improved without yield loss.

Fininsa and Tefera (2007) evaluated eight (8) genotypes [EMP 219, TY 3396-6, TY 3396-7, TY 3396-12, RAB 404, ARA 21, TAR 3, BZ 1289-12] with multiple resistance for anthracnose, angular leaf spot and common bacterial blight and two commercial checks in field performance trials. It was revealed that genotypes with multiple resistance were late maturing and the commercial check Ayenew had the highest 100-seed weight (46 g), but the multiple resistant genotype TY 3396-12 had better yield (4.5 t ha⁻¹) implying no yield penalty.

Ragagnin *et al.* (2009) bred for multiple resistance to anthracnose, angular leaf spot and rust in common beans using marker assisted gene pyramiding techniques. Results revealed that pyramided lines with all the target resistance genes were as productive as the best 'carioca-type' cultivars without gene pyramids.

Souza *et al.* (2009) pyramided rust resistance genes in advanced common bean lines and no penalty was revealed in relation to the recurrent parent after evaluating traits such as grain yield (kg/ha), plant height, seeds per pod and pods per plant.

Souza *et al.* (2014) pyramided rust resistance genes *Ur-5*, *Ur-11* and *Ur-14* and found that the F₇ lines harbouring the three-gene pyramid were as productive as the recurrent parent and high performing control cultivars grown in Brazil.

Mulanya *et al.* (2014) bred for multiple resistance to rust, anthracnose and angular leaf spot in snap beans and reported four lines with multiple resistance to the three diseases that had better pod yield and pod quality compared with existing commercial varieties in Kenya.

Mukankusi *et al.* (2016), in their effort to develop multiple disease resistance breeding parents with pyramided resistance genes to anthracnose, *Pythium* root rot, angular leaf spot and Bean Common Mosaic Virus (BCMV) diseases, observed negative correlations between the number of pyramided genes and several agronomic traits implying a possible yield penalty.

Miklas *et al.* (2017) examined effects of two quantitative trait loci (QTLs) from tepary bean on agronomic and canning quality traits. Results revealed that the QTLs *BC420* and *SU91* did not cause a yield penalty.

The review under this section shows that there is evidence that disease resistance causes a yield penalty/ cost on the plant. However, several resistance gene pyramiding studies have reported contrary findings. This lack of clarity needs to be further investigated.

CHAPTER THREE

Pathogenic variation of *Colletotrichum lindemuthianum* in Uganda

Abstract

Colletotrichum lindemuthianum is a highly variable pathogen of common beans that easily overcomes resistance in cultivars bred with single-gene resistance. Samples of common bean tissues with anthracnose symptoms were collected in eight districts of Uganda, namely Kabarole, Sironko, Mbale, Oyam, Lira, Kapchorwa, Maracha and Kisoro and 51 isolates which sporulated successfully on Potato Dextrose Agar and Mathur's media were used to inoculate 12 differential cultivars under controlled conditions. Five plants per cultivar were inoculated with each isolate and evaluated for their reaction using the 1 – 5 severity scale¹ (Inglis *et al.*, 1988). Races were classified using the binary nomenclature system proposed by Pastor Corrales (1991). Variation due to cultivar and isolate effects was significant ($P \leq 0.001$) for severity. The 51 isolates from eight districts grouped into 27 different races. Sironko district had the highest number of races followed by Mbale and Kabarole. Races 2047 and 4095 were the most frequently found, each with 10 isolates grouped under them. Race 4095 was the most virulent and caused a susceptible (S) reaction on all 12 differential cultivars and the susceptible check followed by races 2479, 2047 and 2045 respectively. Two races, 4094 and 2479, caused a susceptible reaction on the differential cultivar G2333, which nevertheless, showed the most broad spectrum resistance followed by cultivars Cornell 49-242, TU, and AB136 respectively which were recommended for use in breeding programs aimed at breeding for broad spectrum resistance to bean anthracnose in Uganda.

Key words: Resistance, races, variability, differential cultivars

3.1 Introduction

The pathogen *Colletotrichum lindemuthianum* has a wide variation with various races reported in major bean producing regions of Meso-America and Latin America (Balardin and Kelly, 1998) that are the center of origin of common beans (Pastor-Corrales *et al.*, 1995). The highest diversity and variation of *C. lindemuthianum* was reported in Latin America (Pastor-Corrales *et al.*, 1995). The East African highland region is regarded as a secondary center of diversity of common beans (Schwartz and Pastor-Corrales, 1989) and is expected to have a high variability of *C. lindemuthianum* due to host-pathogen co-evolution. Mahuku and Riascos (2004) assessed virulence and molecular diversity of 200 *C. lindemuthianum* isolates collected from Andean and Mesoamerican bean cultivars and they reported high levels of pathotypic diversity with up to 90 pathotypes identified.

In Uganda, three key studies on characterization and diversity of *C. lindemuthianum* were conducted by Leaky and Simbwa-Bunnya (1972), Nkalubo (2006) and Mwesigwa (2008) and a high variability of races revealed. However, a long period of time has passed since the last study (2008) was conducted and change in diversity of the pathogen could have taken place due to increase in bean production, introduction of new germplasm from different gene pools and movement of bean seed within the country and across borders necessitating another study of pathogenic variability. This, therefore, necessitated another study so as to understand the current variability of the pathogen. The objective of this part of the study was to determine the current pathogenic variation of *C. lindemuthianum* in Uganda.

3.2 Materials and methods

3.2.1 Collection of *C. lindemuthianum* samples

Bean pods with symptoms of anthracnose disease were collected from different cultivars growing in farmers' fields from eight districts of Uganda (Figure 3.1). A sampling quadrant measuring 1M² was used in the farmers' fields to select plants from a given part of the field, which were used for sample collection. Data were collected on disease incidence and severity.

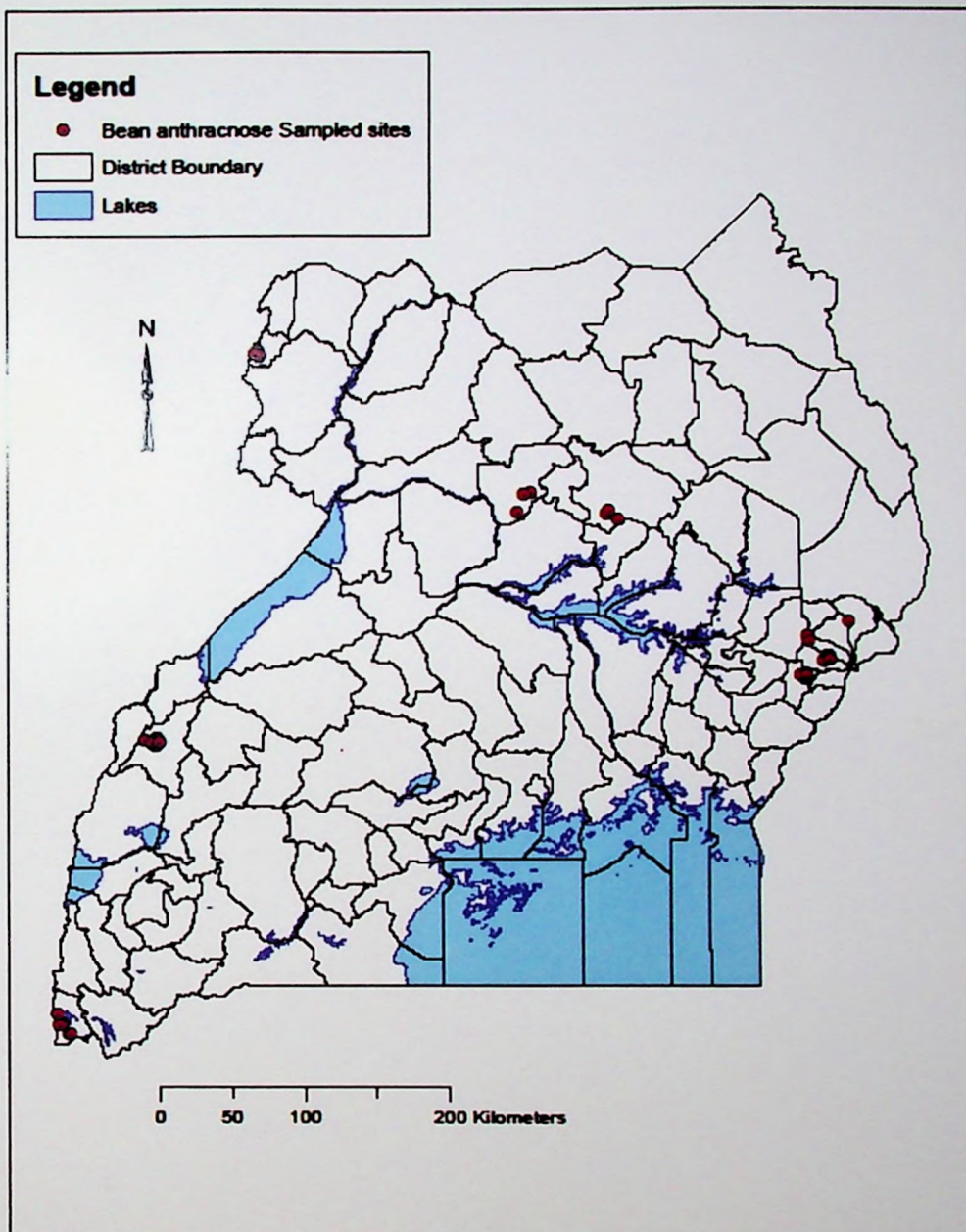


Figure 3.1: Map of Uganda indicating sampling areas for bean anthracnose

Diseased bean pod samples were collected from the sampled fields, placed in polythene bags and stored in boxes. A Global Position System (GPS) device was used to determine altitude and coordinates of fields where samples were picked.

3.2.2 Isolation of *C. lindemuthianum*

Isolation of the fungus was done at the NaCRRRI Biosciences laboratory complex according to the method described by Balardin *et al.* (1997). Infected tissues from the bean pods were cut into small pieces of up to 5cm long and the tissues were placed into a small beaker. Ten milliliters of Sodium hypochlorite (Jik) bleach was added and after two minutes, the bleach was removed and 10ml of ethanol was added for two minutes. The ethanol was decanted and the tissues rinsed with sterilized water. The tissues were placed on filter paper to remove excess water. The tissues were placed on Potato Dextrose Agar media and incubated in darkness at 22 - 24°C for four days before sub-culturing onto modified Mathur's Agar media (500g) made up of 4g of Dextrose, 1.25g of Magnesium Sulfate, 1.35g of Potassium Phosphate, 1.2g of Neopeptone, 1g of Yeast extract and 8g of Agar, to get pure isolates and increase sporulation (Champion *et al.*, 1973).

A second sub-culturing was done by placing single-spore isolates on fresh Mathur's agar (Plate 3.1) in a Petri dish and incubated at 22-24°C for 7 to 10 days to allow the fungus enough time to produce conidial spores (Balardin *et al.*, 1997).

3.2.3 Characterization of *C. lindemuthianum*

Inoculum was prepared by scrapping off germinated conidia (Plate 3.2) on the growth media into a jar with small amounts of distilled water to form a suspension.

Using a hemocytometer, the concentration was adjusted to 1.2×10^6 conidia ml^{-1} (Inglis *et al.*, 1988) and 0.1% Tween 20 was added as a surfactant. Characterization of *C. lindemuthianum* was performed using a set of 12 differential bean cultivars (CIAT, 1988). Four of the differentials were large seeded varieties of Andean origin while the other six were small seeded middle-American genotypes (Table 3.1).

Table 3.1: Differential cultivars used to characterize *C. lindemuthianum*, their binary codes, resistance genes and gene pool

Differential Cultivar	Seed Type ^a	Notation (n)	Binary Code (2 ⁿ)	Resistance Gene	Gene Pool ^b
Michelite	S	0	1	<i>Co-11</i>	MA
Michigan Dark Red Kidney	L	1	2	<i>Co-1</i>	A
Perry Marrow	L	2	4	<i>Co-1</i> ³	A
Cornell 49-242	S	3	8	<i>Co-2</i>	MA
Widusa	L	4	16	<i>Co-1</i> ⁵	A
Kaboon	L	5	32	<i>Co-1</i> ²	A
Mexico 222	S	6	64	<i>Co-3</i>	MA
PI 207262	S	7	128	<i>Co-4</i> ³ <i>Co-9</i>	MA
TO	S	8	256	<i>Co-4</i>	MA
TU	S	9	512	<i>Co-5</i>	MA
AB 136	S	10	1024	<i>Co-6</i> , <i>co-8</i>	MA
G2333	S	11	2048	<i>Co-4</i> ² <i>Co-5</i> ² <i>Co-7</i>	MA

^a S = Small seeded; L = Large seeded. ^b MA= Middle American; A= Andean ; Source: Awale *et al.*, (2007)

Five seeds of each of the 12 differential cultivars and one known susceptible cultivar K132 were pre-germinated and later soaked for 30 minutes in the inoculum before sowing in trays with sterilized soil-saw dust composite in a controlled screen house. Each seed represented a replication and the experiment was set up in a Randomized Complete Block design (RCBD). The humidity chamber conditions were maintained at a minimum of 95% relative humidity and temperature of 22±2°C (Plate 3.3). Disease severity was scored 10 – 14 days after planting using a 1-5 scale (Inglis *et al.*, 1988) where; 1 = no symptoms (resistant), 2-3 = very small lesions mostly on primary leaves (resistant) and 4-5 = numerous enlarged lesions or sunken cankers on the lower side of the leaves or hypocotyls (susceptible). Data was analyzed using GenStat Discovery 12th Edition (Anonymous, 2009).

3.2.4 Race determination

To identify *C. lindemuthianum* races, the binary system of nomenclature (Pastor-Corrales, 1991) was used based on the sum of the binary values assigned to each of the 12 differential cultivars described in Table 3.1 above on which the unknown race is pathogenic. Each differential cultivar has an assigned number 2ⁿ where *n* corresponds to the place occupied by the cultivar within the differential series. The designation of a race number was obtained by summing the numerical values of all differential cultivars exhibiting susceptible (S) reactions to the isolate used for inoculation (Pastor-Corrales, 1991). Isolates with similar reactions on the differentials were grouped to form a race.

3.3 Results

3.3.1 *C. lindemuthianum* samples and isolates collected

Fifty one of the diseased bean samples collected from eight districts are presented in Table 3.2. Isolates of *C. lindemuthianum* after successful culturing are presented in Plates 3.1 and 3.2, while bean anthracnose symptoms on seedlings of differential cultivars are presented in Plate 3.3.

Table 3.2: *C. lindemuthianum* samples collected in different parts of Uganda.

District	Sub-county	Village	Longitude	Latitude	Altitude	Sample/ Isolate I.D*
Kabarole	Mugusu	Kibede	00°36.152N	030°12.976E	1596	025A
Kabarole	Mugusu	Kirugu	00°37.461N	030°13.135E	1605	028A
Kabarole	Mugusu	Kisaru	00°37.506N	030°12.528E	1562	34A
Kabarole	Karambi	Karambi	00°38.107N	030°13.785E	1563	36A
Kabarole	Karambi	Karambi	00°38.107N	030°13.785E	1563	37A
Kabarole	Karambi	Karambi	00°38.107N	030°13.785E	1563	38A
Kabarole	Karambi	Karambi	00°38.107N	030°13.785E	1563	40A
Kabarole	Karambi	Karambi	00°38.100N	030°14.842E	1553	41A
Kabarole	Karambi	Karambi	00°38.100N	030°14.842E	1553	44A
Kabarole	Karambi	Karambi	00°37.923N	030°14.643E	1553	46A
Kabarole	Karambi	Karambi	00°37.923N	030°14.643E	1555	52A
Kabarole	Karambi	Karambi	00°37.923N	030°14.643E	1558	55A
Kapchorwa	Binyinyi	-	01°25.042N	034°31.741E	1929	77A
Kisoro	Busanza	Nyakababi	01°10.066S	029°35.152E	1689	001A
Kisoro	Nyarubuye	Mwalo	01°15.127S	029°38.902E	1790	007A
Kisoro	Nyarubuye	Mwalo	01°15.127S	029°38.902E	1790	008A
Kisoro	Nyarusiza	Nyamushungwa	01°18.691S	029°40.856E	1985	012A
Kisoro	Nyarusiza	Buhangura	01°19.457S	029°40.987E	2045	016A
Lira	Amac	Acuma-roma	02°09.939N	033°01.885E	1071	56A
Lira	Amac	Adyeri	02°05.683N	033°02.392E	1060	57A
Lira	Amac	Corner Ariti	02°06.751N	032°59.934E	1060	59A
Maracha-Terego	Oluvu	Anderu	03°12.316N	030°51.911E	1221	64A
Maracha-Terego	Oluvu	Anderu	03°13.747N	030°50.747E	1238	65A
Maracha-Terego	Oluvu	Anderu	03°13.628N	030°51.030E	1254	66A
Maracha-Terego	Oluvu	Anderu	03°12.427N	030°52.328E	1203	67A
Maracha-Terego	Oluvu	Asuru	03°12.529N	030°51.891E	1212	69A
Maracha-Terego	Oluvu	Asuru	03°12.041N	030°50.730E	1240	71A

Maracha-Terego	Oluvu	Asuru	03°12.305N	030°51.912E	1228	72A
Maracha-Terego	Oluvu	Asuru	03°12.111N	030°52.182E	1218	73A
Mbale	Budwale	Bukumi	01°03.581N	034°15.572E	1862	91A
Mbale	Wanare	Bugeheme	01°03.158N	034°14.834E	1891	92A
Mbale	Wanare	Bugeheme	01°03.204N	034°14.618E	1871	94A
Mbale	Wanare	Bugeheme	01°03.451N	034°14.960E	1842	95A
Mbale	Budwale	Bukumi	01°03.520N	034°15.375E	1518	96A
Mbale	Wanare	Bugeheme	01°03.266N	034°14.982E	1854	97A
Mbale	Bungokho	Namashere	01°03.383N	034°13.907E	1187	98A
Mbale	Wanare	Bugeheme	01°03.197N	034°14.626E	1887	99A
Mbale	Budwale	Bukumi	01°03.621N	034°15.413E	1837	100A
Oyam	Loro	Alut-kot	02°13.793N	032°28.989E	1058	61A
Oyam	Loro	Adyeda	02°14.953N	032°33.087E	1051	62A
Oyam	Loro	Awe-ikwo	02°13.822N	032°29.041E	1061	63A
Sironko	Bukhalu	Bwerucra	01°17.537N	034°17.481E	1089	75A
Sironko	Bukhalu	Buyaga	01°16.478N	034°16.453E	1132	76A
Sironko	Masifwa	Jewa	01°10.236N	034°21.437E	1292	81A
Sironko	Masifwa	Lubunya	01°11.207N	034°22.697E	1549	82A
Sironko	Masifwa	Bugibulungi	01°09.068N	034°22.517E	1690	83A
Sironko	Masifwa	Bugimwera	01°11.472N	034°22.926E	1685	84A
Sironko	Masifwa	Gimbubuni	01°10.643N	034°22.584E	1485	85A
Sironko	Masifwa	Bunagani	01°10.529N	034°22.109E	1314	86A
Sironko	Masifwa	Lubunya	01°11.329N	034°22.810E	1690	88A
Sironko	Masifwa	Bunamono	01°09.173N	034°22.675E	1364	90A

51 *C. lindemuthianum* isolates that sporulated successfully. * A signifies Anthracnose

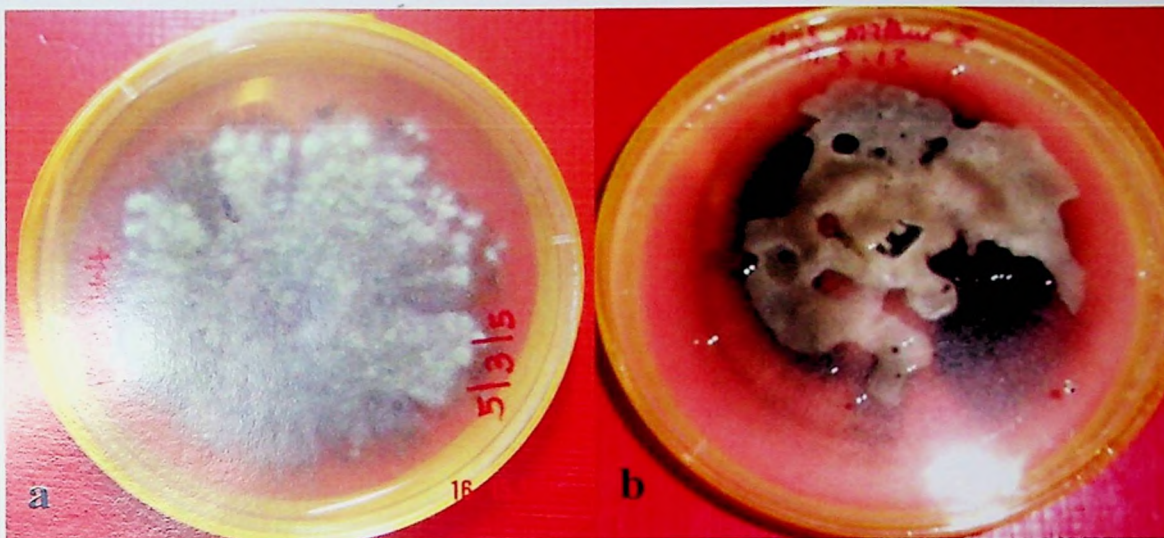


Plate: 3.1: *Colletotrichum lindemuthianum* in culture; **a** = Isolate Kis44 on PDA media;
b = isolate N5 on Mathur's media

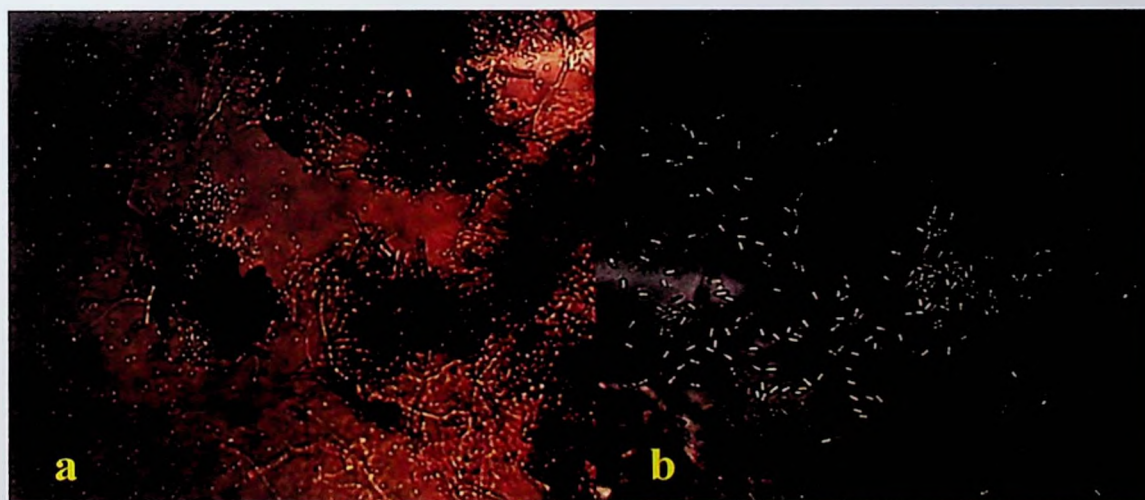


Plate 3.2: Conidia of *Colletotrichum lindemuthianum*; **a** = Conidia of isolate N2; **b** = Conidia of isolate Kis33A



Plate: 3.3: Bean anthracnose symptoms on seedlings; **a** = Arrows pointing at anthracnose symptoms appearing on leaves of a susceptible cultivar; **b** = Arrows pointing at anthracnose symptoms on the cotyledon and hypocotyl of a susceptible cultivar

3.3.2 Isolate and cultivar variability

The results of analysis of variance (Table 3.3) revealed highly significant differences ($P \leq 0.001$) among the cultivars and isolates.

Table 3.3: Analysis of variance for bean anthracnose severity on the 12 differential cultivars

Source of variation ^a	d.f	Severity	
		Mean square	% ^b Contribution
Rep	4	1.10	1.51
Cultivar	12	33.78*	46.20
Isolate	50	36.41*	49.79
Cultivar x Isolate	600	1.33*	1.82
Residual	2,647	0.50	0.69

^a Source of variation; d.f = degree of freedom; S.S = sum of squares; M.S = mean square; F pr = Probability value associated with the F statistic

^b Percentage contribution of each source of variation to the total variation

* Significant at $P \leq 0.001$

Variation due to the interaction between cultivars and isolates was also highly significant ($P \leq 0.001$). Isolate contributed the highest percentage (49.8%) to the total observed variation followed by cultivars (46.2%).

The reactions of 51 isolates on the 12 differential cultivars are presented in the Table 3.4. The overall mean severity score was 2.64.

Table 3.4: Reactions of *Colletotrichum lindemuthianum* isolates on the differential cultivars

Isolate ID	Differential cultivars* and their resistance genes													Mean Sev
	1 ^b	2 ^a	3 ^a	4 ^b	5 ^a	6 ^a	7 ^b	8 ^b	9 ^b	10 ^b	11 ^b	12 ^b	13 ^{ac}	
	<i>Co-11</i>	<i>Co-1</i>	<i>Co-1¹</i>	<i>Co-2</i>	<i>Co-1¹</i>	<i>Co-1²</i>	<i>Co-3</i>	<i>Co-4¹, Co-9</i>	<i>Co-4</i>	<i>Co-5</i>	<i>Co-6, co-8</i>	<i>Co-4², Co-5², Co-7</i>		
12A	1.80	4.20	2.40	1.40	2.00	2.00	2.40	1.40	1.40	1.80	3.20	1.40	2.00	2.11
34A	1.60	1.60	1.20	1.80	1.80	1.60	4.00	2.80	1.80	2.60	1.20	1.40	3.00	2.03
91A	3.00	2.80	3.20	2.00	2.40	2.00	1.80	2.20	1.60	1.40	2.60	1.00	2.80	2.22
92A	2.20	1.20	1.20	1.40	2.60	2.00	2.80	1.40	1.20	1.60	1.80	1.40	2.60	1.80
88A	2.20	2.60	3.00	1.40	2.20	2.80	3.40	2.20	2.40	1.80	2.00	1.20	2.20	2.26
65A	3.00	3.80	3.60	3.60	3.20	3.00	3.60	3.80	3.20	3.00	2.40	1.40	3.40	3.15
94A	1.80	2.40	2.20	2.00	2.40	2.20	2.00	1.60	2.60	1.40	2.40	1.00	2.60	2.05
56A	1.80	1.40	1.80	1.60	2.20	1.80	1.60	1.40	2.20	2.20	1.40	1.20	2.40	1.77
38A	2.00	1.40	1.60	1.40	1.40	1.40	1.40	2.00	1.60	2.00	1.60	1.20	1.80	1.60
08A	3.00	3.00	2.60	2.60	3.00	2.40	2.40	3.00	3.20	2.60	2.40	1.80	3.20	2.71
66A	3.20	3.60	3.20	3.00	3.60	2.80	3.80	3.60	3.20	3.00	2.80	2.20	3.60	3.20
90A	3.20	3.20	3.20	3.00	2.50	2.40	2.00	2.80	2.20	1.40	2.60	1.60	3.00	2.55
72A	2.40	1.60	2.00	1.80	2.20	1.60	1.40	2.60	2.00	1.40	2.60	1.20	2.60	1.95
64A	3.40	3.00	3.40	3.60	2.60	3.00	3.00	3.80	3.20	3.20	2.60	1.60	3.80	3.09
81A	2.00	2.40	1.80	2.20	2.00	2.40	1.00	1.80	2.60	2.40	2.20	1.00	3.00	2.06
100A	3.20	2.80	2.60	2.40	2.40	2.40	2.00	2.40	2.40	2.00	2.60	1.20	3.40	2.45
82A	2.20	1.60	2.00	1.40	2.00	1.80	1.80	1.20	2.00	1.20	1.60	1.00	2.20	1.69
59A	1.40	1.80	1.40	1.60	1.20	1.40	1.20	1.40	1.40	1.40	1.20	1.00	1.40	1.37
57A	1.40	1.20	1.60	1.40	1.80	1.40	1.40	1.40	1.60	1.20	1.60	1.00	1.60	1.43
63A	3.60	3.60	3.00	3.00	3.20	3.60	3.20	3.60	3.00	2.80	3.60	2.40	4.20	3.29
25A	1.20	1.40	1.20	1.20	1.40	1.00	1.00	1.20	1.20	1.20	1.20	1.00	1.40	1.20
44A	3.60	2.80	4.00	3.40	3.80	2.60	3.60	4.40	2.20	3.80	3.40	2.40	3.80	3.37
75A	3.00	3.20	2.80	3.40	3.40	3.40	3.20	3.80	2.20	3.00	2.80	2.00	4.20	3.11
84A	1.40	1.00	1.40	1.40	1.00	2.40	2.40	1.40	2.20	1.20	1.20	1.00	1.60	1.51
69A	2.80	3.00	3.00	2.60	3.60	3.20	3.20	3.00	2.00	3.40	1.60	1.00	3.80	2.78
41A	3.60	3.60	4.00	4.00	3.20	2.80	3.80	4.00	2.00	2.40	3.40	1.80	4.20	3.29
98A	3.00	2.60	3.20	2.80	2.60	3.20	3.00	2.80	3.00	2.60	3.20	1.60	3.80	2.88
52A	3.00	3.20	2.40	3.20	2.60	2.80	2.80	2.80	3.00	3.00	3.00	1.40	3.40	2.82
86A	2.60	2.00	2.00	2.60	1.60	1.80	2.40	2.20	1.60	2.20	1.80	1.20	2.60	2.05
67A	3.40	4.00	3.80	4.20	3.40	3.00	3.00	3.20	3.20	2.80	3.60	2.20	4.40	3.40
55A	4.00	3.60	3.20	3.80	3.00	3.40	3.60	4.00	3.80	3.80	2.80	1.80	4.00	3.45
95A	4.40	2.00	4.20	3.20	2.60	3.00	3.60	2.80	4.20	4.00	3.00	1.60	2.00	3.12
76A	4.40	4.20	4.20	4.80	4.60	4.20	3.80	4.20	4.60	4.20	4.20	2.60	4.00	4.15

28A	4.20	3.80	4.00	3.80	4.00	3.20	2.40	3.40	3.40	2.40	3.60	2.20	3.40	3.37
62A	4.60	2.20	2.40	2.80	3.00	3.00	3.00	2.80	3.40	4.00	1.60	1.00	3.40	2.86
37A	5.00	3.40	3.60	4.20	3.80	2.60	2.80	4.00	2.80	2.80	2.40	1.40	2.40	3.17
16A	3.60	2.60	3.60	1.80	2.60	3.40	2.20	3.60	4.20	1.40	3.00	1.00	3.40	2.80
83A	2.80	2.40	4.40	1.40	1.00	4.20	1.20	2.60	3.80	1.60	1.60	2.20	3.40	2.51
97A	3.20	2.60	4.20	1.00	2.60	4.40	2.40	3.40	2.80	2.60	4.60	1.80	3.20	2.98
99A	4.20	2.80	2.40	1.40	3.80	3.80	3.00	2.60	2.80	1.80	3.60	2.00	3.80	2.92
001A	4.80	4.40	4.60	4.60	4.80	4.40	4.60	5.00	4.40	4.80	5.00	3.20	4.60	4.55
77A	3.40	4.00	4.00	3.40	3.80	4.00	3.20	3.60	4.00	3.80	4.00	2.60	4.40	3.71
73A	3.40	4.00	4.00	3.40	3.80	4.00	3.20	3.60	4.00	3.80	4.00	2.60	4.40	3.71
46A	2.80	2.60	2.60	2.60	2.60	3.00	2.60	3.40	2.80	2.60	2.80	1.80	4.00	2.78
61A	2.00	2.40	2.00	2.00	2.00	2.00	1.60	2.20	2.20	1.80	2.00	1.20	3.40	2.06
40A	3.20	2.00	2.40	2.60	3.00	2.40	2.80	2.80	3.40	3.00	2.20	1.80	3.40	2.69
71A	2.80	2.40	3.40	3.60	3.00	3.60	3.00	3.60	2.60	2.80	3.00	1.60	4.40	3.06
007A	3.00	3.00	3.00	2.00	1.80	2.60	2.40	2.60	2.60	3.00	2.40	1.20	3.20	2.52
85A	1.80	2.20	2.00	2.40	1.60	2.60	2.00	1.20	1.20	1.60	1.40	1.00	3.40	1.88
36A	1.80	1.40	1.60	1.60	1.80	1.60	1.60	2.20	1.40	2.00	1.60	1.20	2.60	1.72
96A	3.00	3.20	3.40	3.60	3.40	3.20	3.40	3.20	3.20	2.80	3.20	2.20	4.00	3.22
Mean														
Sev	2.91	2.69	2.82	2.58	2.66	2.72	2.61	2.78	2.65	2.48	2.58	1.58	3.19	2.64
S.e	0.13	0.13	0.13	0.14	0.13	0.12	0.12	0.14	0.13	0.13	0.13	0.08	0.12	0.10

*Differential cultivar: 1 = Michelite; 2 = MDRK; 3 = Perry Marrow; 4 = Cornell 49-242; 5 = Widusa; 6 = Kaboon; 7 = Mexico 222; 8 = PI 207262; 9 = TO; 10 = TU; 11 = AB136; and 12 = G 2333

^a Andean gene pool; ^b Mesoamerican gene pool; ^c Susceptible local check K132

Mean severity scores on cultivars across 51 isolates ranged from 1.58 to 3.19. The cultivar G2333 had the lowest mean score (1.58) followed by cultivars Tu (2.48), Cornell 49-242 (2.58) and AB136 (2.58), while the susceptible check K132 had the highest mean severity score (3.19). The isolate 001A had the highest mean severity (4.15) across cultivars followed by isolates 76A (4.15), 77A (3.71), 73A (3.71) and 67A (3.40), while the isolate 25A had the lowest mean severity (1.20) across cultivars.

3.3.3 Race determination and distribution

Results of race determination through screening of bean differential cultivars are presented in Table 3.5. Using the binary system, twenty seven (27) races were identified out of the 51 isolates. Races 2047 and 4095 were the most abundant.

Race 4095 was the most virulent causing a susceptible reaction on all the 12 differential cultivars and the susceptible check. This was followed by races 2047, 2045 2039 and 2023.

Table 3.5 Characterization of *C. lindemuthianum* pathotypes using differential cultivars

Isolates	Differential cultivars ¹ and their respective resistance genes ²												Race*
	1	2	3	4	5	6	7	8	9	10	11	12	
	Co-11	Co-1	Co-1 ³	Co-2	Co-1 ⁴	Co-1 ⁵	Co-3	Co-4 ⁶ , Co-9	Co-4	Co-5	Co-6 ⁷ , Co-8	Co-4 ⁸ , Co-7	
38A, 59A, 57A, 25A	R	R	R	R	R	R	R	R	R	R	R	R	0
82A	S	R	R	R	R	R	R	R	R	R	R	R	1
85A	R	S	R	S	R	S	R	R	R	R	R	R	42
92A	S	R	R	R	R	S	R	R	R	R	R	R	81
36A	R	R	R	R	R	R	R	S	R	R	R	R	128
84A	R	R	R	R	R	R	R	S	R	R	R	R	352
61A	R	S	R	R	R	R	R	S	S	R	R	R	386
88A	S	S	S	R	R	S	S	S	S	R	R	R	503
34A	R	R	R	R	R	R	R	S	R	S	R	R	704
86A	S	R	R	S	S	R	S	S	R	S	R	R	713
69A	S	S	S	S	S	S	S	S	R	S	R	R	767
56A	R	R	R	R	R	R	R	S	S	S	R	R	784
62A	S	S	S	S	S	S	S	S	S	S	R	R	1023
12A	R	S	S	R	R	R	S	R	R	R	S	R	1094
72A	S	S	R	R	R	R	R	S	R	R	S	R	1169
91A	S	S	S	R	R	R	R	S	R	R	S	R	1175
94A	R	S	S	S	S	S	R	R	S	R	S	R	1334
90A, 100A	S	S	S	S	S	S	S	S	S	R	S	R	1471
16A, 99A	S	S	S	R	S	S	S	S	S	R	S	R	1527
41A	S	S	S	S	S	S	S	S	R	S	S	R	1791
81A	R	S	S	R	R	S	R	R	S	S	S	R	1834
007A	S	S	S	S	R	S	S	S	S	S	S	R	2023
97A	S	S	S	R	R	S	S	S	S	S	S	R	2039
95A, 40A	S	R	S	S	S	S	S	S	S	S	S	R	2045
65A, 08A, 64A, 75A, 98A, 52A, 55A, 37A, 46A, 71A	S	S	S	S	S	S	S	S	S	S	S	R	2047
83A	S	S	S	S	R	S	R	S	S	R	R	S	2479
66A, 63A, 44A, 67A, 76A, 28A, 001A, 77A, 73A, 96A	S	S	S	S	S	S	S	S	S	S	S	S	4095

* Races characterized using the binary system (Pastor Corralles, 1991): R=Resistant reaction; S=Susceptible reaction

¹ Position numbers and Binary codes: Michelite (0)1; MIDRK (1)2; Perry Marrow (2)4; Cornell 49-242 (3)8; Widusa (4)16; Kaboon (5)32; Mexico 222 (6)64; PI 207262 (7)128; TO (8)256; TU (9)512; AB136 (10)1024; and G 2333 (11)2048.

Race 2047 comprised of 10 isolates collected from five districts namely Kabarole, Kisoro, Maracha, Mbale and Sironko.

Race 2479 comprised of one isolate collected from Sironko district, while race 4095 comprised of 10 isolates collected from seven districts of Kabarole, Kapchorwa, Kisoro, Oyam, Mbale, Maracha and Sironko making race 4095 the most widely distributed followed by race 2047. Sironko and Mbale districts had the highest number of races including the most virulent ones followed by Kabarole district and Kisoro district. These districts are all in high altitude bean growing regions ranging from 1429 – 1860m above sea level and therefore offer favorable conditions for the development and spread of bean anthracnose disease.

Results of incidences and severity of bean anthracnose in the sampled districts are presented in Table 3.6.

Table 3.6: Incidence, severity and races of *C. lindemuthianum* by district

District	Incidence (%)	Severity (1-5)	Cultivars sampled	Races identified
Lira	23	2.3	Variety mixtures (3)	0, 784
Sironko	76	4.3	Unknown local varieties (10)	1, 42, 352, 503, 713, 1471, 1834, 2047, 2479, 4095
Mbale	75	4.0	Unknown local varieties (9)	81, 1175, 1334, 1471, 1527, 2039, 2045, 2047, 4095
Oyam	31	1.5	Variety mixtures (2) Bweyale-yellow	1023, 4095 386
Maracha	45	2.6	Ofuta ofuta, Agrupia, Wande-wande, Mvugupia, K132	2047, 4095 4095 767 1169
Zombo	0	0.0	Cogudibi, Lau lau, Mixed varieties, Nyar adranga, Ocidu, Nyar awora	- - -
Kabarole	-	-	Variety mixtures (12)	0, 128, 704, 1791, 4095, 2045, 2047
Kisoro	-	-	Variety mixtures (5)	1094, 1527, 2023, 2047, 4095

which grouped into three subgroups using RAPD markers. This high variability reported in *C. lindemuthianum* (Mahuku and Riascos, 2004) is attributed to sexual recombination in the teleomorphic phase of *C. lindemuthianum* called *Glomerella cingilata* f.sp *phaseoli* (Souza *et al.*, 2010). However, Barcelos *et al.* (2014) and Mota *et al.* (2016) refuted that *Glomerella spp* is a teleomorphic phase of *C. lindemuthianum* arguing that it instead co-inhabits anthracnose lesions. Another mechanism of variability of the pathogen is asexual recombination through conidial anastomosis tubes (CATs) (Roca *et al.*, 2007; Ishikawa *et al.*, 2012). Ogallo (1991) also attributed pathogen variability to exchange of genetic materials to somatic cells, mutation and extra chromosomal DNA.

Only three races in this study were similar to races reported earlier in Uganda namely race 0 and 128 (Mwesigwa, 2008) and race 767 (Nkalubo, 2006). None of the races in this study were similar to races reported by Leaky and Simbwa-Bunnya (1972). Race 767 was reported by Nkalubo (2006) as the most abundant and aggressive race while (Mwesigwa, 2008) reported races 1024, 1536, 1538, 1856, 1857, 1989, 3086 and 4033 as the most aggressive. This study, however, revealed that races 4095, 2047, 2045, 2039 and 2023, which had not been reported before in Uganda were the most aggressive (Table 3.5). This lack of consistency may be attributed to use of different sets of differentials in the case of Leaky and Simbwa-Bunnya (1972), who used differentials namely Emerson 847, Perry Marrow, Michelite, Dark Red Kidney (Hubberling, 1961) Imuna, Kaboon, Coco de la crème, Widusa and Cornell 49-242 (Bannerot, 1965). The occurrence of pathogenic variability among plant pathogens has direct implications in breeding for resistance, therefore, the methodologies employed in race determination should be uniform (Buruchara, 1991).

The Race 2047 was reported by de Lima Castro *et al.* (2017) as a Mesoamerican race. It is reported in Brazil to be one of the most aggressive of *C. lindemuthianum* races that can overcome anthracnose resistance conferred by seven resistance genes namely *Co-1*, *Co-2*, *Co-3*, *Co-4*, *Co-5*, *Co-6* and *Co-11*; and five alleles namely *Co-1*², *Co-1*³, *Co-1*⁵, *Co-3*³ and *Co-4*³ (Darben *et al.*, 2017). Mahuku and Riascos (2004) isolated race 2047 in Colombia from materials obtained from Costa Rica implying its wide distribution.

The anthracnose disease was not observed in Zombo district of West Nile despite the fact that it is a highland locale with favorable conditions for the disease. This could be due to sampling in first rain season (March – June), which has less rain and humidity in the West Nile zone than the second rain season (August to November) (<http://Weatherspark.com>).

The differential cultivar G2333, Cornell 49-242, TU and AB 136 respectively showed the most broad-spectrum resistance against *C. lindemuthianum* races in Uganda (Table 3.7) implying that the genes *Co-4²*, *Co-5²*, *Co-7* from cultivar G2333, *Co-2* from cultivar Cornell 49-242, *Co-5* from cultivar TU and *Co-6*, *co-8* from cultivar AB136 are the best resistance genes against bean anthracnose disease in Uganda. Bigirimana *et al.* (2000) reported the cultivars TU, AB136 and G2333 to be highly resistant against 12 isolates from major bean growing areas in Burundi. Nkalubo (2006) reported cultivars G2333 and AB136 as the most resistant. Mwesigwa (2008) reported cultivar Widusa (*Co-1⁵*), which did not succumb to any of the 41 isolates, as the most resistant followed by G2333. Davide and Souza (2009) reported cultivars TU, AB136, G333 and Cornell 49-242 as the most effective resistance. The highly effective resistance in cultivar G2333 is attributed to its naturally occurring three gene pyramid *Co-4²+Co-5²+Co-7* (Young *et al.*, 1998). The allele *Co-4²* is recognized as being among the most effective resistance genes against *C. lindemuthianum* described in common beans (Silverio *et al.*, 2002).

The exceptional performance by cultivar Cornell 49-242 (*Co-2* gene) against the 27 races can be attributed a complex cluster of resistance genes at the end of linkage group Pv11 (Campa *et al.*, 2014) corresponding to the previously described anthracnose resistance cluster *Co-2* (David *et al.*, 2009). Campa *et al.* (2014) identified race specific resistance genes in this cluster and another independent gene with a complementary mode of action on linkage group Pv09.

It should be noted that all the six genes above are from the Mesoamerican gene pool. When considering gene pyramiding for more effective resistance to *C. lindemuthianum* Kelly *et al.* (1994) recommended combining resistance genes from both Mesoamerican

and Andean gene pools. The Andean gene *Co-1* is multiple allelic having four different alleles namely *Co-1*, *Co-1*², *Co-1*³ and *Co-1*⁵ from cultivars Michigan Dark Red Kidney, Kaboon, Perry marrow and Widusa respectively. Based on results of this study the alleles *Co-1*³ & *Co-1*⁵ were more effective.

It was observed, however, that, G2333 succumbed to two races namely 2479 and 4095. Mwesigwa (2008) also reported two races 3086 and 4033 that caused symptoms on cultivar G2333. The cultivars Widusa (*Co-1*⁵), Mexico 222 (*Co-3*), Tu (*Co-5*) and AB136 (*Co-6*, *co-8*) showed resistance to race 2479, implying that any of the genes in these cultivars could be combined with the genes in cultivar G2333 to form new highly effective pyramids. Although, Leaky and Simbwa-Bunnya (1972) observed an immune nature of resistance in the cultivar Cornell 49-242 with a single dominant gene *Co-2*, this study showed that this cultivar had anthracnose symptoms with 11 races. It still ranked second after G2333 in conferring resistance to *C. lindemuthianum* in Uganda

The cultivars Michelite (*Co-11*), MDRK (*Co-1*) and PI207262 (*Co-4*³, *Co-9*) had the lowest number of resistant reactions implying that the resistance genes they carry are less broad-spectrum. These genes could still be useful in breeding programs targeting resistance gene pyramiding for broad spectrum resistance and/or specific resistance to races 0, 42, 128, 352, 386, 704, 784, 1094, 1334 and 1834 for cultivar Michelite; and races 0, 1, 42, 81, 352, 784, 1094, 1334 and 1834 for cultivar PI207262. The cultivar PI207262 was the most susceptible to *C. lindemuthianum* rather than the earlier reported cultivar Michelite. Mwesigwa (2008) observed the same about Michelite, which is usually reported as a universal susceptible differential cultivar. The resistance in Michelite is attributed to the resistance gene designated as *Co-11* (Gonçalves-Vidigal *et al.*, 2007), which appears to have some level of resistance to *C. lindemuthianum* races in Uganda. Bigirimana *et al.* (2000) ranked the cultivar PI207262 second lowest after Michelite out of the 12 differential cultivars. According to Kelly and Vallejo (2004) the cultivar PI207262 containing a natural resistance gene pyramid *Co-4*³+*Co-9* possesses a very specific breeding value against Andean races of *C. lindemuthianum* and was recommended for use only to diversify the resistance in new gene pyramids.

3.5 Conclusion

Colletotrichum lindemuthianum had a high pathogenic variability in Uganda with 27 races identified. Pathogen variability was highest in the Eastern and South Western highland regions of Uganda. Sironko and Mbale districts had the highest concentration of aggressive races. Two races 2479 and 4095 were highly aggressive causing a susceptible reaction on the most resistant cultivar G2333, rarely reported to succumb to any known race in the world. This points to existence of new and highly aggressive races harboured in Uganda. The cultivars G2333, Cornell 49-242, TU and AB136 respectively showed broad-spectrum resistance against the 27 *C. lindemuthianum* races.

3.6 Recommendations

New varieties with broad-spectrum resistance should be developed and deployed especially in Eastern and South Western Uganda for urgent management and control of bean anthracnose disease. The cultivars G2333, Cornell 49-242, TU and AB136 are recommended for use in breeding as sources of broad-spectrum resistance to bean anthracnose. Further studies should be conducted to; identify and test other sources of effective resistance against *C. lindemuthianum* races in Uganda; assess effectiveness and fitness of *Co-4*², *Co-5*², *Co-5*, *Co-3*, *Co-6 co-8* Mesoamerican and, *Co-1*³, *Co-1*⁵ Andean single resistance genes; and use these single genes in different pyramid combinations and assess effectiveness against *C. lindemuthianum* in Uganda.

CHAPTER FOUR

Pyramiding of resistance genes and their effectiveness in conferring disease resistance to anthracnose in common bean

Abstract

Bean anthracnose is one of the most devastating diseases that constrain common bean production in Uganda. This study investigated the effectiveness of pyramided and single genes against bean anthracnose disease. SCAR markers were used to facilitate the process of pyramiding three anthracnose resistance genes using a cascading pedigree pyramiding scheme. Detached leaf trifoliates of 40 $F_{4,6}$ families were screened with four *C. lindemuthianum* races and severity scored using Balardin *et al.* (1997) scale. Disease severity data were subjected to ANOVA. Correlation and path analysis were done to establish relationships between number of pyramided genes and the yield traits. Races, genotypes and Race x Genotype interaction were all significant ($P < 0.001$). $Co-4^2 + Co-5 + Co-9$ and $Co-4^2 + Co-5$ pyramids had the lowest severity scores. The group $Co-4^3 + Co-9$ had the highest severity score. The $Co-4^2$ and $Co-5$ genes conferred resistance to all the four races 352, 713, 767 and 2047. The single gene $Co-4^2$ was not significantly different from the best pyramids $Co-4^2 + Co-5 + Co-9$ and $Co-4^2 + Co-5$ ($P < 0.01$). Similarly the $Co-5$ gene was not significantly different from $Co-4^2 + Co-5$, $Co-4^2 + Co-9$ and $Co-5 + Co-9$ pyramids ($P < 0.01$). The $Co-9$ gene showed antagonism in all pyramids. Number of pyramided genes negatively correlated with seed weight per plant (-0.17), number of pods per plant (-0.24, $P < 0.05$) and number of seeds per plant (-0.19, $P < 0.1$) and had a significant ($P < 0.05$) negative indirect effect (-0.25) on seed weight per plant via number of seeds per plant. Therefore, the effectiveness of pyramided genes highly depended on the individual genes combined. The single genes $Co-4^2$ and $Co-5$ had factors that promote broad-spectrum resistance to *C. lindemuthianum*. Pyramiding of resistance genes had negative direct and indirect effects on yield traits.

Key words: SCAR markers, *Colletotrichum lindemuthianum*, broad-spectrum resistance

4.1 Introduction

Gene pyramiding is a technique aimed at combining multiple desirable genes from multiple parents into a single genotype (Ye and Smith, 2008). Using this technique, it is possible to combine more than one anthracnose resistance genes into a susceptible bean variety.

Different authors have recommended the use of gene pyramiding as a strategy to increase broad-spectrum resistance to bean anthracnose. Kelly *et al.* (1994) recommended pyramiding *Co-1* Andean and *Co-2* Mesoamerican genes against all known *C. lindemuthianum* races in North America. Young and Kelly (1996) suggested pyramiding of major genes such as *Co-6* and *Co-5* in combination with the *Co-1* gene for durable resistance against anthracnose in common beans. For successful resistance gene pyramiding, however, there is need to understand the nature of resistance conferred by single genes and the different pyramided gene combinations under a diverse *C. lindemuthianum* population. The purpose of this study therefore was to; a) develop advanced bean populations/ lines with pyramided anthracnose resistance genes; and b) to assess the effectiveness of single and pyramided resistance genes in conferring broad resistance to diverse *C. lindemuthianum* races.

4.2 Materials and methods

4.2.1 Genetic materials

Research on *C. lindemuthianum* was conducted at the National Crop Resources Research Institute (NaCRRRI), Namulonge, located 00° 32' N of the Equator and 32° 37' E, 27Km North of Kampala and elevated at 1,150 meters above sea level (asl); and at the International Center for Tropical Agriculture (CIAT), based at the National Agricultural Research Laboratories (NARL), Kawanda, located 0° 24' 38.15" N and 32° 32' 14.06" E and elevated at 1,147 meters above sea level.

All the cultivars used as parents in this study were obtained from the Legumes Program, NaCRRI and are presented with their characteristics in Table 4.1.

All crosses and advancement from F₁ to F₄ generations were conducted under screen house conditions at NaCRRI, while Marker Assisted Selection (MAS) for allele selection during gene pyramiding, fixation of alleles from F₄ up to F₆, genotypic and phenotypic screening of advanced lines were conducted at CIAT

Table 4.1: Parents used in the gene pyramiding scheme

Cultivar	Pedigree	Growth habit	Gene pool	Anthracnose Resistance genes	Response to anthracnose
K132 ^a	Calima-2 x Argentino 1	B	A	-	Susceptible to anthracnose (Nkalubo <i>et al.</i> , 2009)
NABE 4 ^a	Sug 47 x Cal 103	B	A	-	Susceptible to anthracnose (Mwesigwa, 2008)
NABE 13 ^a	RWR 1946	B	A	-	Susceptible to anthracnose
NABE 14 ^a	RWR 2075	B	A	-	Susceptible to anthracnose
G 2333 ^{b,c}	Colorado de Teopisca	C	MA	<i>Co-4</i> , <i>Co-5</i> & <i>Co-7</i>	Resistant to anthracnose (Kelly and Vallejo, 2004)
PI 207262	Tlalnepantla 64	C	MA	<i>Co-4</i> & <i>Co-9</i>	Resistant to some races of anthracnose (Kelly and Vallejo, 2004)
KK-22 ^d	RWR719	B	MA	-	Donor for Pythium root rot resistance (Otsula <i>et al.</i> , 1998)

^a Variety officially released in Uganda

^b Landrace from Mexico.

^c Variety officially released in Rwanda as Umubano and in Uganda as NABE10C

^d Variety officially released in Kenya

A = Andean; MA = Mesoamerica; B = Bush; C = Climber

4.2.2 Molecular markers used in Marker Assisted Selection

The target genes, linked DNA markers used to tag the genes, their sizes, orientation and primer sequences are presented in Table 4.2.

Table 4.2: PCR-based markers used in Marker Assisted Selection

Marker	Locus/ Gene tagged	Original marker*	Linkage group	Size (bp)/ orientation	Primer Sequence	Reference
<i>SBB14</i>	<i>Co-4</i> ²	BB14	B8	1150/1050, codominant	Forward: GTG GGA CCT GTT CAA TAA TAC Reverse: GTG GGA CCT GGG TAG TGT AGA AAT	Awale and Kelly (2001)
<i>SAS13</i>	<i>Co-4</i>	AS13	B8	950, <i>Cis</i>	Forward: CAC GGA CCG AAT AAG CCA CCAACA Reverse: CAC GGA CCG AGG ATA CAG TGA AAG	Young <i>et al.</i> (1998)
<i>SAB3</i>	<i>Co-5</i>	AB-3	B7	400, <i>Cis</i>	Forward: TGG CGC ACA CAT AAG TTC TCA CGG Reverse: TGG CGC ACA CCA TCA AAA AAG GTT	Vallejo and Kelly, (2001)
<i>SB12</i>	<i>Co-9</i>	B-12	B4	350, <i>Cis</i>	Forward: CCT TGA CGC ACC TCC ATG Reverse: TTG ACG ATGGG TTG GCC	Mendez de Vigo <i>et al.</i> (2002)
<i>PYAA19₈₀₀</i>	<i>Prr</i>	AA19	-	800, <i>Cis</i>	Forward: TTA GGC ATG TTA ATT CAC GTT GG Reverse: TGA GGC GTG TAA GGT CAG AG	Muhuku <i>et al.</i> (2007)

*Original markers were Random Amplified Polymorphic DNA (RAPDs) markers converted to SCARs

Sequence Characterized Amplified Regions (SCAR) markers were used to tag and track the resistance genes of interest. The primers were obtained from the Department of Molecular and Cellular Biology, University of Cape Town, Randebosch, South Africa. Later batches in premix form were obtained from Bioneer Corporation, Munpyeong-dong, Daejeon, South Korea. A 25/100 base pairs (bps) mixed DNA molecular weight

marker (Ladder), specifically designed for determining the size of double strand DNA from 25 to 2,000 bps, was used.

4.2.2.1 DNA extraction and storage

Leaf samples were picked from 14-day old plants raised in a screen house. Genomic DNA was extracted using the Cetyltrimethylammoniumbromide (CTAB) method adapted from Doyle and Doyle (1987). Fifty milligram samples of young leaf tissues were ground to a fine powder in liquid nitrogen. The powder was placed in labelled 1.5 mls Eppendorf microtubes. 500 μ l of 2% CTAB extraction buffer [20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2% CTAB, plus 0.4% beta-mercaptoethanol added just before use] was added to the microtubes containing the crushed leaf samples. The solution was incubated at 55°C for 45 min while gently mixing by inversion every 15 min. 500 μ l of chloroform-isoamylalcohol (24:1) was added to the tubes and gently mixed for one (1) minute. Samples were centrifuged for 10 minutes at 12,000 revolutions per minute (rpm). 600 μ l of the supernatant was then transferred to a fresh labelled tube following the addition of 500 mL chloroform-isoamylalcohol (24:1). This procedure was repeated twice. 500 mL of the supernatant was then transferred to a fresh tube with 700 μ l of cold isopropanol (-20°C). Samples were gently mixed by inversion and centrifuged at 12,000 rpm for 10 minutes making it possible to visualize the DNA adhered to the bottom of the tube. The liquid solution was then released and the DNA pellet washed with 700 μ l of 70% ethanol to eliminate salt residues adhered to the DNA. The DNA was left to dry for approximately 12 hours with the tubes inverted over a filter paper, at room temperature. The DNA pellet was then re-suspended in 100 μ l TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) plus 5 μ l ribonuclease (RNase 10 mg mL⁻¹) in each tube. The DNA solution was incubated at 37°C for 1h and thereafter stored at -20°C.

4.2.2.2 DNA amplification, gel electrophoresis and imaging

The reagents used for preparing the Polymerase Chain Reaction (PCR) master mix, their volumes and concentrations are presented in Table 4.3.

Table 4.3: Ingredients of a PCR master mix using PCR reagents and a Bioneer PCR premix

PCR Reagents	Reaction Volume (μ l)	Stock concentration	Final concentration
ddH ₂ O	10.6		
dNTPs	0.8	10mM	0.1mM
Primer 1	1.0	10mM	0.1 μ M
Primer 2	1.0	10mM	0.1 μ M
MgCl ₂	1.2	50mM	1.5mM
PCR Buffer	2.0	10X	1X
<i>Taq</i> Polymerase enzyme	0.4	5U/ μ l	0.2U
PCR Premix			
ddH ₂ O	17		
Primer 1	1.0	10mM	0.1 μ M
Primer 2	1.0	10mM	0.1 μ M

μ l = microliters; mM = millimolar; μ M = micromolar; X = stock solution concentration ranging from 1 to 10; Unit (U) = amount of enzyme that catalyzes the conversion of 1 micromole of substrate per minute.

To prepare a PCR master mix using PCR reagents, double distilled water (ddH₂O), deoxy nucleoside triphosphates (dNTPs including dATP, dGTP, dCTP and dTTP), forward and reverse primers, MgCl₂, PCR buffer and *Taq* polymerase enzyme were mixed based on the concentrations in Table 4.3 and the reaction volumes were multiplied by the number of samples to be amplified to get to total master mix volume. Nineteen microliters (19 μ l) of the master mix were pipetted into individual PCR tubes and 1.0 μ l of 40ng plant DNA were added to each tube to make a total PCR reaction volume of 20 μ l.

When the PCR Bioneer Premix (Bioneer Inc, Korea) was used instead of the PCR reagents, the PCR master mix was prepared by adding ddH₂O to a 1.5ml Eppendorf tube, followed by the forward and reverse primers according to the working concentrations in Table 4.3. The total master mix volume was derived by multiplying the individual reaction volumes of the ingredients by the number of samples to be amplified. Nineteen (19) microliters were pipetted into the PCR tubes containing the premix pellet, which is made up of 2mM MgCl₂, 0.2mM dNTPS, 0.2U Top DNA polymerase and a tracking dye. One microliter (1.0 μ l) of 40ng DNA was added to make a total reaction volume of 20 μ l. Amplification of DNA was carried out in a Bioneer Thermal cycler (Bioneer Inc, Korea) with an initial denaturation step at 95°C for 5 minutes and 35 cycles each of a denaturation step at 94°C for 20 seconds, an annealing step at 64°C (*SBB14*), 68°C (*SAS13*), 65°C (*SAB3*), 65°C (*SB12*), 64°C (*SH18*) and 63°C (*PYAA19₈₀₀*) for 40 seconds;

an extension step at 72°C for one (1) minute followed by a final extension for 10 minutes at 72°C. The amplicons were resolved on 1.5% agarose gels in 1X TBE (0.045 M Tris–borate and 1 mM EDTA, pH 8.2) at 100V for 90 minutes and stained with 0.5µg/ml ethidium bromide for 10 minutes. Gel images were captured using the SynGene G: BOX gel documentation system (Syngene, Frederick, MD, USA).

4.2.3 Development of populations

A cascading pedigree gene pyramiding scheme (Servin *et al.*, 2004), was used to develop populations with pyramided resistance genes against bean anthracnose (Figure 4.1). In this breeding scheme, only one cross was made at each generation beginning with two founding parents and followed by an intermediate genotype and one founding parent. The minimum population size to ensure at a predetermined probability of 95% or 99%, that at least one desired genotype is present in a population was derived from the equation; $N_q = \ln(1-q) / \ln(1-p)$ (Ye and Smith, 2008) where; N = minimum population size, p = number of individuals of the desirable genotype in a population of size N and q = predetermined probability. A root genotype is an intermediate genotype in which all the desired genes have been introgressed but still in heterozygous state (Servin *et al.*, 2004). After obtaining the root genotype, the fixation of these genes was achieved through selfing.

Pedigree breeding method was used to advance selected individuals that possessed the desired number and combination of resistance genes. Marker Assisted selection (MAS) was used to select individuals with the desired single and pyramided genes during the pedigree and fixation steps of the breeding scheme.

4.2.3.1 Pedigree steps of the pyramiding scheme

The gene pyramiding and marker assisted selection scheme used is illustrated in Figure 4.1. The donor parents for anthracnose resistance G2333 and PI207262 were crossed in a screen house to combine the target anthracnose resistance alleles *Co-4²/Co-4³*, *Co-5*, *Co-9* in F_{1a} plants (G2333 x PI207262). In order to improve background of the populations with resistance to Pythium root rot, the F_{1a} plants were crossed with RWR719 to produce F_{1b} plants [(G2333 x PI207262) x RWR719].

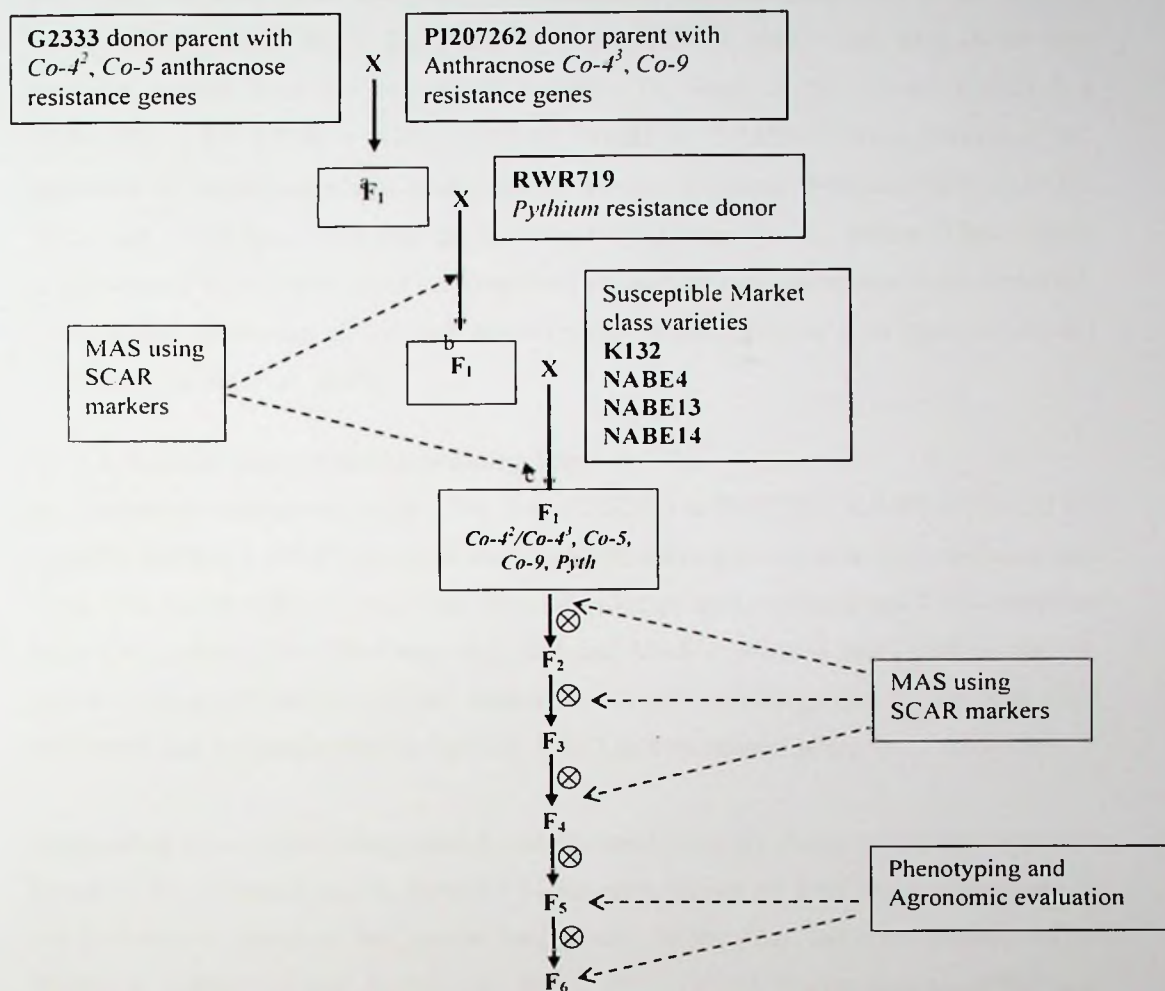


Figure 4.1: Cascading gene pyramiding scheme used to develop advanced common bean populations pyramided with anthracnose and *Pythium* resistance genes. G2333, PI207262, RWR719, K132, NABE4, NABE13 & NABE14 are Founding parents; F1a and F1b are Intermediate genotypes; F1c is the Root genotype

The F_{1b} seeds of the cross were planted as single potted plants along with varieties K132, NABE4, NABE13 and NABE14. The SCAR markers *SAS13*, *SBB14*, *SAB3* and *SB12* were run on extracted DNA of 105 F_{1b} plants to identify plants that possessed all the target genes before crossing with the susceptible varieties. DNA of 35 F_{1b} plants

positively amplified all the three desired anthracnose resistance genes (*Co-4²/Co-4³*, *Co-5* and *Co-9*). Six (6) of the 35 F_{1b} plants were crossed with plants from each of the four recurrent parents from the susceptible varieties. F_{1c} seeds of the crosses [(G2333 x PI207262) x RWR719) x K132, NABE4, NABE13, NABE14] were harvested and replanted as individual plants under screen house conditions. Markers *SBB14*, *SAB3*, *SB12* and *PYAA19₈₀₀* were run on DNA extracted from 46 F_{1c} plants. Three plants possessing all three anthracnose and one *Pythium* root rot resistance gene were identified. These plants possessing all the four desired genes in heterozygous state became the root genotype (Servin *et al.*, 2004).

4.2.3.2 Fixation steps of the pyramiding scheme

F_{1c} seed of the root genotype from the cross [(G2333 x PI207262) x RWR719) x K132, NABE4, NABE13, NABE14] were sowed and raised under screen house conditions and allowed to self-fertilize. F_2 seed from the root genotype was replanted and DNA extracted from 69 F_2 plants. The DNA was amplified and MAS conducted using *SBB14*, *SAS13*, *SAB3*, *SB12* and *PYAA19₈₀₀* SCAR markers to identify and categorize F_2 plants that did not inherit any resistance gene or that had 1, 2, 3 or 4 resistance genes.

Segregating plants were categorized based on number of resistance genes inherited and based on the recurrent parent. Selected plants were advanced with varying numbers of fixed resistance genes in the genetic background of the four recurrent parents K132, NABE 4, NABE 13 and NABE 14. At F_3 DNA of 156 plants was amplified and individual plants possessing different numbers of resistance genes were selfed to F_6 to obtain homozygosity.

4.2.4 Raising the bean populations for phenotypic screening

Advanced F_6 lines were used for phenotypic screening. Seed of selected F_6 lines were sown in five liter plastic pots filled with top soil mixed with sand and sterilized manure in the ratio of 5:3:2 respectively in the greenhouse. Diammonium Phosphate (DAP) fertilizer was applied prior to sowing and watering was done daily.

4.2.5 Phenotyping for anthracnose resistance among F₆ lines

4.2.5.1 F₆ lines used for phenotyping

The advanced lines were categorized into 10 groups based on number and specific resistance gene inherited

4.2.5.2 *C. lindemuthianum* races and inoculum preparation

Four (4) *C. lindemuthianum* races were cultured and inoculum prepared for phenotypic screening of the F₆ advanced populations. The choice of these four races was based on their robustness in growth and sporulation and their ability to show differences in infection on the susceptible and resistant controls. To confirm sporulation, *C. lindemuthianum* samples were prepared on slides and viewed under X10 and X40 magnification of a compound microscope. Photographs of *C. lindemuthianum* in sporulation were taken using Optikam Pro 3 microscope digital camera at X40 magnification.

Single-spore isolates were placed on fresh Mathur's agar medium in a Petri dish and incubated at 22-24°C for 7 days to allow the fungus enough time to produce conidial spores. Conidial spores were scrapped off the growth medium into a small amount of water to make a suspension. Using a hemocytometer the concentration was adjusted to 1.2×10^6 conidia ml⁻¹. 0.1% Tween 20 was added as a surfactant.

4.2.5.3 Inoculation and disease scoring

The detached leaf technique (Tu, 1986) for screening beans in vitro against anthracnose was used to differentiate the resistant and susceptible bean families. Leaf trifoliates were detached starting from 14 days after planting and inoculated by immersion in the inoculum. Individual plants from which trifoliates were picked were considered as replicates and three plants per genotype were used as replications. The experiment was designed in a Randomized Complete Block Design (RCBD). The inoculated leaf trifoliates were placed in transparent plastic containers with moistened paper towels and covered with transparent covers, which were tightly placed to allow for humidity build-

up. The containers were placed on wooden shelves fitted with Phillips^R TLT 18-20W/75RS Fluorescents tubes that supplied approximately $50\mu\text{moles m}^{-2}\text{s}^{-1}$ of light so as to enable prolonged physiological processes of the detached leaves up to between 14 to 28 days.

A light timer was connected to the fluorescent tubes to enable 12-hour day light and 12-hour night regime. Room temperatures were maintained, with the help of an air conditioner, between 22°C and 25°C, which is recommended for successful infection of *C. lindemuthianum* on the bean host (Awale *et al.*, 2007).

Disease symptoms were scored after a seven day incubation period using a modified 1 – 9 scale by Balardin *et al.* (1997) where: 1 = no symptoms (resistant), 2 – 3 = very small lesions mostly on primary leaves (resistant), 4 – 9 = numerous enlarged lesions or sunken cankers on the lower side of the leaves or hypocotyls (susceptible); and plants were categorized as either Resistant (R) or Susceptible (S).

4.2.6 Data analysis

Disease severity data were subjected to Analysis of Variance (ANOVA) using GenStat Discovery, 12th Edition (Anonymous, 2009). To determine whether pyramid group means were significantly different with respect to anthracnose resistance levels, a Tukey's Honest Significant Difference (HSD) test was carried out to test the null hypothesis H_0 ; all gene pyramid group means are equal; $H_0 : \mu_i = \mu_j$, $H_a : \mu_i \neq \mu_j$. Tukey Test statistic; $HSD = q\sqrt{MSE/n_c}$. Where; q = value from studentized range table, MSE = Mean Square for Error from ANOVA table, n_c = number of replicates per treatment.

Standard error of pyramid group means (SEM) was computed using the formula: $SEM = s/\sqrt{n}$, where; s = sample standard deviation and n = sample size. Sample standard deviation (s) was computed using the formula; $s = \sqrt{1/N-1 \sum_{i=1}^N (x_i - \bar{x})^2}$, where; $x_1, \dots, x_N$ = the sample data set, $\bar{x}$ = mean value of the data set, N = size of sample data set.

4.3 Results

4.3.1 Inheritance profile of resistance genes among F_{4,6} lines

Gene groups based on number of resistance genes inherited and number of F₆ common bean families under each group are presented in Table 4.4. Advanced F₆ families, pedigree, resistance genes inherited and respective molecular markers are presented in Table 4.5.

A total of 53 F_{4,5} and 69 F_{4,6} families with varying resistance gene profiles were obtained as a result of the cascading gene pyramiding scheme. Out of 69 F_{4,6} families, nine (9) families inherited all the three (3) target anthracnose genes (*Co-4*²/*Co-4*³, *Co-5* and *Co-9*), 17 families inherited two anthracnose resistance genes, 28 families inherited a single anthracnose resistance gene; while 12 families did not inherit any of the target genes (Table 4.5). Twenty seven (27) families inherited the *Co-4*² allele, eight (8) families inherited the *Co-4*³ allele, 33 families inherited the *Co-5* gene, while 21 families inherited the *Co-9* gene.

Table 4.4: Grouping of the F₆ advanced lines based on number and type of genes inherited

Group	Genes inherited	Number of resistance genes inherited	Number of families with similar inheritance profile
Group 1	<i>Co-4</i> ² + <i>Co-5</i> + <i>Co-9</i>	3	9
Group 2	<i>Co-4</i> ² + <i>Co-5</i>	2	8
Group 3	<i>Co-4</i> ² + <i>Co-9</i>	2	1
Group 4	<i>Co-4</i> ³ + <i>Co-9</i>	2	1
Group 5	<i>Co-5</i> + <i>Co-9</i>	2	7
Group 6	<i>Co-5</i>	1	9
Group 7	<i>Co-4</i> ²	1	11
Group 8	<i>Co-4</i> ³	1	5
Group 9	<i>Co-9</i>	1	3
Group 10 ^a	No gene	0	15
Total			69

^a Group 10 comprised of families that did not inherit any of the four anthracnose resistance gene s/ alleles and was designated as the 'No gene' group

Table 4.5: Sixty nine (69) F_{4:6} families with their profile of inherited resistance genes

Pedigree	Family codes	Markers and Genes screened				
		<i>SAS13</i>	<i>SBB14</i>	<i>SAB3</i>	<i>SB12</i>	<i>PYAA19₈₀₀</i>
		<i>Co-4³</i>	<i>Co-4²</i>	<i>Co-5</i>	<i>Co-9</i>	<i>Prr</i>
12x8xRWR719xK132	A44.5.7.1.1.1.1	-	-	-	+	-
12x8xRWR719xK132	A44.5.7.1.1.1.4	-	-	-	+	-
12x8xRWR719xK132	A44.5.7.1.1.1.7	-	-	-	+	-
12x8xRWR719xK132	A16.1.2.1.6.3.1	-	-	-	-	-
12x8xRWR719xK132	A16.1.2.1.6.3.2	-	-	-	-	-
12x8xRWR719xK132	A16.1.2.1.6.3.3	-	-	-	-	-
12x8xRWR719xK132	A 16.1.2.1.6.3.5	-	-	-	-	-
12x8xRWR719xK132	A 16.1.2.3.7.1.1	-	-	-	-	+
12x8xRWR719xK132	A 16.1.2.3.7.1.3	-	-	-	-	+
12x8xRWR719xK132	A 16.1.2.3.7.1.4	-	-	-	-	+
12x8xRWR719xK132	A 16.6.1.6.25.1.2	+	+	-	-	-
12x8xRWR719xK132	A 44.5.2.1.26.1.4	+	+	+	-	-
12x8xRWR719xK132	A 44.5.2.3.28.1.1	+	+	+	+	-
12x8xRWR719xK132	A 44.5.2.3.28.1.2	+	+	+	+	-
12x8xRWR719xK132	A 44.5.2.3.28.4.3	+	+	+	+	-
12x8xRWR719xK132	A 44.5.2.3.28.4.5	+	+	+	+	-
12x8xRWR719xK132	A 44.5.2.3.28.4.6	+	+	+	+	-
12x8xRWR719xK132	A 44.5.2.3.28.4.8	+	+	+	+	-
12x8xRWR719xNABE4	B44.7.9.8.102.1.1	+	+	+	+	-
12x8xRWR719xNABE4	B44.7.5.8.112.1.2	-	-	-	-	-
12x8xRWR719xNABE4	B44.7.5.8.112.1.3	-	-	-	-	-
12x8xRWR719xNABE4	B44.7.5.8.112.1.4	-	-	-	-	-
12x8xRWR719xNABE4	B44.7.5.8.112.2.1	-	-	-	-	-
12x8xRWR719xNABE4	B89.5.2.7.117.1.1	+	+	-	+	-
12x8xRWR719xNABE4	B89.5.2.10.118.15.2	+	-	-	+	-
12x8xRWR719xNABE4	B44.7.2.2.76.1.1	+	+	+	+	+
12x8xRWR719xNABE4	B44.7.2.2.76.4.1	+	+	+	+	+
12x8xRWR719xNABE4	B89.5.1.1.81.1.1	+	+	-	-	+
12x8xRWR719xNABE4	B89.5.1.1.81.2.1	+	+	-	-	+
12x8xRWR719xNABE4	B44.7.8.2.91.10.1	+	+	-	-	-
12x8xRWR719xNABE4	B44.7.8.2.91.10.3	+	+	-	-	-
12x8xRWR719xNABE4	B44.7.8.2.91.10.4	+	+	-	-	-
12x8xRWR719xNABE4	B44.7.8.2.91.11.2	+	+	-	-	-
12x8xRWR719xNABE4	B44.7.8.2.91.11.3	+	+	-	-	-
12x8xRWR719xNABE4	B44.7.9.5.92.2.3.4.6	+	+	-	-	-
12x8xRWR719xNABE4	B44.7.8.3.92.3.2	+	+	-	-	-
12x8xRWR719xNABE4	B44.7.8.3.92.3.5	+	+	-	-	-
12x8xRWR719xNABE13	C44.1.6.7.130.1.1	-	-	-	-	-

12x8xRWR719xNABE13	C44.1.6.7.130.3.2	-	-	-	-	-
12x8xRWR719xNABE13	C16.1.3.8.136.1.4	+	-	-	-	+
12x8xRWR719xNABE13	C16.1.3.8.136.2.5	+	-	-	-	+
12x8xRWR719xNABE13	C16.1.3.8.136.2.7	+	-	-	-	+
12x8xRWR719xNABE13	C16.1.3.8.136.2.8	+	-	-	-	+
12x8xRWR719xNABE13	C16.1.3.8.136.2.9	+	-	-	-	+
12x8xRWR719xNABE13	C44.1.4.3.141.1.2	-	-	+	+	+
12x8xRWR719xNABE13	C44.1.4.3.141.1.3	-	-	+	+	+
12x8xRWR719xNABE13	C44.1.4.3.141.1.4	-	-	+	+	+
12x8xRWR719xNABE13	C44.1.4.3.141.2.1	-	-	+	+	+
12x8xRWR719xNABE13	C44.1.4.5.142.3.6	-	-	+	-	+
12x8xRWR719xNABE13	C44.1.4.5.142.4.2	-	-	+	-	+
12x8xRWR719xNABE13	C44.1.4.5.142.4.4	-	-	+	-	+
12x8xRWR719xNABE13	C44.1.4.5.142.4.6	-	-	+	-	+
12x8xRWR719xNABE13	C44.1.4.5.142.4.7	-	-	+	-	+
12x8xRWR719xNABE13	C44.1.4.7.143.1.4	-	-	+	-	-
12x8xRWR719xNABE13	C44.1.4.7.143.1.8	-	-	+	-	-
12x8xRWR719xNABE14	D16.3.3.1.151.1.1	-	-	+	-	+
12x8xRWR719xNABE14	D16.3.3.1.151.1.2	-	-	+	-	+
12x8xRWR719xNABE14	D16.3.3.2.152.1.2.1	-	-	-	-	-
12x8xRWR719xNABE14	D16.3.3.2.152.1.2.2	-	-	-	-	-
12x8xRWR719xNABE14	D16.3.3.8.157.3.3	+	+	+	-	+
12x8xRWR719xNABE14	D16.3.3.9.158.1.2	+	-	+	-	+
12x8xRWR719xNABE14	D16.3.3.9.158.1.3	+	-	+	-	+
12x8xRWR719xNABE14	D16.3.3.10.159.6.2	+	+	+	-	+
12x8xRWR719xNABE14	D16.3.3.11.160.2.1	+	+	+	-	+
12x8xRWR719xNABE14	D16.3.3.11.160.2.2	+	+	+	-	+
12x8xRWR719xNABE14	D16.3.3.11.160.2.3	+	+	+	-	+
12x8xRWR719xNABE14	D16.3.3.11.161.1	-	-	+	+	+
12x8xRWR719xNABE14	D16.3.3.11.161.3	-	-	+	+	+
12x8xRWR719xNABE14	D16.3.3.11.161.4	-	-	+	+	+

12 = Donor parent G2333

8 = Donor parent PI 207262

+ = presence of band on gel

- = absence of band on gel

A = pedigree 12x8xRWR719xK132

B = pedigree 12x8xRWR719xNABE4

C = pedigree 12x8xRWR719xNABE13

D = pedigree 12x8xRWR719xNABE14

4.3.2 Specific molecular marker analysis and polymorphism

Gel photographs of the four dominant SCAR markers *SAS13*, *SAB3*, *SB12*, *PYAA19₈₀₀* and one codominant marker *SBB14* are presented on Plate 4.2.

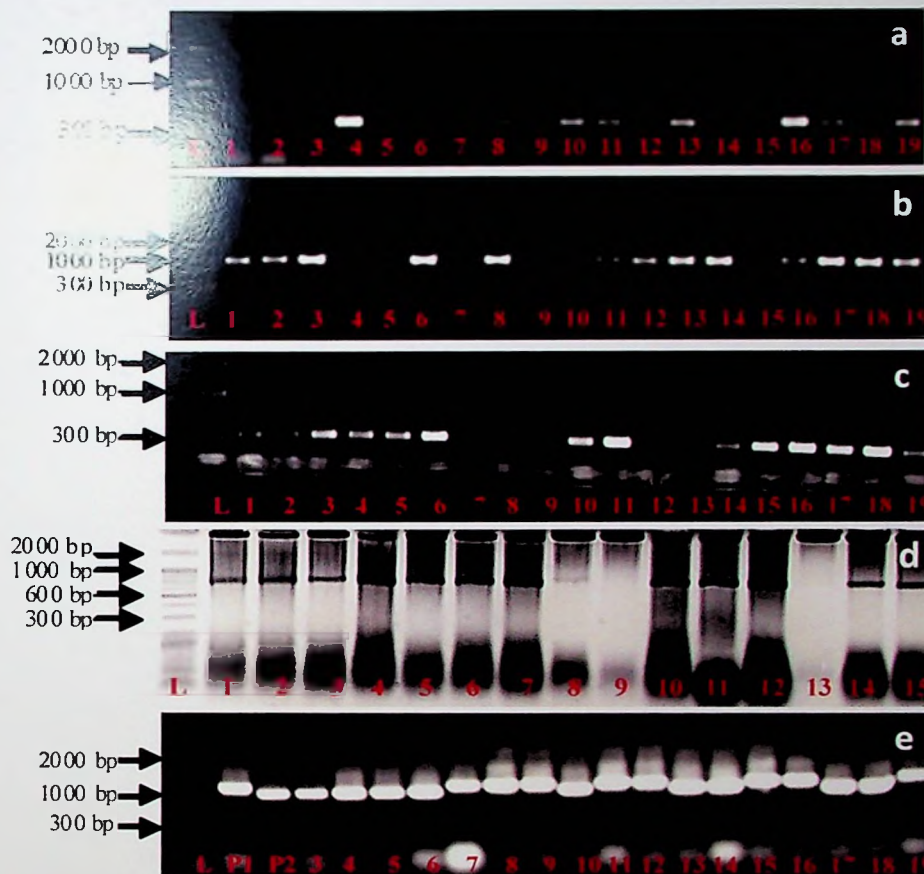


Plate 4.2: Gel photos showing banding patterns of DNA fragments amplified with the different SCAR markers;

a = DNA fragment at 400bps amplified with *SAB3* marker linked to *Co-5* gene

b = DNA fragment at 950bps amplified with *SAS13* marker linked to *Co-4* locus

c = DNA fragment at 350bps amplified with *SB12* marker linked to *Co-9* gene

d = DNA fragment at 800bps amplified with *PYAA19₈₀₀* marker linked to *Prr* gene

e = Two DNA fragments at 1150bps for upper fragment and 1050bps for lower fragment amplified with *SBB14* codominant marker linked to allele *Co-4²*

L = Lane for 2000bps molecular weight marker ladder

P1 = Donor parent; **P2** = Susceptible parent; **1 – 19** = Lanes for segregating lines

The markers were polymorphic between the resistant and susceptible parents and were successfully used to distinguish genotypes where the target gene was present from those where the gene was absent (Plate 4.2). A total of 1,704 PCR reactions were performed with the five markers in four MAS cycles out of which 893 were positive reactions. *SAS13* was the most frequent marker with 64.2% of 372 reactions followed by *SB12* (58.6% of 372 reactions), *SBB14* (47.0% of 317 reactions), *PYAA19₈₀₀* (45.4% of 271 reactions) and *SAB3* (44.1% of 372 reactions). Molecular data for SCAR marker *SB12* revealed presence of *Co-9* anthracnose resistance gene in parent RWR719.

4.3.3 Phenotypic evaluation of F₆ lines for resistance to bean anthracnose disease

4.3.3.1 *C. lindemuthianum* Isolates used

The four *C. lindemuthianum* races used for phenotypic evaluation of F₆ families are shown on plates 4.3A and 4.3B. These races were robust in growth and sporulation and showed clear morphological differences on growth media and under microscope.

4.3.3.2 Evaluation of families with pyramided genes

The reactions of 36 advanced F_{4:6} families and seven (7) parental cultivars to four *C. lindemuthianum* races and four SCAR markers are presented in Table 4.6. A pictorial depiction of reaction of advanced bean lines with single and pyramided resistance genes to *C. lindemuthianum* is shown on Plates 4.4A and 4.4B. Five (5) families with three (3) pyramided resistance genes *Co-4²+Co-5+Co-9* were resistant to all the four races. Three (3) out of four (4) families possessing *Co-4²+Co-5* gene pyramid were also resistant to all four (4) races, while two families with the same pyramid succumbed to the aggressive races 2047 and 767. Four (4) out of five (5) families possessing single gene *Co-4²* were resistant to all the four races, while families with only *Co-4³* allele succumbed to races 2047 and 767 (Plate 4.3B). The two families that possessed single gene *Co-9* and the family that possessed *Co-4³+Co-9* pyramid succumbed to three aggressive races 2047, 713 and 767 and showed resistance to only race 352 (Plate 4.3A). The donor parents PI207262 possessing the *Co-4³+Co-9* pyramid was only resistant to race 352, while the donor parent G2333 possessing *Co-4²+Co-5+Co-7* pyramid was resistant to all four races. All six families that did not inherit any resistance gene for anthracnose were

susceptible to all four races. Three of four families possessing *Co-5* single gene were resistant to all the four races.

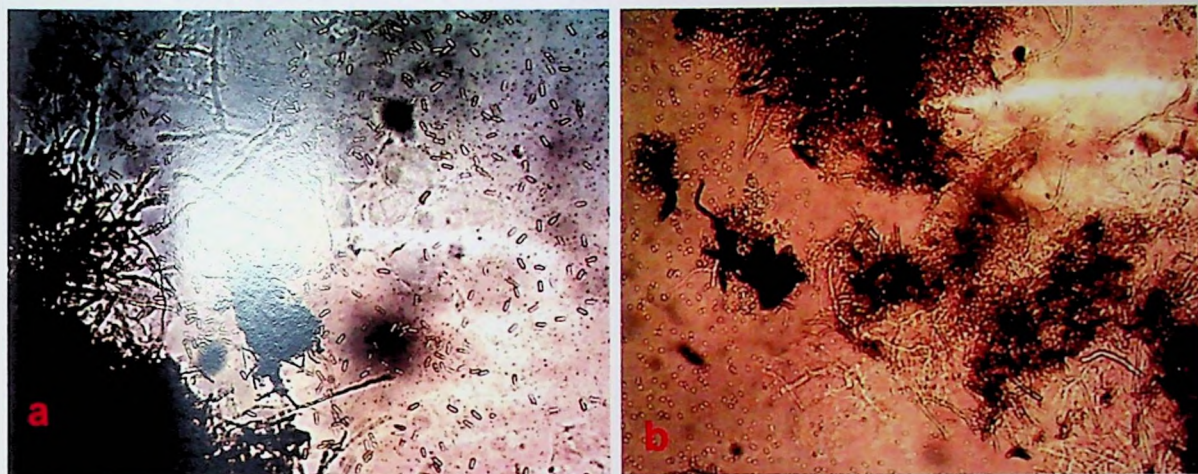


Plate 4.3A: Conidia of *C. lindemuthianum* in culture; a = Race 352; b = Race 713

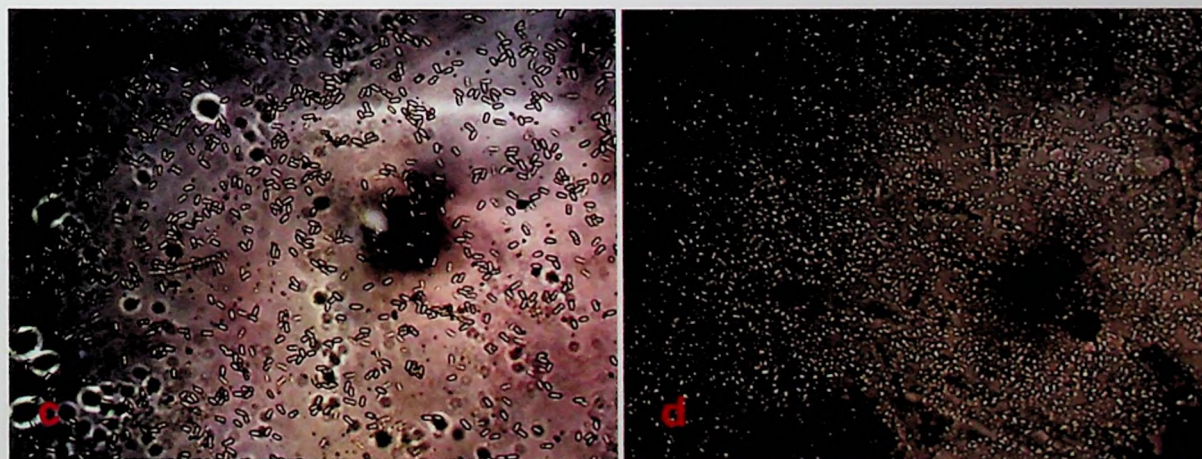


Plate 4.3B: Conidia of *C. lindemuthianum* in culture; c = Race 767; d = Race 2047

Table 4.6: Reaction of F₆ advanced lines and parents to four races of *C. lindemuthianum* and four SCAR markers

Family code	Phenotypic data				Genotypic data			
	<i>C. lindemuthianum</i> races				Molecular markers			
	2047	713	767	352	SAS13	SAB3	SB12	PYAA19 ₈₀₀
A44.5.7.1.1.1.1	S	S	S	R	0	0	1	0
A16.1.2.1.6.3.3	S	S	S	S	0	0	0	0
A16.1.2.1.6.3.5	S	S	S	S	0	0	0	0
A16.1.2.3.7.1.4	S	S	S	S	0	0	0	1
A16.6.1.6.25.1.2	S	R	R	R	1	0	0	0
A44.5.2.1.26.1.4	S	S	S	S	0	0	0	0
A44.5.2.3.28.1.1	S	R	S	R	0	1	1	0
A44.5.2.3.28.1.2	S	R	S	R	0	1	1	0
A44.5.2.3.28.4.5	R	R	R	R	1	1	1	0
A44.5.2.3.28.4.8	R	R	R	R	1	1	1	0
B44.7.2.2.76.1.1	R	R	R	R	1	1	1	1
B44.7.2.2.76.4.1	R	R	R	R	1	1	1	1
B89.5.1.1.81.1.1	R	R	R	R	1	0	0	1
B44.7.8.2.91.10.3	R	R	R	R	1	0	0	0
B44.7.8.2.91.10.4	R	R	R	R	1	0	0	0
B44.7.9.5.92.2.3.4.6	R	R	R	R	1	0	0	0
B44.7.9.8.102.1.1	R	R	R	R	1	1	1	0
B44.7.5.8.112.1.3	S	S	S	S	0	0	0	0
B89.5.2.7.117.1.1	S	R	R	R	1	0	1	0
B89.5.2.10.118.15.3	S	S	S	R	1*	0	1	0
C44.1.6.7.130.1.1	S	S	S	S	0	0	0	0
C16.1.3.8.136.1.4	R	R	S	R	1*	0	0	1
C16.1.3.8.136.2.7	S	R	S	R	1*	0	0	1
C16.1.3.8.136.2.8	S	R	S	R	1*	0	0	1
C44.1.4.3.141.1.2	R	R	R	R	0	1	1	1
C44.1.4.3.141.1.4	R	R	R	R	0	1	1	1
C44.1.4.5.142.4.2	R	R	R	R	0	1	0	1
C44.1.4.5.143.1.4	R	R	R	R	0	1	0	0
D16.3.3.1.151.1.1	S	S	S	S	0	1	0	1
D16.3.3.1.151.1.2	R	R	R	R	0	1	0	1
D16.3.3.2.152.1.2.2	S	S	S	S	0	0	0	0
D16.3.3.9.158.1.2	R	R	R	R	1	1	0	1
D16.3.3.9.158.1.3	R	R	R	R	1	1	0	1
D16.3.3.11.160.2.2	S	R	R	R	1	1	0	1
D16.3.3.11.160.2.3	R	R	R	R	1	1	0	1
D42.12.4.1.161.4	S	S	S	R	0	0	1	1
Parents								
PI207262	S	S	S	R	1*	0	1	0
Nabe 4	S	S	S	S	0	0	0	0
RWR719	S	S	S	S	0	0	0	1
G2333	R	R	R	R	1	1	0	0
K132	S	S	S	S	0	0	0	0
Nabe 13	S	S	S	S	0	0	0	1
Nabe 14	S	S	S	S	0	0	0	1

* *Co-4*³ allele; R = resistant reaction, S = Susceptible reaction; 1 = marker present, 0 = marker absent; SAS13 marker tags *Co-4* locus (both *Co-4*² and *Co-4*³ alleles), SAB 3 marker tags *Co-5* gene, SB12 marker tags *Co-9* gene and PYAA19₈₀₀ marker tags *Pythium* resistance gene. A = 12x8xRWR719XK132; B = 12x8xRWR719xNABE4; C = 12x8xRWR719xNABE13; D = 12x8xRWR719xNABE14

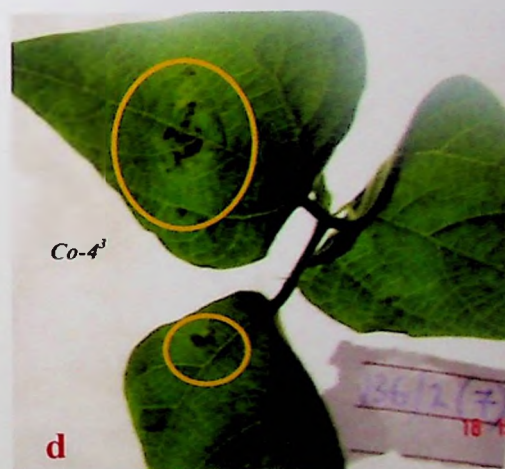
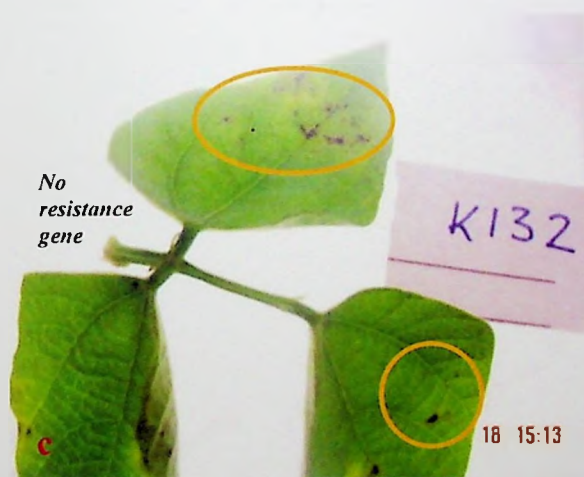
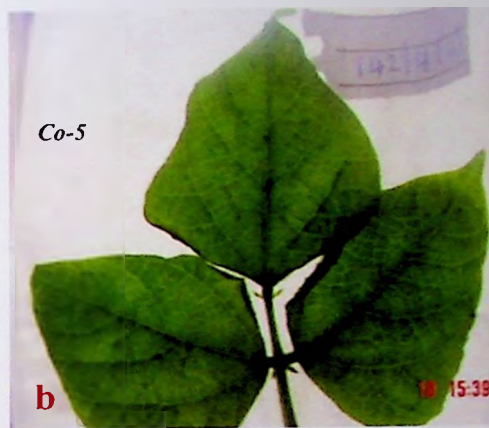


Plate 4.4A: Leaf trifoliates of three F₆ cultivars with single resistance genes in comparison with one susceptible cultivar, seven days post inoculation; **a** = F₆ cultivar with *Co-4*² single gene showing no symptoms; **b** = F₆ cultivar with *Co-5* single gene showing no symptoms; **c** = Susceptible commercial cultivar showing symptoms; **d** = F₆ cultivar with *Co-4*¹ single gene showing mild symptoms;

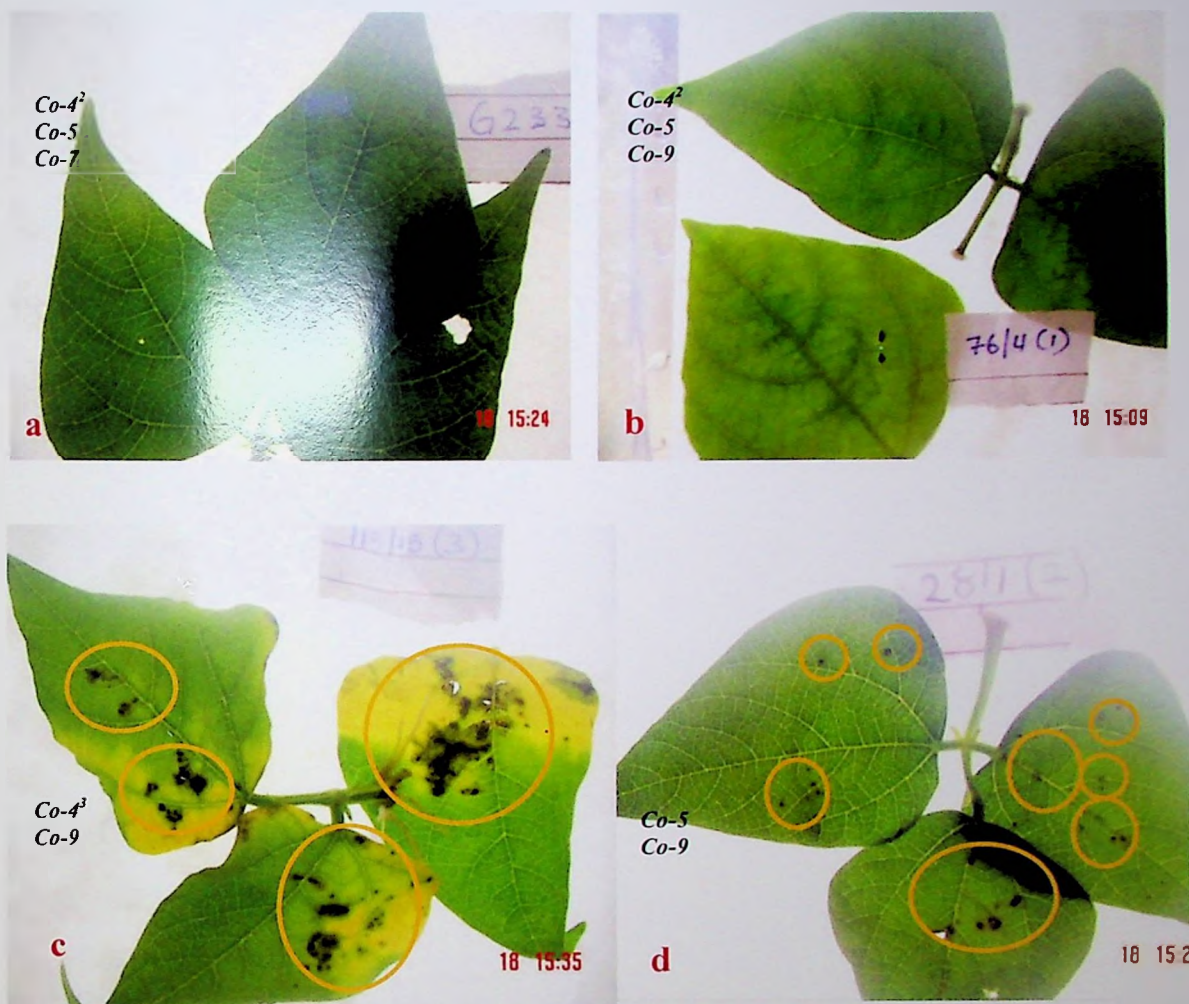


Plate 4.4B: Leaf trifoliates of three F_6 cultivars with pyramided resistance genes in comparison with a known resistant cultivar seven days post inoculation; **a** = resistant donor parent G2333 with no symptoms; **b** = cultivar with a three-gene pyramid *Co-4²+Co-5+Co-9* showing no symptoms; **c** = cultivar with a two-gene pyramid *Co-4³+Co-9* showing severe symptoms; **d** = cultivar with a two-gene pyramid *Co-5+Co-9* showing mild symptoms

4.3.4 Effectiveness of single and pyramided genes in conferring broad resistance to diverse *C. lindemuthianum* races

Analysis of variance for anthracnose disease severity is presented in Table 4.7 and severity scores of cultivars in the different gene groups inoculated with diverse *C. lindemuthianum* races are presented in Table 4.8.

Table 4.7: Analysis of variance for severity of four races on different Gene-groups

Source of variation ^a	d.f.	S.S	M.S	%Contribution	F pr.
Rep	2	0.175	0.087	0.2	
Gene-groups	9	276.121	30.680*	52.6	<.001
Race	3	76.547	25.516*	43.7	<.001
Gene-group x Race	27	53.581	1.985*	3.4	<.001
Residual	78	9.060	0.116	0.2	

^a Source of variation; d.f = degree of freedom; S.S = sum of squares; M.S = mean square; F pr = Probability value associated with the F-statistic. *Highly significance ($P < 0.01$)

Table 4.8: Mean severity scores of cultivars in the different gene groups inoculated with diverse *C. lindemuthianum* races

Gene group	Gene combination	Races				Overall Group mean
		352	713	767	2047	
1	<i>Co-4</i> ² + <i>Co-5</i> + <i>Co-9</i>	1.0±0.00	1.1±0.07	1.1±0.07	1.1±0.07	1.1±0.03
2	<i>Co-4</i> ² + <i>Co-5</i>	1.1±0.13	2.2±0.12	1.9±0.18	3.1±0.24	2.1±0.41
3	<i>Co-4</i> ² + <i>Co-9</i>	1.0±0.00	2.0±0.00	2.0±0.00	4.3±0.33	2.3±0.69
4	<i>Co-4</i> ³ + <i>Co-9</i>	1.5±0.29	5.8±0.17	6.3±0.44	7.0±0.29	5.2±1.24
5	<i>Co-5</i> + <i>Co-9</i>	1.4±0.07	2.3±0.07	3.3±0.29	4.3±0.29	2.8±0.63
Mean		1.2±0.10	2.7±0.81	2.9±0.92	4.00±0.96	2.7±0.68
6	<i>Co-5</i>	2.0±0.00	2.5±0.23	2.8±0.07	3.0±0.27	2.6±0.22
7	<i>Co-4</i> ²	1.0±0.00	1.4±0.12	1.2±0.12	2.4±0.12	1.5±0.31
8	<i>Co-4</i> ³	1.9±0.10	3.3±0.49	4.3±0.38	4.2±0.11	3.4±0.56
9	<i>Co-9</i>	2.3±0.17	5.2±0.17	5.5±0.00	4.8±0.17	4.5±0.73
Mean		1.8±0.28	3.1±0.80	3.5±0.93	3.6±0.55	3.0±0.63
10	No-gene group	5.4±0.07	5.9±0.07	6.3±0.12	6.3±0.09	5.9±0.21
Overall Mean for Races		1.9±0.42	3.2±0.57	3.5±0.64	4.1±0.56	3.1±0.50

Lsd (0.05) = 0.55, S.e.d = 0.26, C.V = 7.8%

Gene-groups, races and interaction between Gene-groups and races were all highly significant ($P < 0.01$) with Gene-groups contributing highest (52.6%) to the total variation followed by Races (43.7%) and Gene-group by Race interaction (3.4%).

Out of the four *C. lindemuthianum* races, race 2047 had the highest overall mean score (4.1 ± 0.56) followed by races 767 (3.5 ± 0.64), 713 (3.2 ± 0.57) and 352 (1.9 ± 0.42). The *no-gene* group had the highest mean score (5.9 ± 0.21) across the four races, followed by the single-gene groups (3.0 ± 0.63), while the pyramid gene groups had the lowest mean score (2.7 ± 0.68) across the four races. However, the mean scores 3.0 ± 0.63 and 2.7 ± 0.68 for pyramid and single -gene groups respectively were not significantly different from each other.

There were five pyramid gene groups with two and three resistance genes which were inoculated with the four races of *C. lindemuthianum* (Table 4.8). The three-gene pyramid group $Co-4^2 + Co-5 + Co-9$ had the lowest severity score of all pyramid groups. This was evident in the amount of leaf symptoms expressed on leaves of plants in two-gene pyramid groups, such as $Co-4^2 + Co-5$ and $Co-5 + Co-9$, in comparison with those with the three-gene pyramid. Among the two-gene pyramid groups, the $Co-4^3 + Co-9$ pyramid group had the highest overall mean severity score (5.2 ± 1.24) (Plate 4.3A) across the four races and it succumbed to all races except race 352 which was the least aggressive. It was followed by $Co-5 + Co-9$ (2.8 ± 0.63) and $Co-4^2 + Co-9$ (2.3 ± 0.69), both of which succumbed to race 2047. The pyramid group $Co-4^2 + Co-5$ had the lowest severity score and did not succumb to any race (≥ 3.1).

4.3.5 Pairwise comparison of severity of *C. lindemuthianum* between gene groups

Pair-wise comparisons of group means are presented in Table 4.9. The mean severity score 1.1 ± 0.03 for the three-gene pyramid $Co-4^2 + Co-5 + Co-9$ was significantly lower ($P < 0.01$) than all the scores of the two-gene pyramid groups. The mean score 2.1 ± 0.41 for $Co-4^2 + Co-5$ pyramid group was significantly lower ($P < 0.01$) than scores 2.8 ± 0.63 and 5.2 ± 1.24 for the $Co-5 + Co-9$ and $Co-4^3 + Co-9$ pyramid groups respectively, but was not significantly different from score 2.3 ± 0.69 for $Co-4^2 + Co-9$ pyramid group.

The mean scores 2.8 ± 0.63 ($Co-5+Co-9$) and 5.2 ± 1.24 ($Co-4^3+Co-9$) were significantly different from each other. The mean score 2.3 ± 0.69 for the $Co-4^2+Co-9$ pyramid group was significantly lower than score 5.2 ± 1.24 ($Co-4^3+Co-9$) but was not significantly different ($P < 0.01$) from 2.8 ± 0.63 ($Co-5+Co-9$).

Table 4.9: Pair-wise comparison of mean severity scores of the different gene groups

Gene group	Means	Co-4 ² + Co-5 ÷ Co-9	Co-4 ² +Co-5	Co-4 ² +Co-9	Co-4 ³ +Co-9	Co-5 5 ÷ Co-9	Co-5	Co-4 ²	Co-4 ³	Co-9	No-gene
Co-4 ² + Co-5 5 ÷ Co-9	1.1										
Co-4 ² +Co-5	2.1	P<0.01									
Co-4 ² +Co-9	2.3	P<0.01	n/s								
Co-4 ³ +Co-9	5.2	P<0.01	P<0.01	P<0.01							
Co-5 5 ÷ Co-9	2.8	P<0.01	P<0.05	n/s	P<0.01						
Co-5	2.6	P<0.01	n/s.	n/s	P<0.01	n/s					
Co-4 ²	1.5	n/s	n/s	P<0.01	P<0.01	P<0.01	P<0.01				
Co-4 ³	3.4	P<0.01	P<0.01	P<0.01	P<0.01	n/s	P<0.01	P<0.01	P<0.01		
Co-9	4.5	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	
No-gene	5.9	P<0.01	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Critical values HSD _{0.05} = 0.63, HSD _{0.01} = 0.78, n/s = not significant											

Critical values $HSD_{0.05} = 0.63$, $HSD_{0.01} = 0.78$, n/s = not significant

There were four single-gene groups namely $Co-4^2$, $Co-4^3$, $Co-5$, $Co-9$ and one no-gene group. The $Co-4^2$ gene group had the lowest severity score (1.5 ± 0.31) followed by $Co-5$ (2.6 ± 0.22), $Co-4^3$ (3.4 ± 0.56) and $Co-9$ (4.5 ± 0.73) gene groups. The no-gene group had the highest severity score (5.9 ± 0.21). Overall severity scores of single-gene groups were significantly different ($P < 0.01$) from each other and from the no-gene group. The mean severity score of $Co-9$ gene group (4.5 ± 0.73) was significantly higher than mean scores of all the single-gene groups and was the only group with severity score falling in the susceptible range (4.0 – 9.0).

The mean severity score of the pyramid groups was 2.7 ± 0.68 while the mean severity score of the single-gene groups was 3.0 ± 0.63 (Table 4.8). These two means were not significantly

different from each other although both were significantly different from the 5.9 ± 0.21 mean score of the *No-gene* group ($P < 0.01$, $HSD_{0.05} = 0.58$, $HSD_{0.01} = 0.72$).

The mean score, 1.5 ± 0.31 , of the *Co-4²* single-gene group was not significantly different from the mean scores, 1.1 ± 0.03 and 2.1 ± 0.41 , of the *Co-4²+Co-5+Co-9* and *Co-4²+Co-5* pyramid groups respectively, but was significantly lower than the mean scores of the *Co-4²+Co-9* (2.3 ± 0.69), *Co-4³+Co-5* (5.2 ± 1.24) and *Co-5+Co-9* (2.8 ± 0.63) pyramid groups.

The mean score, 2.6 ± 0.22 , of the *Co-5* single-gene group was not significantly different from mean scores 2.1 ± 0.41 , 2.3 ± 0.69 and 2.8 ± 0.63 of the *Co-4²+Co-5*, *Co-4²+Co-9* and *Co-5+Co-9* pyramid groups respectively, but was significantly higher than the score 1.1 ± 0.03 for the three-gene pyramid *Co-4²+Co-5+Co-9* ($P < 0.01$, $HSD_{0.05} = 0.63$, $HSD_{0.01} = 0.78$).

The mean score 3.4 ± 0.56 of the *Co-4³* single-gene group was not significantly different from 2.8 ± 0.63 for *Co-5+Co-9* the pyramid but was significantly less than 5.2 ± 1.24 for the *Co-4³+Co-9* pyramid group. The mean score 4.5 ± 0.73 of the *Co-9* gene group was significantly higher than scores for all the pyramid gene groups.

4.4 Discussion

4.4.1 Gene pyramiding and development of advanced populations

Developing elite breeding populations and varieties requires plant breeders to combine desirable traits from multiple parents in gene pyramiding, which can be accelerated by the use of molecular markers. In this study four parents (K132, NABE4 NABE13 and NABE14) susceptible to anthracnose were crossed with G2333, PI207262 and RWR719 in a three-way cross cascading gene pyramiding scheme. The SCAR markers *SAS13*, *SH18*, *SBB14*, *SAB3* and *SB13* were used for selection of anthracnose resistance genes. SCAR marker *PYAA19₈₀₀* was used to track the *Pythium* resistance gene for background improvement against *Pythium* root rot disease among the populations.

4.4.2 Marker analysis and polymorphism during gene pyramiding

Frequency of appearance of a marker in a population increased as the linkage distance of the marker from the target gene decreased. Among the four markers, *SAB3* was reported by Campa *et al.* (2005) to be the most distant marker from the gene of interest at 14.4cM, followed by *SBB14* linked to the *Co-4*² allele at 5.89cM (Awale and Kelly, 2001). *SB12* was reported to be linked to the *Co-9* gene at a distance of 2.9cM (Mendez de Vigo *et al.*, 2002) while *SAS13* was reported to be the most tightly linked to the *Co-4* locus at 0.01cM (Young *et al.*, 1998). According to Byrne and Richardson (2005), 1cM of genetic distance is approximately equal to 1% recombination and hence, as the distance between the marker and the gene of interest increases, there is a greater chance of recombination between gene and marker. This implies that, in this study, the chances of recombination leading to false positives were highest with *SAB3* and least with *SAS13*.

The donor parents G2333 and PI207262 possess different alleles *Co-4*² and *Co-4*³ at locus *Co-4*. The two alleles were both detected by the *SAS13* marker without differentiation because the marker amplifies consensus sequences common to all alleles at the locus. The co-dominant marker *SBB14* specifically amplified the *Co-4*² allele which agrees with the findings of Awale and Kelly (2001), while no marker was available to specifically amplify the *Co-4*³ allele. This challenge was overcome by running both *SAS13* and *SBB14* markers on the same samples, which made it possible to differentiate between genotypes possessing *Co-4*² allele and those possessing *Co-4*³ allele.

4.4.3 Effectiveness of single and pyramided genes in conferring broad resistance to bean anthracnose

Analysis of variance (ANOVA) revealed high significance among Gene-groups (genotypes) and Races ($P < 0.01$) implying that number of genes/ gene combinations and *C. lindemuthianum* races significantly affected disease severity and are therefore of relative importance in the development and progress of the bean anthracnose disease. The interaction 'Gene-group x Race' was highly significant indicating that the effect of *C. lindemuthianum* races on disease severity highly depended on number and combination of resistance genes in the bean cultivars. This finding is in agreement with Davide and Souza (2009) who also reported specific genotype x race interactions to be an indicator of vertical or major gene resistance.

The races 2047 and 767 were the most aggressive and resulted in the highest disease scores on cultivars. They both caused high host susceptibility on cultivars possessing the $Co-4^3+Co-9$ and moderate susceptibility on cultivars possessing $Co-4^2+Co-9$ and $Co-5+Co-9$ pyramids and $Co-4^3$ and $Co-9$ single genes. This implies that these gene pyramids and single genes do not effectively confer resistance to the two races. Darben *et al.* (2017) reported race 2047 to be one of the most aggressive *C. lindemuthianum* races in Brazil that can overcome anthracnose resistance conferred by seven resistance genes $Co-1$, $Co-2$, $Co-3$, $Co-4$, $Co-5$, $Co-6$ and $Co-11$; and five alleles namely $Co-1^2$, $Co-1^3$, $Co-1^5$, $Co-3^3$ and $Co-4^3$. Nkalubo (2006) found race 767 to be the most widespread out of eight identified studies in Uganda

The race 713 caused moderate symptoms on cultivars possessing $Co-4^3+Co-9$ pyramid and $Co-9$ single gene implying that the pyramid combination and the single gene $Co-9$ were not effective against it. The race 352 did not cause symptoms on any cultivar apart from cultivars with no resistance gene suggesting that all the single genes and the different pyramid combinations were effective against it.

The single genes $Co-4^2$ and $Co-5$ conferred resistance to all the four races 352, 713, 767 and 2047. The single gene $Co-4^3$ conferred resistance to races 352 and 713 but was overcome by races 767 and 2047. The $Co-9$ group conferred resistance to only race 352 while the *No-gene* group was overcome by all the four races. These results reveal that $Co-4^2$ gene was the most effective single gene in conferring broad resistance to bean anthracnose, followed by $Co-5$ and $Co-4^3$ genes.

Results showed that the single gene $Co-4^2$ was as effective as the best pyramid combinations $Co-4^2+Co-5$ and $Co-4^2+Co-5+Co-9$ pyramids and the single gene $Co-5$ was as effective as pyramid combinations $Co-4^2+Co-5$ and $Co-4^2+Co-9$. This implies that the two single genes possess factors that promote broad-spectrum resistance and that these factors are higher in $Co-4^2$ gene. These two genes would confer effective and broad-spectrum resistance in single deployment against a diverse *C. lindemuthianum* population.

The *Co-4*² gene is recognised as the broadest based resistance genes against *C. lindemuthianum* in common beans (Young and Kelly, 1996; Balardin and Kelly, 1998; Awale and Kelly, 2001; Silverio *et al.*, 2002; Kelly and Vallejo, 2004). It is located at the *Co-4* locus which has control of up to 97% of all currently identified races of *C. lindemuthianum* (Melotto *et al.*, 2000). Young *et al.* (1998) reported the *Co-4* locus to be a complex gene family with three anthracnose resistance alleles residing at this locus namely *Co-4*² in cultivars G2333 and SEL 1308, *Co-4* allele in cultivar TO (Young *et al.*, 1998) and *Co-4*³ in cultivar PI207262 (Alzarte-Marin *et al.*, 2007). Melotto and Kelly (2001) fine mapped the complex *Co-4* locus to reveal an open reading frame designated as *COK-4* that encodes for protein kinase and was strongly associated with anthracnose resistance. The predicted amino-acid sequence of *COK-4* had a high degree of similarity with expressed sequences generated by resistance genes in other crops such as *Pto* in *Lycopersicon pimpinellifolium* and *L. esculentum*; and *Xa21* in *O. Sativa*. Further genetic analysis by Melotto and Kelly (2001) revealed that other genes may be tightly clustered with *Co-4*², although segregation data indicated a single gene. These tightly clustered genes could be responsible for the broad spectrum resistance associated with the *Co-4* gene.

In this study, the *Co-4*³ allele showed a mildly susceptible reaction to races 767 and 2047 with mean scores of 4.3 ± 0.38 and 4.2 ± 0.11 respectively. Its overall mean score of 3.4 ± 0.56 across all four races, however, was still within the resistance range implying that it is still a highly beneficial allele to specific races and has the potential to add value if combined with other compatible single genes in pyramid. The *Co-4*³ allele was, however, reported to possess a narrower anthracnose resistance spectrum than the *Co-4* allele in cultivar TO and the *Co-4*² allele in cultivar G2333 (Alzarte-Marin *et al.*, 2007). Balardin and Kelly (1998) also alluded to the fact that certain anthracnose resistance genes were more effective than others in controlling multiple races of the *C. lindemuthianum*.

The *Co-5* gene was reported to be among the most effective genes in Central America and Mexico due to its wide resistance spectrum conferring resistance to 31 races (Balardin *et al.*, 1997). Young and Kelly 1997 and Vallejo and Kelly (2001) reported the *Co-5* locus to be a resistance cluster, which in part explains its broad spectrum resistance. The gene has been used

by breeders to develop pyramids combining Meso and Andean gene pools (Young and Kelly, 1997).

However, the *Co-9* gene was the least effective against the pathogen. It was also associated with increased severity when combined with other genes. This implies that the *Co-9* gene was antagonistic with other genes and therefore, should be avoided in gene pyramiding programs. Its presence in the three gene pyramid *Co-4*²+*Co-5*+*Co-9*, however, was not antagonistic probably because of the presence and combined effectiveness of the *Co-4*² and *Co-5* genes reducing symptoms to a mean score of 1.1 ± 0.03 as compared to the score of 2.1 ± 0.41 in *Co-4*²+*Co-5* pyramid where *Co-9* was absent. Kelly and Vallejo (2004) reported that the *Co-9* gene is not a widely effective resistance gene and only possesses a very specific breeding value against Andean races of *C. lindemuthianum*. Alzate-Marin *et al.* (2003) reported the *Co-9* gene to be susceptible to weak Mesoamerican races 65 and 69. The cultivar PI 207262 possessing the *Co-9* gene was reported to be overcome by many anthracnose races (Kelly, 2004). This could explain the poor resistance spectrum observed with the *Co-9* gene in this study. Kelly and Vallejo (2004) recommended its use only to diversify resistance in gene pyramids because of its independence and potential value in controlling Andean races.

The *Co-4*²+*Co-5*+*Co-9* and *Co-4*²+*Co-5* pyramid had the lowest severity scores and did not succumb to any of the races implying that the combination and number of genes in these pyramids was highly effective in conferring broad-spectrum resistance to *C. lindemuthianum* in Uganda. The *Co-4*²+*Co-9* and *Co-5*+*Co-9* pyramid groups conferred resistance to races 352, 713 and 767 (score 1.0-3.3) but were overcome by race 2047 (> 4.0) while the *Co-4*³+*Co-9* pyramid group was overcome by races 713 (5.8 ± 0.17), 767 (6.3 ± 0.44) and 2047 (7.0 ± 0.29) but conferred resistance to only race 352 (1.5 ± 0.29). These results when compared with the mean severity scores of *Co-4*² and *Co-5* further reveal the poor complementarity of the *Co-9* gene.

The results revealed that gene pyramids were not consistent in conferring broad-spectrum resistance to *C. lindemuthianum* races with the exception of the three-gene pyramid *Co-4*²+*Co-5*+*Co-9* and the two-gene pyramid *Co-4*²+*Co-5* (Figure 4.1). For instance the pyramid *Co-4*³+*Co-9* was overcome by races 713, 767 and 2047 while the pyramids *Co-5*+*Co-9* and *Co-4*²+*Co-9* were both overcome by the race 2047. This could be the effect of presence of *Co-9*

gene in the pyramids and results have indicated that it may have an antagonistic effect in combination with other genes. For this reason, genotypes with *Co-9* gene should be avoided in breeding programs since the gene will negatively affect resistance. The presence of *Co-4*² gene in a pyramid always reduced disease severity on the leaves. In contrast, the single genes *Co-4*² and *Co-5* were not overcome by any of the four races. This implies that some gene combinations interact negatively and fail to confer effective resistance against a diverse *C. lindemuthianum* race population.

It was observed that a higher number of resistance genes in a pyramid significantly reduced the amount of symptoms on the host (Table 4.8) and according to Wheeler and Diachun (1983); Schafer and Roelfs (1985); and Kolmer *et al.* (1991) pyramids with higher resistance gene numbers are most likely to remain effective over long periods of time (durable resistance) before breaking down. Their argument is based on the probability hypothesis (Mundt, 1991) which states that "*cultivars possessing multiple race-specific resistance genes (pyramided genes) owe their durable resistance to a low probability of the pathogen independently mutating to virulence at multiple avirulent loci corresponding to the host resistance genes*". According to this hypothesis the pyramids *Co-4*²+*Co-5*+*Co-9* and *Co-4*²+*Co-5*, which were the most effective in conferring resistance, are most likely to remain effective over a longer period of time than single genes and other pyramid combinations. Mundt (1990,1991), however, argues contrary to the probabilities hypothesis basing on the lack of supportive empirical data, the assumption that mutations to virulence at different loci are necessarily independent and that mutation is the only source of genetic variation for plant pathogens. Mundt (1991, 2014) maintained that the influence of specific virulence combinations on pathogen fitness and the effect of mutation to multiple virulence on fitness may be at least as important as the probability that a change to multiple virulence genes will occur. The *Sr6* resistance gene for wheat stem rust is one the single resistance gene cited by Mundt (1991) to be more closely associated with broad-spectrum/ durable. Similar observations were made in the current study where the single anthracnose resistance gene *Co-4*² was associated with increased degree and spectrum of resistance, implying, according to Mundt (1990) that other factors other than gene numbers account for spectrum/ durability of resistance. It is, therefore, very pertinent that plant breeders

investigate fitness of single resistance genes before deploying them or using them in gene pyramiding programs against *C. lindemuthianum*.

Balardin and Kelly (1998) proposed pyramiding genetically diverse but complementary resistance genes in pyramids and deploying them in different regions as the most practical and realistic approach to provide effective long term anthracnose resistance. Kelly (2004) proposed combination of *Co-4²*, *Co-5* and *Co-6* genes in North America and *Co-1²* and *Co-4²* gene pair for Central America. The *Co-1²* gene confers resistance against Andean races while *Co-4²* confers resistance against Mesoamerican races. Resistance gene pyramids which incorporate at least two unique modes of action are reported to delay the evolution of virulent pathotypes (Roush, 1998).

The ability of the single genes *Co-4²*, *Co-5* and *Co-4³* to confer broad-spectrum resistance as effective as pyramided genes, and in some cases better than pyramided genes, could be as a result of factors at their respective loci that promote broad-spectrum and durable resistance (Mundt, 2014); while in some pyramids, the genes interact antagonistically to promote increased disease severity as was the case in this study when *Co-9* gene was combined with another gene. This implies that, for environments in which the disease occurs at moderate or low severity, a single resistance gene may be adequate for a very long period of time (Mundt, 2014). The results suggest that a gene pyramiding program would benefit from first understanding the nature of the individual resistance genes in a given *C. lindemuthianum* population and pyramid only those with broad-spectrum complementarity (Mundt, 2014).

4.5 Conclusions

Resistance gene pyramiding was effective in conferring broad-spectrum resistance in some cases and was ineffective in other cases. Effectiveness of pyramided resistance genes heavily depended on the fitness of individual resistance genes combined. The most effective pyramid combinations were *Co-4²+Co-5+Co-9* and *Co-4²+Co-5*, while the least effective pyramid combination was *Co-4³+Co-9*. Some single resistance genes such as *Co-4²* and *Co-5* were the most effective and exhibited broad-spectrum resistance, while the single gene *Co-9* was the least effective and exhibited antagonism when combined with other single genes.

4.6 Recommendations

Resistance gene combinations $Co-4^2+Co-5+Co-9$ and $Co-4^2+Co-5$ are recommended as effective pyramids for use in Uganda. The single genes $Co-4^2$ and $Co-5$ are recommended for deployment in cultivars specifically developed for areas where race variability is not high to avoid easy breakdown. The single genes and pyramid gene combinations should be monitored periodically and assessed for durability over time. Races 2047 and 767 are recommended for use in artificial inoculation while undertaking studies on resistance to bean anthracnose disease. Further studies should be conducted to identify and assess new effective single genes and pyramid gene combinations using other available sources of anthracnose resistance genes.

CHAPTER FIVE

Genetic effect of pyramided resistance genes on yield traits among common bean populations

Abstract

Pyramiding of resistance genes in commercial varieties would ensure reduction of yield losses resulting from the bean diseases. However, the effect and relationship between pyramided genes and plant yield traits is not well elucidated; and understanding genetic variability of breeding materials is crucial in ensuring and taking advantage of genetic gain among progenies. 53 $F_{4,5}$ and 69 $F_{4,6}$ advanced populations with varying number of resistance genes were evaluated in the field for yield performance. ANOVA was done to assess genetic variability of yield traits among the populations. Correlation and path coefficient analyses were done to uncover relationships between number of pyramided genes and the yield traits. ANOVA revealed significant variability for all yield traits among the populations and further revealed that PCV estimates were higher than GCV estimates. Broad sense heritability and genetic advance among populations were low for number of pods per plant, number of seeds per plant and seed weight per plant indicating importance of non-additive gene action. Number of pyramided genes showed a significant negative correlation with seed weight per plant (-0.17 , $P<0.05$), number of pods per plant (-0.24 , $P<0.05$) and number of seeds per plant (-0.19 , $P<0.1$). Path coefficient analysis revealed a significant negative indirect effect (-0.25 , $P<0.05$) of number of pyramided genes on seed weight per plant via number of seeds per plant. This suggests a possible reduction in yield that may be experienced as a result of pyramiding more resistance genes in a given genetic background. However, the significance of the potential yield loss may be reduced through integrating gene pyramiding with appropriate breeding strategies, which increase yield gain along with enhanced resistance to disease.

Key words: Correlation analysis, path coefficient analysis, pyramided genes, yield

5.1 Introduction

Direct selection for yield may not be effective as it is a complex trait and depends upon the component traits. Understanding the association of traits, therefore, is crucial in developing an efficient breeding program that aims at developing high yielding varieties with effective resistance to bean anthracnose. Additionally, variability among germplasm is essential for the success of breeding programs for all economically important traits (Ramalho *et al.*, 1993). It is recommended that more than one trait be considered in a breeding program (Gonzalves-Vidigal *et al.*, 2003). Correlation is useful in showing relationships among independent traits (Yucel *et al.*, 2006). The estimated correlation measured enables the breeder to understand the changes that occur in a determined trait in function of the selection practiced on another correlated trait (Gonzalves-Vidigal *et al.*, 2003). However, the quantification and interpretation of the magnitude of a correlation can result in errors when indirect selection strategy is used based on the effect of a third trait or of a group of other traits on them (Gonzalves-Vidigal *et al.*, 2003). Path coefficient analysis, therefore, further partitions the correlation coefficients into direct and indirect effects of a set of independent variables on the dependent variable (Guler *et al.*, 2001). It also permits critical examination of the specific forces acting to produce a given correlation and measures the relative importance of each causal factor (Singh *et al.*, 2013). Path coefficient analysis, therefore, plays an important role in determining the degree of relationship between a given trait and yield components (Ciftci *et al.*, 2004).

Gene pyramiding has been successfully used to pyramid resistance genes and create broad-spectrum and durable resistance (Ferreira *et al.*, 2012). However, the relationship between pyramided genes and plant yield traits is not well elucidated. Some studies have indicated that gene pyramiding of resistance genes incurred no yield penalty on the plants. For example, Liebenberg *et al.* (2005) successfully pyramided three bean rust resistance genes *Ur-3+*, *Ur-5*, *Ur-11* in advanced common bean lines and observed no yield reduction. Souza *et al.* (2014) also observed no yield penalty as a result of pyramiding rust resistance genes *Ur-5*, *Ur-11* and *Ur-14* into advanced populations. Other studies have indicated the contrary, such as a 6% yield reduction reported by Singh and Espino (1997) to be associated with pyramiding *Lr34* brown rust resistance gene with other *Lr* genes; and a 9% yield penalty reported by Latter *et al.* (1998) to be associated with three stem rust resistance genes in wheat.

This part of the study, therefore, was to establish the relationship between pyramided resistance genes and yield traits in advanced common bean populations.

5.2 Materials and methods

5.2.1 Field experimental set up and evaluation of advanced populations

Fifty three (53) $F_{4.5}$ and sixty nine (69) $F_{4.6}$ common bean families were developed using a cascading gene pyramiding scheme as indicated in Chapter Four were selected for this study based on number of resistance genes inherited. The resistance genes for anthracnose included $Co-4^2/Co-4^3$, $Co-5$ and $Co-9$. The $F_{4.5}$ families were evaluated in the field during season 1 and $F_{4.6}$ families evaluated during season 2, using a nested randomized complete block design (RCBD) with three replications. Single row plots of 1.5m length were used with a 50cm between row and 15cm within row spacing. The parents used in the breeding scheme were used as checks namely G2333, PI207262, RWR719, K132, NABE 4, NABE 13 and NABE 14. The trials were conducted at the National Agricultural Research Laboratories (NARL), Kawanda. Data were collected on plant vigor scored using a 1 – 5 scale where 1 = low vigor and 5 = high vigor; number of pods per plant, number of seeds per pod, pod length (cm), number of seeds per plant, seed weight per plant (gm), 100-seed weight (gm), days to 50% flowering and days to maturity.

5.2.2 Data analysis

5.2.2.1 Genetic variability and parameter estimation among populations

Analysis of variance (ANOVA) was carried out using GenStat discovery 12th edition (Anonymous, 2009) to partition variability for different characters. Genetic parameters to quantify genotypic variability among the genotypes for the characters under study were estimated as follows: Genotypic variance (σ_g^2) = $MSS_{genotypes} - MSS_{error} / \text{Number of replications}$; Phenotypic variance (σ_p^2) = $\sigma_g^2 + MSS_{error}$; Genotypic coefficient of variability (GCV) = $(\sigma_g / \mu) \times 100$; Phenotypic coefficient of variability (PCV) = $(\sigma_p / \mu) \times 100$ (Wricke and Weber, 2010); Broad sense heritability (h_b^2) = σ_g^2 / σ_p^2 (Griffiths *et al.*, 2000); Genetic advance

$(GA) = h^2 \times k \times \sigma_p$ and Genetic advance as percent of mean $(GAM) = GA / \mu \times 100$ (Johnson *et al.*, 1955).

Where; $MSS_{\text{genotypes}}$ = Mean Square for genotypes
 MSS_{error} = Mean Square for residual
 μ = Grand mean for character
 h_b^2 = Broad sense heritability
 k = Standardized selection differential at 5% selection intensity = 2.063.
 σ_p = Phenotypic standard deviation
 σ_g = Genotypic standard deviation

GCV and PCV values were categorized as low (0 – 10%), moderate (10 – 20%) and high (20 % and above) according to Burton (1952). Heritability categorized according to Dabholker (1992) as low (5 – 10%), medium (10 – 30%) and high (>30%). GA and GAM were categorized as low (<10%), moderate (10 – 20%) and high (>20%) according to Falconer and Mackay (1996).

5.2.2.2 Correlation analysis of pyramided genes and yield traits

The degree of association of different characters was done through correlation analysis with correlation coefficients calculated using the formula by Weher and Moorthy (1952). The standardization of the coefficients based on the standard deviations of the variables is the approach typically used to make coefficients comparable (Grace and Bollen, 2005). Therefore, data were standardized before correlation analysis.

$$r_{xy} = \text{Cov}XY / \sqrt{\sigma_p^2 x \cdot \sigma_g^2 y}$$

Where; r_{xy} = Correlation between characters x and y ;
 $\text{Cov}XY$ = Covariance between characters x and y
 $\sigma_p^2 x \cdot \sigma_g^2 y$ = Phenotypic variance of characters x and y respectively.

5.2.4 Path coefficient analysis of pyramided genes and yield traits

Path coefficient analysis was done according to the method described by Akintunde (2012) using MS Excel, in order to identify yield traits via which number of pyramided genes directly

or indirectly affected plant yield. Path coefficients computation is based on simultaneous equations which express the basic relationships between correlations and path coefficients as indicated below;

$$\begin{aligned}
 r_{x1y} &= P_{x1y} + r_{x1x2}P_{x2y} + r_{x1x3}P_{x3y} + r_{x1x4}P_{x4y} + r_{x1x5}P_{x5y} + r_{x1x6}P_{x6y} + r_{x1x7}P_{x7y} + r_{x1x8}P_{x8y} + r_{x1x9}P_{x9y} + r_{x1x10}P_{x10y} \\
 r_{x2y} &= r_{x2x1}P_{x1y} + P_{x2y} + r_{x2x3}P_{x3y} + r_{x2x4}P_{x4y} + r_{x2x5}P_{x5y} + r_{x2x6}P_{x6y} + r_{x2x7}P_{x7y} + r_{x2x8}P_{x8y} + r_{x2x9}P_{x9y} + r_{x2x10}P_{x10y} \\
 &\dots \\
 r_{x10y} &= r_{x10x1}P_{x1y} + r_{x10x2}P_{x2y} + r_{x10x3}P_{x3y} + r_{x10x4}P_{x4y} + r_{x10x5}P_{x5y} + r_{x10x6}P_{x6y} + r_{x10x7}P_{x7y} + r_{x10x8}P_{x8y} + r_{x10x9}P_{x9y} + \\
 &P_{x10y}
 \end{aligned}$$

Where; r_{x1y} to r_{x10y} denotes the correlation coefficients between independent characters $x1$ to $x10$ and the dependent character y ; r_{x1x2} to r_{x10x9} denotes the correlation coefficients between all possible combinations of independent characters; P_{x1y} to P_{x10y} denotes the direct effects of characters $x1$ to $x10$.

5.3 Results

5.3.1.1 Genetic variability among advanced common bean populations

The results for analysis of variance for the yield traits among advanced bean populations are presented in Table 5.1. Genotypes Mean Squares (M.S) were highly significantly different ($P < 0.01$ - $P < 0.05$) for all the yield traits. Number of seeds/plant contributed most to the total variation (61.9% for season 1 and 81.3% for season 2) while plant vigor contributed the least (0.03% for season 1 and 0.21% for season 2).

During season one, seed weight per plant had the highest coefficient of variation (64.7%) followed by number of seeds per plant (62.2%), pods per plant (60.3%) and plant height (39.2%), while during season two number of seeds per plant had the highest coefficient of variation (40.2%) followed by seed weight per plant (37.3%) and pods per plant (35.0%). Hence variability in the advanced populations was considered for further analysis.

Table 5.1: Analysis of variance Mean Squares for the different yield traits

Traits	Mean squares			%	Prob	C.V (%)
	M.S Replication	M.S Genotype	M.S Error			
<u>Season one (F_{4,5})</u>						
100-seed weight	70.6**	95.8**	26.0	2.3	<.001	18.2
Pod length	0.1**	7.2**	1.0	0.2	<.001	11.1
Pods/ plant	18.6	146.6	99.2	3.5	0.065	60.3
Seeds/ pod	0.6**	2.6**	0.5	0.1	<.001	15.3
Seeds/ plant	197.0**	2588.0**	1.4	61.9	0.008	62.2
Seed weight/ plant	104.6**	239.7**	119.0	5.7	0.003	64.7
Days to 50% flow	-	9.1*	5.2	0.2	0.022	5.4
Days to maturity	-	10.0*	5.7	0.2	0.023	2.8
Plant height	-	1082.5*	580.3	25.9	0.014	39.2
Plant vigor	-	1.3*	0.8	0.0	0.030	23.1
<u>Season 2 (F_{4,6})</u>						
100-seed weight	156.2**	40.0**	10.1	5.7	<.001	11.3
Pods/ plant	11.5**	36.5**	20.3	5.2	0.002	35.0
Seeds/ plant	214.1*	580.0*	400.0	81.3	0.037	40.2
Seed weight/ plant	11.9**	46.9**	26.1	6.6	0.002	37.3
Plant vigor	4.9**	1.5**	0.4	0.2	<.001	16.8

** Highly significant ($P < 0.01$). * significant ($P < 0.05$). prob = probability. C.V% = Coefficient of variation

5.3.1.2 Phenotypic and genotypic coefficient of variability among populations

Parameter estimates from the segregating F_{4,5} and F_{4,6} bean populations are presented in Table 5.2. For all characters PCV estimates were higher than GCV. In season one, days to 50% flowering and days to maturity had low PCV values (<10%). Pod length had a moderate PCV value (10 – 20%). Seeds per pod, 100-seed weight, plant vigor, pods per plant, seeds per plant, seed weight per plant and plant height had high PCV values (>20%). In season two, 100-seed weight had moderate PCV values while all the other traits had high PCV values (>20%).

In season one, days to 50% flowering and days to maturity had low GCV values (0 – 10%). Pod length, seeds per pod, 100-seed weight and plant vigor had moderate GCV values (10 – 20%). Pods per plant, seeds per plant, seed weight per plant and plant height had high GCV values (>20 %).

Table 5.2: Parameter estimation among advanced bean populations

Characters	Genetic Parameters							
	Grand Mean (μ)	GV	PV	GCV	PCV	h_b^2	GA	GAM
Season one (F_5)								
100-seed weight	28.0	23.3	49.3	17.2	25.1	47	7.2	25.7
Pod length	9.1	2.1	3.1	15.7	19.3	68	2.5	27.5
Pods/ plant	16.5	15.8	115.0	24.1	65.0	14	3.0	18.2
Seeds/ pod	4.6	0.7	1.2	18.2	23.8	58	1.4	30.4
Seeds/ plant	59.9	399.7	1788.7	33.4	70.6	22	17.4	29.1
Seed weight/ plant	16.9	40.2	159.2	37.5	74.6	25	7.8	46.2
Days to 50% flow	42.5	4.0	9.1	4.7	7.1	44	2.7	6.4
Days to maturity	84.1	4.3	10.0	2.5	3.8	43	2.8	3.3
Plant height	61.4	502.0	1083.0	36.5	53.6	46	31.4	51.1
Plant vigor	3.7	0.5	1.3	19.1	30.5	38	0.9	24.3
Season two (F_6)								
100-seed weight	27.9	10.0	20.0	11.3	16.1	50	4.6	16.5
Pods/ plant	12.9	5.4	25.7	18.0	39.4	21	2.1	16.3
Seeds/ plant	49.9	60.0	460.0	15.5	43.0	13	4.4	8.8
Seed weight/ plant	13.7	7.0	33.0	19.2	41.8	21	2.4	17.5
Plant vigor	3.8	0.4	0.8	16.0	23.1	50	0.9	23.7

GV = Genetic variance, PV = Phenotypic variance, GCV = Genetic coefficient of variability, PCV = Phenotypic coefficient of variability, h_b^2 = Broad sense heritability (%), GA = Genetic advance, GAM = Genetic advance as a percentage of the grand mean

5.3.1.3 Heritability and genetic advance among advanced bean populations

Pods per plant, seeds per plant and seed weight per plant had low to moderate broad sense heritability values (10% – 30%) while, pod length, seeds per pod, 100-seed weight, days to 50% flowering, days to maturity, plant height and plant vigor had high heritability values (>30%) (Table 5.2). Pod length had the highest broad sense heritability of 68% followed by seeds per pod (58%), 100-seed weight (50%), plant vigor (50%), while seeds per plant (22%) and pods per plant (21%) had the lowest heritability estimates.

Pod length, seeds per pod, 100-seed weight, plant height and plant vigor had high heritability estimates (>30%) and high genetic advance as percent of mean (GAM) (>20%). Seeds per plant and seed weight per plant had moderate heritability estimates (10 – 30%) and high GAM. Pods per plant had a low heritability (5 – 10%) and a moderate GAM. However, days to 50% flowering and days to maturity had high heritability values but low GAM.

5.3.2 Yield traits performance among advanced bean populations

Advanced populations, number of inherited resistance genes and the performance of different yield traits are presented in Table 5.3.

Table 5.3: F₅ Advanced bean populations and their yield performance

Family code	SWP	NPG	PV	PH	DF	DM	SP	PL	NPP	NSP	100SW
A16.1.2.1.6.3	26.2	0	4.0	55.8	40.0	83.5	3.2	9.4	26.3	60.0	40.8
A16.1.2.1.6.2	7.1	0	3.0	26.7	40.0	83.0	2.3	9.1	15.5	31.0	29.6
B44.7.5.8.112.2	15.9	0	4.5	33.5	42.0	81.5	5.3	8.8	18.3	58.5	32.4
B44.7.5.8.112.1	6.8	0	3.5	36.5	44.0	81.0	3.4	7.2	7.3	27.7	22.7
C44.1.6.7.130.1	22.8	0	3.0	85.6	45.7	85.7	5.7	10.0	26.5	98.0	24.2
C44.1.6.7.130.2	18.7	0	3.0	93.8	46.3	85.3	6.1	10.5	16.3	73.0	25.6
C44.1.6.7.130.3	24.5	0	3.3	65.2	46.0	89.0	5.3	10.2	24.8	96.5	25.5
D16.3.3.2.152.1.2	9.0	0	2.0	46.3	44.0	83.5	5.3	11.1	10.2	43.8	19.1
D16.3.3.2.152.2.1.3	12.7	0	3.0	72.0	44.0	85.0	8.0	12.5	10.0	49.0	25.9
A16.1.2.3.7.1	45.8	1	4.5	59.7	41.0	83.0	5.5	10.9	38.8	155.2	30.1
A16.1.2.3.7.2	24.3	1	3.0	65.0	42.0	84.0	3.6	8.8	26.8	87.5	33.5
A16.6.1.6.25.1	12.6	1	3.3	46.2	39.3	80.7	4.7	8.6	14.4	51.6	22.2
A44.5.7.1.1.1	4.3	1	4.0	19.3	38.0	80.0	2.6	6.2	9.6	21.1	22.8
B44.7.8.2.91.1	15.6	1	4.0	77.3	42.3	85.0	4.0	8.4	19.3	54.9	31.3
B44.7.8.2.91.10	22.0	1	4.3	88.3	38.0	83.3	5.4	10.1	19.1	77.7	27.8
B44.7.8.3.92.3	17.0	1	4.5	57.0	40.0	83.3	4.1	8.8	17.9	56.3	30.3
B44.7.8.2.91.11	31.6	1	5.0	77.7	42.0	85.0	6.0	11.9	16.8	89.0	31.2
B44.7.8.3.92.2.3.4	11.5	1	4.0	54.0	43.0	82.0	4.5	9.5	11.8	32.3	33.9
A44.5.2.1.26.2	12.2	2	3.5	24.2	40.0	80.0	4.4	7.9	14.0	43.4	21.4
A44.5.2.3.28.1	9.8	2	3.3	99.2	42.0	85.0	4.9	8.7	12.3	49.6	19.7
A44.5.2.1.26.1	4.1	2	3.0	21.3	38.0	80.0	3.2	6.5	7.5	19.5	20.9
A44.5.1.11.46.1	8.7	2	2.0	57.0	43.3	85.0	4.8	9.1	18.3	59.0	14.3
B89.5.2.10.118.15	4.3	2	2.5	28.0	44.0	85.0	3.4	6.6	7.0	23.7	21.1
B89.5.1.1.81.2	10.4	2	4.3	58.8	43.3	85.0	5.6	10.3	11.4	54.0	18.7
B89.5.1.1.81.3	7.6	2	4.0	52.7	44.0	84.5	5.3	10.2	7.9	32.6	24.2
B89.5.2.7.117.1.1	10.4	2	3.0	43.0	44.0	85.0	3.0	6.5	17.0	46.0	22.5
B89.5.2.7.117.2.2	1.3	2	2.0	17.5	45.0	85.0	1.7	2.5	1.0	5.0	26.6
B44.7.9.5.99.2.3.4	11.7	2	3.5	83.7	45.0	85.5	5.0	8.8	14.0	47.7	25.7
B89.5.1.1.81.1.1	7.8	2	3.0	71.0	45.0	84.0	5.3	9.7	11.0	37.0	21.2
C16.1.3.8.136.1	17.2	2	4.0	59.2	42.0	82.5	4.9	9.9	16.8	52.9	32.2
C16.1.3.8.136.2	26.8	2	5.0	77.7	44.3	88.7	5.1	10.3	17.8	72.2	34.7
C44.1.4.5.142.1	23.9	2	4.0	68.3	46.0	88.3	4.8	9.3	22.3	85.2	28.3
C44.1.4.5.142.3	14.0	2	4.0	48.6	43.7	83.7	3.6	7.7	12.6	44.3	30.2
C44.1.4.5.142.4	27.8	2	4.0	50.5	40.0	84.5	4.5	8.8	26.5	96.0	29.2
C44.1.4.7.143.1	20.6	2	4.0	94.2	43.5	85.0	4.5	8.6	18.1	70.5	28.4
D16.3.3.1.151.1	15.3	2	3.5	33.8	42.0	83.3	5.3	11.9	17.5	52.8	29.5
A44.5.9.1.14.2	2.3	3	2.0	14.0	43.0	83.5	2.3	4.8	2.5	7.5	32.6
A44.5.2.3.28.2	11.0	3	3.3	86.9	42.3	84.7	4.1	7.7	12.6	47.9	20.2
A44.5.2.3.28.4	13.5	3	2.7	73.7	43.0	83.3	4.4	8.3	16.2	61.9	21.8

A44.5.9.1.14.1	13.6	3	3.0	58.0	38.0	82.0	4.2	8.6	13.5	42.0	32.5
C44.1.4.3.141.1	21.9	3	4.7	83.4	41.7	86.7	4.0	7.6	21.7	73.2	31.1
C44.1.4.3.141.2	12.1	3	4.5	53.3	40.0	83.0	3.0	6.4	11.6	37.6	33.2
D16.3.3.8.157.3	17.3	3	4.3	36.1	40.7	81.7	4.8	9.7	12.6	47.8	36.2
D16.3.3.9.158.1	16.3	3	4.0	38.8	40.7	81.0	4.8	10.3	15.3	49.9	32.3
D16.3.3.10.159.6	20.0	3	4.0	68.0	42.0	84.0	5.3	11.8	15.7	67.7	29.1
D16.3.3.11.160.2	23.4	3	4.7	98.4	42.0	83.0	6.0	11.5	14.9	68.2	35.1
D42.12.4.1.161	14.3	3	5.0	94.0	43.0	82.0	3.4	8.1	16.7	46.5	31.3
D16.3.3.11.160.5.6	28.6	3	5.0	69.0	42.0	83.0	6.1	11.7	19.0	84.2	35.0
D16.3.3.11.160.5.8.3	22.7	3	4.0	57.0	44.0	83.0	4.3	11.3	17.0	71.0	32.0
D16.3.3.11.160.5.8	11.1	3	4.0	110.5	44.0	85.0	6.0	12.0	13.0	44.0	25.1
B44.7.9.8.102.1	8.4	4	3.5	56.7	43.5	87.0	4.4	9.5	13.3	29.8	27.9
B44.7.2.2.76.4	7.9	4	3.5	23.4	42.0	85.0	4.0	7.9	7.0	42.5	19.7
B44.7.2.2.76.1.1	1.8	4	4.0	34.0	42.0	87.0	3.0	6.3	2.0	11.0	16.4
Std deviation	8.7	1.2	0.8	24.2	2.1	2.1	1.2	2.0	6.8	26.5	5.7

Parents											
K132	36.1	0	3.3	22.2	41.4	80.0	4.0	11.7	24.0	86.0	42.0
NABE 4	19.4	0	4.5	30.0	40.7	81.3	3.6	8.5	13.3	46.5	41.2
NABE 13	22.2	1	4.6	28.8	38.8	81.8	4.8	10.0	13.0	58.4	36.5
NABE 14	14.1	1	5.0	25.9	39.3	82.7	4.6	11.4	9.1	38.4	36.2
RWR719	34.3	1	3.8	55.5	43.5	84.0	7.0	10.8	27.7	161.3	21.1
PI207262	19.8	2	4.6	70.4	38.2	81.0	5.5	7.5	26.1	99.3	20.2
G2333	37.1	3	4.4	243.0	44.5	90.0	8.2	11.2	23.5	196.9	19.2
Std deviation	9.4	1.1	0.6	79.2	2.4	3.3	1.7	1.6	7.5	60.1	10.3

A = pedigree 12x8xRWR719xK132; B = pedigree 12x8xRWR719xNABE4; C = pedigree 12x8xRWR719xNABE13; D = pedigree 12x8xRWR719xNABE14; SWP = Seed weight per plant; NPG = Number of pyramided genes; PV = Plant vigor; PH = Plant height; DF = Days to flowering; DM = Days to maturity; SP = Seeds per pod; PL = Pod length; NPP = Number of pods per plant; NSP = Number of seeds per plant; 100SW = 100-seed weight.

The overall best five (5) lines/ genotypes in terms of seed weight per plant were line A16.1.2.3.7.1 (45.8gms) with single inherited gene, parent G2333 (37.1gm), parent K132 (36.1gm), parent RWR719 (34.3gm) and line B44.7.8.2.91.11 (31.6gm) with a single inherited gene. Three lines with a four-gene pyramid namely B44.7.9.8.102.1 (8.4gm), B44.7.2.2.76.4 (7.9gm) and B44.7.2.2.76.1 (1.8gm) had a yield range of 1.8gm – 8.4gm per plant. Fourteen genotypes with a three-gene pyramid had a yield range of 11.0gm and 28.4gm. Eighteen genotypes with a two-gene pyramid had a yield range of 1.8gm and 27.8gm; nine genotypes with a single gene had a yield range of 4.3gm and 45.8gm; and nine genotypes with no-gene had a yield range of 6.8 – 26.2gms.

Among the pyramided lines, line D16.3.3.11.160.5.6 with a three-gene pyramid performed best in terms of yield per plant (28.6gm) followed by C44.1.4.5.142.4 (27.8gm) and C16.1.3.8.136.2 (26.8gms) both with a two-gene pyramid. These three lines were among the overall best ten genotypes in terms of seed weight per plant. In terms of seed yield per plant, populations derived from cultivar NABE 13 performed best with an average yield of 20.9gms followed by NABE 14 derived populations (17.3gms), while NABE 4 derived populations performed least (11.3gms) (Table 5.4).

Among the recurrent parents K132 performed best (36.1) followed by NABE 13 (22.2gms), NABE 4 (19.4gms) and NABE 14 (14.1). Derived populations on average yielded less than their respective parents except those of NABE 14 (17.3gms).

Table 5.4: Comparison of performance between parents and their derived advanced populations/ lines

Genotypes	SWP (gms)	PV	SP	NPP	NSP	100SW (gms)
K132 Parent	36.1	3.3	4.0	24.0	86.0	42.0
K132 derived populations	14.0	3.2	3.9	16.3	52.7	25.9
NABE 4 parent	19.4	4.5	3.6	13.3	46.5	41.2
NABE 4 derived populations	11.3	3.7	4.3	11.9	42.7	25.5
NABE 13 parent	22.2	4.6	4.8	13.0	58.4	36.5
NABE 13 derived populations	20.9	4.0	4.7	19.5	72.7	29.3
NABE 14 parent	14.1	5.0	4.6	9.1	38.4	36.2
NABE 14 derived populations	17.3	4.0	5.4	14.7	56.8	30.1

SWP = Seed weight per plant; PV = Plant vigor; SP = Seeds per pod; NPP = Number of pods per plant; NSP = Number of seeds per plant; 100SW = 100-seed weight

There was a general decline, however, of 100-seed weight among derived populations for all the four recurrent parents. Declining trends in seed weight per plant (Figure 5.1) and number of pods per plant (Figure 5.2) were observed as number of pyramided genes increased.

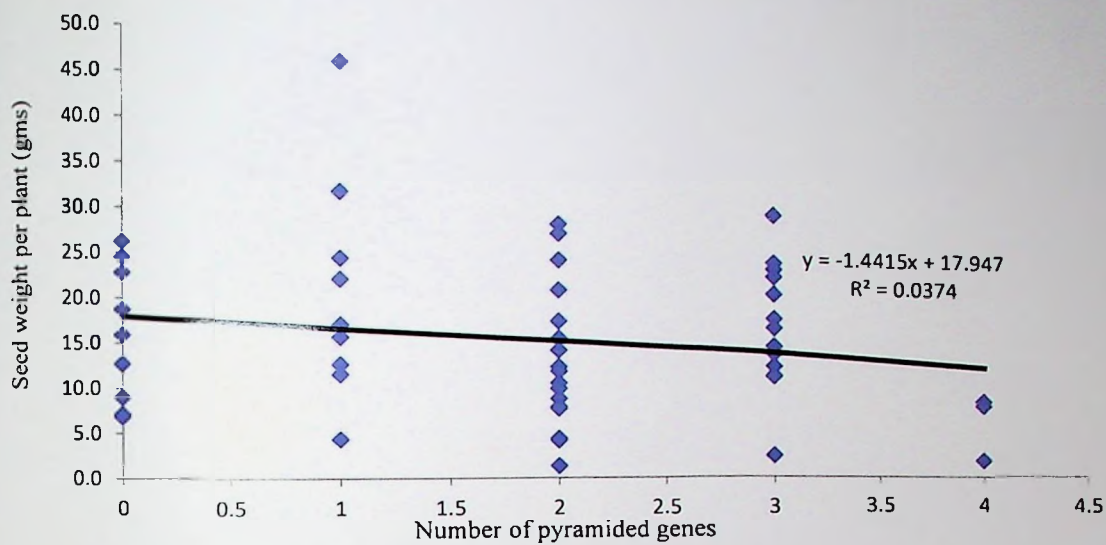


Figure 5.1: Trend of Seed weight per plant (SWP) with increasing number of pyramided genes

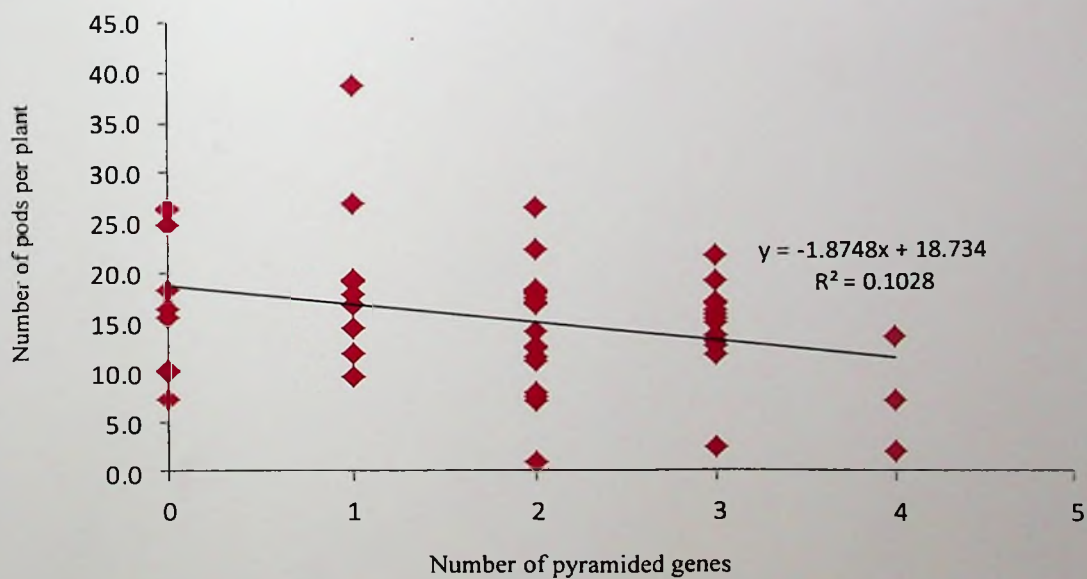


Figure 5.2: Trend of Number of pods per plant (NPP) with increasing number of pyramided genes

5.3.3 Correlation between number of pyramided genes and yield associated traits

The results of correlation between number of pyramided genes (NPG) and yield traits of the advanced bean families are presented in Table 5.5. A highly significant negative correlation was observed between number of pyramided genes and seed weight per plant (-0.17 , $P < 0.01$), while significant negative correlations were observed between number of pyramided genes and number of pods per plant (-0.24 , $P < 0.05$) and number of seeds per plant (-0.19 , $P < 0.1$).

Table 5.5: Correlation matrix for seed weight per plant and different plant growth and yield characters

Variables	SWP	NPG	PV	PH	DF	DM	NSP	PL	NPP	NSPT	100 SW
SWP	1.00										
NPG	-0.17**	1.00									
PV	0.56**	-0.02ns	1.00								
PH	0.45**	0.00	0.33**	1.00							
DF	-0.03ns	-0.09ns	-0.24*	0.30*	1.00						
DM	0.17ns	0.08ns	-0.03ns	0.39**	0.63**	1.00					
NSP	0.46**	-0.18ns	0.25*	0.56**	0.25*	0.18ns	1.00				
PL	0.56**	-0.17ns	0.36**	0.50**	0.13ns	0.11ns	0.86**	1.00			
NPP	0.82**	-0.24*	0.39**	0.40**	-0.05ns	0.15ns	0.31*	0.45	1.00		
NSPT	0.92**	-0.19ns	0.39**	0.49**	0.07ns	0.25*	0.52**	0.56**	0.85**	1.00	
100SW	0.42**	-0.02ns	0.42**	0.12ns	-0.19	-0.12ns	0.01ns	0.23ns	0.03ns	-0.09ns	1.00

** Highly significant ($P < 0.01$). * Significant ($P < 0.05$). ns = not significant. SWP = seed weight per plant, NPG = number of pyramided genes, PV = plant vigor, PH = plant height, DF = days to 50% flowering, DM = days to maturity, NSP = number of seeds per pod, PL = pod length, NPP = number of pods per plant, NSPT = number of seeds per plant and 100SW = 100-seed weight.

5.3.4 Path coefficient analysis of number of pyramided genes and yield traits

The results of estimates of direct and indirect path coefficients using seed weight per plant as the response variable are presented in Table 5.6.

Number of seeds per plant (NSPLT) and 100-seed weight (100-SW) were the only two yield traits with significant direct effects (0.975 , $P < 0.01$ and 0.276 , $P < 0.05$ respectively) on Seed weight per plant (SWP), the response variable. Number of pyramided genes (NPG) had a negative but non-significant (-0.012) direct effect on Seed weight per plant (SWP). However, a negative and significant indirect effect (-0.25 , $P < 0.05$) of Number of pyramided genes on Seed weight per plant was observed via Number of seeds per plant). The overall (direct and indirect)

effect of Number of pyramided genes on Seed weight per plant was negative and significant (-0.194, $P < 0.1$).

Table 5.6: Direct and indirect path coefficients for seed weight per plant

Variables	NPG	PV	PH	DF	DM	NSP	PL	NPP	NSPLT	100SW	SWP (r)
NPG	<u>-0.012</u>	0.013	-0.000	0.004	0.002	0.012	-0.011	0.050	-0.246**	-0.006	-0.194*
PV	-0.002	<u>0.082</u>	-0.006	0.010	-0.001	-0.017	0.022	-0.052	0.371***	0.143	0.549***
PH	-0.000	0.027	<u>-0.018</u>	-0.012	0.010	-0.038	0.031	-0.063	0.478***	0.033	0.447***
DF	0.001	-0.020	-0.005	<u>-0.040</u>	0.016	-0.017	0.008	0.008	0.070	-0.054	-0.034
DM	-0.001	-0.002	-0.007	-0.025	<u>0.025</u>	-0.012	0.007	-0.024	0.240**	-0.034	0.165
NSP	0.002	0.021	-0.010	-0.010	0.005	<u>-0.067</u>	0.053	-0.048	0.511***	0.002	0.456***
PL	0.002	0.030	-0.009	-0.005	0.003	-0.058	<u>0.061</u>	-0.070	0.548***	0.063	0.565***
NPP	0.002	0.026	-0.007	0.002	0.004	-0.021	0.027	<u>-0.157</u>	0.882***	0.105	0.867***
NSPLT	0.003	0.031	-0.009	-0.003	0.006	-0.035	0.035	-0.142	<u>0.975***</u>	0.076	0.937***
100SW	0.000	0.042	-0.002	0.008	-0.003	-0.000	0.014	-0.060	0.268**	<u>0.276**</u>	0.544***

*** Highly significant ($P < 0.01$), ** Significant ($P < 0.05$), * Significant ($P < 0.1$). SWP (r) = seed weight per plant, NPG = number of pyramided genes, PV = plant vigor, PH = plant height, DF = days to 50% flowering, DM = days to maturity, NSP = number of seeds per pod, PL = pod length, NPP = number of pods per plant, NSPLT = number of seeds per plant and 100SW = 100-seed weight. The sum of direct and indirect path coefficients is bolded in the last column. Direct path coefficients appear diagonally in bold and underlined. The rest of the coefficients are indirect coefficients.

5.4 Discussion

5.4.1 Genetic variability among the advanced populations

The success of genetic advance under selection depends on three factors namely genetic variability, heritability and selection intensity (Allard, 1960). In this study, ANOVA revealed significant variations for all yield traits among the advanced populations indicating a high amount of variability among the genotypes for all traits. This high variability is an indication of a wide genetic base available to breeders for progress (Day, 1973) in common bean improvement. All traits except days to 50% flowering and days to maturity had moderate to high PCV and GCV estimates implying a high phenotypic and genotypic variability for all traits except days to 50% flowering and days to maturity. For all traits, PCV estimates were higher than GCV estimates indicating importance of environmental effects in the phenotypic expression and improvement of these traits (Wricke and Weber, 2010). The low PCV and GCV

of days to 50% flowering and days to maturity suggests a low response to selection for these traits as compared to number of pods per plant, number of seeds per plant and seed weight per plant, which had the highest estimates. These results are consistent with findings of Singh *et al.* (1994), Raffi and Nath (2004), Negash (2006), Andriani *et al.* (2015) and Rasel *et al.* (2018).

The high broad sense heritability estimates observed for pod length, 100-seed weight, 50% flowering, days to maturity, plant vigor, plant height, pod length and seeds per pod indicated that a large proportion of total observed variance was due to genotypic variance and therefore these traits could be improved through selection because of a close correspondence between genotype and phenotype (Singh, 2009). Low to moderate heritability estimates were observed for number of pods per plant, number of seeds per plant and seed weight per plant indicating a low correspondence between genotype and phenotype and that these yield traits are highly influenced by environment (Singh, 2009). Therefore, genetic improvement through selection would be difficult. According to Amin *et al.* (1992) however, high broad sense heritability is not always associated with high genetic advance and relying on it alone may not be effective in ensuring genetic gain through selection. Johnson *et al.* (1955) suggested use of broad sense heritability estimates in conjunction with predicted genetic advance for effective selection.

High heritability estimates (>30%) and high genetic advance as percent of mean (GAM) (>20%) were observed for pod length, number of seeds per pod, 100-seed weight, plant height and plant vigor implying that these yield traits respond well to selection (Singh, 2009) and that the genotype is closely associated with the observed phenotype (Govindaraj *et al.*, 2011). High heritability but low to moderate GAM estimates were observed for days to 50% flowering, days to maturity and number of pods per plant implying low response to selection and the need to explore other breeding methods for improvement of such traits (Singh, 2009; Brown and Caligari, 2011; Govindaraj *et al.*, 2011; and Kute and Kumar, 2013).

The low to moderate heritability (0.2-0.3) observed with pods per plant, number of seeds per plant and seed weight per plant indicates these yield traits are influenced by environment and controlled by non-additive genes making improvement by selection difficult. Both additive and non-additive gene effects are reported to control yield traits (Gonzalves-Vidigal *et al.*, 2003)

making heterotic breeding a viable strategy to enhance yield and limit possible negative effects on yield that could arise due to resistance gene pyramiding.

5.4.2 Relationship between number of pyramided genes and yield

Number of pyramided genes had a significant negative correlation with seed weight per plant (-0.17) and number of pods per plant (-0.24) implying a yield penalty through reduction of seed weight per plant and number of pods per plant. The reduction in seed weight per plant was also evident in the reduction of 100-seed weight observed when recurrent parents K132, NABE 4, NABE 13 and NABE 14 were compared with their respective derived populations with pyramided genes (Table 5.4).

Path coefficient analysis further revealed no significant direct effect of number of pyramided genes on seed weight per plant, but a significant negative indirect effect (-0.25, $P < 0.05$) of number of pyramided genes on seed weight per plant via number of seeds per plant implying an indirect yield penalty). The overall (direct and indirect) effect of number of pyramided genes on seed weight per plant, however, was significantly negative implying an overall yield penalty due to pyramided resistance genes at $P < 0.1$ significance level. The positive indirect effects that number of pyramided genes had on seed weight per plant through plant vigor (0.013), days to flowering (0.004), days to maturity (0.002), number of seeds per pod (0.012) and number of pods per plant (0.050) seemed to compensate for the significant negative indirect effect (0.12, $P < 0.05$) observed via number of seeds per plant. Singh and Espino (1997) associated a 6% yield reduction with *Lr34* gene pyramided with other *Lr* genes which provided durable resistance to brown rust of wheat, while Latter *et al.* (1998) associated a 9% yield penalty with three stem rust resistance genes wheat.

Contrary to the negative relationship observed between number of pyramided genes and yield, a number of studies such as Coyne *et al.* (2000), Liebenberg *et al.* (2005), Fininsa and Tefera (2007), Ragagnin *et al.* (2009), Souza *et al.* (2014), Mulanya *et al.* (2014) and Miklas *et al.* (2017) reported no yield penalty as a result of pyramiding resistance genes in common beans. It should be noted, however, that these studies focused on the evaluation of advanced lines with pyramided resistance genes in comparison with available commercial varieties included as

checks, but did not go deeper to investigate the relationship between yield and pyramided genes through correlation and path analyses. Using the same approach used in the above studies, this part of the study evaluated yield performance of advanced lines with and without pyramided resistance genes alongside susceptible commercial varieties and results could not clearly reveal a yield penalty as three advanced lines with three-gene and two-gene pyramided resistance genes were among the top 10 out of over 50 advanced lines evaluated (Table 5.3). The lines were D16.3.3.11.160.5.6 (28.6gm seed weight per plant) with a three-gene pyramid followed by C44.1.4.5.142.4 (27.8gm) and C16.1.3.8.136.2 (26.8gms) with two-gene pyramids. These results indicate that through segregation and advancement of populations it is still possible to have lines that will favorably combine resistance and yield genes to have lines with pyramided resistance genes but with outstanding yield performance.

There is compelling evidence, however, that disease resistance affects crop performance (Bergelsen and Purrington, 1996; Heil, 2007). In order to establish resistance, plants have to cope with metabolic efforts that can cause considerable costs depending on plant growth stage and resource availability (Heil, 2007). The concept of fitness costs assumes that resistant plants have lower reproduction than less resistant plants when compared under disease free conditions (Simms and Fritz, 1990). If resistance has a substantial cost, therefore, it has commercial significance because it may hinder the more important objective of increasing yield (Brown, 2002). According to Brown (2002), certain deleterious genes may be also be linked to a resistance gene and affect yield through linkage drag, which makes it worthwhile for plant breeders to analyze such linkages and try to break them when the yield penalty is commercially significant.

5.5 Conclusion

There was high phenotypic and genotypic variability for the yield traits among advanced common bean populations. This high variability coupled with the high broad-sense heritability of most yield traits points to a high potential to identify and select from the populations superior genotypes with higher yields and enhanced broad-spectrum resistance to bean anthracnose. The relationship between number of pyramided resistance genes and seed weight per plant, number of pods per plant and number of seeds per plant was significantly negative, which translates in

to a yield penalty. The indirect effect of number of pyramided resistance genes on seed yield was significantly negative via number of seeds per. This was a further confirmation of existence of a yield penalty due to pyramided anthracnose resistance genes.

5.6 Recommendations

The advanced lines D16.3.3.11.160.5.6, C44.1.4.5.142.4 and C16.1.3.8.136.2 possessing pyramided anthracnose resistance genes are recommended for further development, evaluation and release as commercial varieties or/ and used as elite donor parents for anthracnose resistance genes. Further studies should be conducted to investigate specific combining ability (SCA) and general combining ability (GCA) of common bean elite germplasm to identify cultivars possessing high breeding potential for use as founding parents so as to maximize yield gain and reduce possible yield penalty effects in gene pyramiding programs.

CHAPTER SIX

General discussion, conclusions and recommendations

The study was undertaken with the overall objective to enhance broad-spectrum resistance to anthracnose disease among common bean varieties for increased productivity in Uganda.

The study investigated the pathogenic variation of *Colletotrichum lindemuthianum* in Uganda through screening of differential cultivars with isolates from eight districts. It was found that; *C. lindemuthianum* diversity was high with 27 races characterized from 51 isolates; races 2047 and 4095 were the most widely distributed in the sampled regions and races 4095, 2479 and 2047 respectively were the most virulent with race 4095 causing a susceptible reaction on all the 12 differentials. The pathogenic variability of *C. lindemuthianum* was highest in the Eastern and South Western highland regions of Uganda implying the need to deploy pyramided genes in these regions for effective management of bean anthracnose. The cultivars G2333, Cornell, TU, and ABI36 showed the most broad-spectrum resistance to the 27 races implying that they are best suited as sources of effective resistance in breeding programs in Uganda.

The study undertook a gene pyramiding scheme for bean anthracnose resistance genes with the help of SCAR markers and assessed the effectiveness of both single and pyramided genes. SCAR markers *SBB14*, *SAS13*, *SAB3* and *SB12* successfully tagged *Co-4²*, *Co-4³*, *Co-5* and *Co-9* anthracnose resistance genes. Thirty eight (38) populations with two anthracnose resistance genes; nine (9) populations with three anthracnose resistance genes; and 25 populations with single anthracnose genes were successfully identified with SCAR markers. Effectiveness of resistance against *C. lindemuthianum* conferred by different gene pyramids differed with *Co-4²+Co-5+Co-9* and *Co-4²+Co-5* combinations emerging as the best. The effectiveness of single genes against *C. lindemuthianum* also differed with *Co-4²* and *Co-5* single genes emerging the best and with good complementarity with each other and with other genes. But *Co-9* resistance gene was antagonistic with other genes and was always associated with increased symptoms.

Analysis of genetic variability among advanced populations revealed; a high genetic variability for all yield traits in the advanced bean populations indicating high potential for genetic improvement; PCV estimates higher than GCV estimates indicating the importance of environment in the improvement of these traits; low to moderate heritability estimates for number of pods per plant, number of seeds per plant and seed weight per plant; and low to moderate GAM estimates for days to 50% flowering and days to maturity and number of pods per plant implying that these traits are controlled by non-additive genes effects, respond poorly to selection and therefore heterosis breeding is beneficial for their improvement. Assessing the relationship between number of pyramided genes and yield traits revealed a significantly negative relationship with seed weight per plant, number of pods per plant and number of seeds per plant, and a significantly negative indirect effect with seed weight per plant via number of seeds per plant, implying yield penalty. The advanced lines D16.3.3.11.160.5.6, C44.1.4.5.142.4 and C16.1.3.8.136.2 with pyramided genes were among the top 10 yield performing lines.

6.1 General conclusion

Colletotrichum lindemuthianum had a high pathogenic variability in Uganda with 24 new races identified and the highest variability observed in the Eastern and South Western highland regions of Uganda. Two new highly aggressive races 2479 and 4095 are hereby reported. Resistance gene pyramiding was effective in conferring broad-spectrum resistance in some cases and was ineffective in other cases.

Effectiveness of pyramided resistance genes heavily depended on the fitness of individual resistance genes combined. $Co-4^2+Co-5+Co-9$ and $Co-4^2+Co-5$ were the most effective pyramid combinations while $Co-4^2$ and $Co-5$ were the most effective single genes. Single gene $Co-9$ exhibited antagonism when combined with other single genes.

High phenotypic and genotypic variability and moderate to high broad-sense heritability were observed for yield traits among advanced common bean populations. The relationship between number of pyramided resistance genes and seed weight per plant suggested a yield penalty arising from pyramiding higher number of anthracnose resistance.

6.2 General recommendation

New varieties with broad-spectrum resistance should be developed and deployed especially in Eastern and South Western Uganda in urgent response to diverse and aggressive *C. lindemuthianum* and management of bean anthracnose disease. The cultivars G2333, Cornell 49-242, TU and AB136 are recommended for use as sources of broad-spectrum resistance to bean anthracnose in Uganda. Further studies should be conducted to; identify and test other sources of effective resistance against *C. lindemuthianum* races in Uganda; assess effectiveness and fitness of *Co-4*², *Co-5*², *Co-5*, *Co-3*, *Co-6* *co-8* Mesoamerican and, *Co-1*³, *Co-1*⁵ Andean single resistance genes; and use these single genes in different pyramid combinations and assess effectiveness against *C. lindemuthianum* in Uganda.

Resistance gene combinations *Co-4*²+*Co-5*+*Co-9* and *Co-4*²+*Co-5* are recommended as effective pyramids for use in Uganda. The single genes *Co-4*² and *Co-5* are recommended for deployment in cultivars specifically developed for areas where race variability is not high to avoid easy breakdown. Cultivars with single genes and pyramid gene combinations should be monitored periodically and assessed for durability of resistance over time. Races 2047 and 767 are recommended for use in artificial inoculation while undertaking studies on resistance to bean anthracnose disease. Further studies should be conducted to identify and assess new effective single genes and pyramid gene combinations using other available sources of anthracnose resistance genes.

The advanced lines D16.3.3.11.160.5.6, C44.1.4.5.142.4 and C16.1.3.8.136.2 possessing pyramided anthracnose resistance genes are recommended for further development, evaluation and release as commercial varieties or/ and used as elite donor parents for anthracnose resistance genes. Further studies should be conducted to investigate specific combining ability (SCA) and general combining ability (GCA) of common bean elite germplasm to identify cultivars possessing high breeding potential for use as founding parents so as to maximize yield gain and reduce possible yield penalty effects in gene pyramiding programs.

REFERENCES

- Agrios, G.N. 1997. Control of plant diseases. In: Plant Pathology, 4th Edition. Academic Press, San Diego, California:pp200-216.
- Akintunde, A. 2012. Path analysis step by step using MS Excel. Journal of Technical Science and Technologies 1(1):9-15.
- Allard, R.W. 1960. Principles of plant breeding. John Wiley and Sons. New York: 485.
- Allen, D.J. 1983. The pathology of tropical food legumes: disease resistance in crop improvement. Chichester, UK: John Wiley and Sons.
- Alzate-Marin, A. L. and Sartorato, A. 2004. Analysis of the pathogenic variability of *Colletotrichum lindemuthianum* in Brazil. Annual Report of the Bean Improvement Cooperative 47:241-242.
- Alzate-Marin, A.L., Barros, E.G. and Moreira, M.A. 1999. Co-evolution model of *Colletotrichum lindemuthianum* (Melanconiaceae, Melanconiales) roots that occur in some Brazilian regions. Genetics and Molecular Biology 22(1):115-118.
- Alzate-Marin, A.L., de Moraes Silva, M.A., de Oliveira, E.J., Moreira, M.A. and de Barros, E.G. 2003. Identification of the second anthracnose resistance gene present in the common bean cultivar PI207262. Annual report. Bean Improvement Cooperative 46:177-178.
- Alzate-Marin, A.L., de Souza, K.A., Silva, M.G.M., Oliveira, E.J., Moreira, M.A. and Barros, E.G. 2007. Genetic characterization of anthracnose resistance genes *Co-4*³ and *Co-9* in common bean cultivar Tlalnepantla 64 (PI 207262). Euphytica 154:1-8.
- Amin, M.R., Barma, N.C.D. and Razzaque, M.A. 1992. Variability, heritability, genetic advance and correlation study in some quantitative characters in durum wheat. Rachis Newsletter 11:30-32.
- Andrus, C.F. and Wade B.L. 1942. The factorial interpretation of anthracnose resistance in beans. Technical Bulletin. USDA Washington D.C. 810.
- Andriani, V., Soegianto, A. and Kuswanto. 2015. Evaluation of genetic variability and genetic advance to qualitative and quantitative for selection of expected lines of purple common bean (*Phaseolus Vulgaris* L). Journal of Agriculture and Veterinary Science 8(1):44-50.
- Anonymous. 2009. GenStat Discovery, 12th Edition. Lawes Agricultural Trust.

Ansari, K.I., Palacios, N., Araya, C., Langin, T., Egan, D., Doohan, F.M. 2004. Pathogenic and genetic variability among *Colletotrichum lindemuthianum* isolates of different geographic origins. Plant Pathology 53(5):635-642. doi.org/10.1111/j.0032-0862.2004.01057.x.

Asensio, S., Manzanera, M.C., Asensio, C. and Singh, S.P. 2005. Introgressing resistance to bacterial and viral diseases from the middle American to Andean common bean. Euphytica 143(1-2):223-228. doi:10.1007/s10681-005-3860-9.

Awale, H., E. Falconi, J.C. Villatoro and J. D. Kelly. 2007. Control and characterization of *Colletotrichum lindemuthianum* Isolates from Ecuador and Guatemala. Annual Report. Bean Improvement Cooperative 50:85:86.

Awale, H.E. and Kelly, J.D. 2001. Development of SCAR markers linked to *Co-4²* gene in common bean. Annual Report. Bean Improvement Cooperative 44:119-120.

Balardin, R.S. and Kelly, J.D. 1996. Identification of race 65-epsilon of bean anthracnose (*Colletotrichum lindemuthianum*) in Michigan. Plant Diseases 80:712. doi: 10.1094/PD-80-0712B.

Balardin, R.S. and Kelly, J.D. 1998. Interaction among races of *Colletotrichum lindemuthianum* and diversity in *Phaseolus vulgaris*. Journal of American Society of Horticultural Sciences 123:1038-1047.

Balardin, R.S., Jarosz, A. and Kelly, J.D. 1997. Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central and North America. Phytopathology 87:1184 - 1191.

Banneroth, H. 1965. Results of infection of a collection of beans by six phy-siological races of anthracnose. Annals of Plant Breeding 15(2):201-222.

Barcelos, Q.L., Pinto, J.M., Vaillancourt, L.J. and Souza, E.A. 2014. Characterization of Glomerella strains recovered from anthracnose lesions on common bean plants in Brazil. PLoS One 9(6): e90910. doi.org/10.1371/journal.pone.0090910

Barrus, M.F. 1911. Variation of varieties of beans in their susceptibility to anthracnose. Phytopathology 1:190-195.

Beebe, S. 2012. Common beans breeding in the Tropics. Plant Breeding Reviews. Volume 36. First Edition. Edited by Jules Janick 2012 Wiley-Blackwell. Published 2012 by John Wiley & Sons, Inc:357-427.

Benard-Capelle, J., Soubeyrand, S. Neema, C. 2006. Reproductive consequences of *Colletotrichum lindemuthianum* (Ascomycota) infection on wild bean plants (*Phaseolus vulgaris*). Canadian Journal of Botany 84(10):1542-1547. doi.org/10.1139/b06-114.

Bergelsen, J., Purrington, C.B. 1996. Surveying patterns in the cost of resistance in plants. *American Naturalist* 148:536-558.

Bigirimana, J., Fontaine, R. and Hofte, M. 2000. Bean anthracnose: virulence of *Colletotrichum lindemuthianum* isolated from Burundi, Central Africa. *Plant Disease Notes* 84:491. doi.org/10.1094/PDIS.2000.84.4.491C.

Blondet, A. 1963. Bean anthracnose - French Physiological Breeds Study of *Colletotrichum lindemuthianum*. Thesis, Faculty of Science, Paris, France:160.

Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P. and Vanderleyden, J. 2003. Beans (*Phaseolus spp*) model food legume. *Plant Soil* 252:55-128. doi:10.1023/A:1024146710611.

Brown, J. and Caligari, P. 2008. An introduction to plant breeding. Blackwell Publishing Ltd, Oxford, UK.

Brown, J.K.M, 2002. Yield penalties of disease resistance in crops. *Current Opinion in Plant Biology* 5: Elsevier Science Ltd. doi 10.1016/S1369-5266(02)00270-4.

Burkholder, W.H. 1923. The gamma strain of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Brio. & Cav. *Phytopathology* 13:316-323.

Burton, G.W., 1952. Quantitative inheritance in grasses. *Proceedings of the International Grassland Congress* 1: 277-283.

Buruchara, R., Mukankusi, C., Ampofo, K. 2010. Bean diseases and pest identification and management. International Center for Tropical Agriculture (CIAT); Pan-African Bean Research Alliance (PABRA)-Handbook for Small Scale Seed Producers.

Buruchara, R.A. 1991. Use of soil amendments in the management of root rots. CIAT African Workshop Series No. 17. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia:6.

Byrne, P. and Richardson, K. 2005. Marker Assisted Selection: objectives and overview. *Plant and Soil Science eLibrary*. <http://croptechnology.unl.edu>.

CAB International. 2004. Crop protection compendium. 2004 Edition. CAB International Publishing. Wallingford, UK.

Campa, A., Rodriguez-Suarez, C., Paneda, A., Giraldez, R. and Ferreira, J. 2005. The bean anthracnose resistance gene *Co-5* is located in linkage group B7. *Annual report* 48(48):68-69.

Campa, A., Rodríguez-Suárez, C., Giraldez, R., Ferreira, J.J. 2014. Genetic analysis of the response to eleven *Colletotrichum lindemuthianum* races in a RIL population of common bean

(*Phaseolus vulgaris* L.). BioMed Central Plant Biology 14:115. doi: 10.1186/1471-2229-14-115.

Caporalino, C., Palloix, A., Fazari, A., Marteu, N., Barbary, A., Abad, P., Sage-Palloix, A.M., Mateille, T., Risso, S., Lanza, R., Taussig, C. and Castagnone-Sereno, P. 2014. Pyramiding, alternating or mixing: comparative performances of deployment strategies of nematode resistance genes to promote plant resistance efficiency and durability. Biomedical Central Plant Biology 14:53. doi: 10.1186/1471-2229-14-53.

Champion, M.R., Brunet, D., Maudit, M.L., Ilami, R. 1973. Method of controlling the resistance of bean varieties to anthracnose (*Colletotrichum lindemuthianum* (Sacc & Magn.) Briosi et Cav. C. R. Hebd). Seances Academy of Agriculture, France 59: 951-958.

Chiorato, A.F., Carbonell, S.A.M., de Moura, R.R., Ito, M.F. and Colombo, C.A. 2006. Co-evolution between physiological races of *Colletotrichum lindemuthianum* and common bean. Bragantia 65(3). 381-388. doi.org/10.1590/S0006-87052006000300003.

CIAT.1988. Annual report: Bean program. Work document 72. CIAT, Cali, Colombia.

CIAT, 1997. Bean program annual report 1995. Working Document No. 163, 1997.

CIAT. 2001. Centro Internacional de Agricultura Tropical. Common bean: The nearly perfect food. In: CIAT in focus. 2001. www.ciat.cgiar.org/ciatinfocus/index.htm.

Ciftci, V., Togay, N., Togay, Y. and Dogan, Y. 2004. Determining relationships among yield and some yield components using path coefficient analysis in chickpea (*Cicer arietinum* L.). Asian Journal of Plant Sciences 3(5):632-635.

Cortes, A.J., F.A. Monserrate, J. Ramirez-Villegas, S. Madrinan, and M. Blair. 2013. Drought tolerance in wild plant populations: the case of common beans (*Phaseolus vulgaris* L.). PLoS ONE 8, e6289. doi:10.1371/journal.pone.0062898.

Coyne, D.P., Nuland, D.S., Lindgren, D.T., Steadman, J.R.; Smith, D.W.; Gonzales, J., Schild, J., Reiser, J. Sutton, L., Carlson, C., Stavely, J.R. and Miklas, P. 2000. 'Weihsing' Great Northern disease-resistant dry bean. Agronomy and Horticulture: Paper 638.

Dabholker, A.R. 1992. Elements of biometrical genetics. Concepts Publishing Company, New Delhi, India:431.

Darben, L.M., Gonela, A., Elias, H.T., Pastre, H.H. and Goncalves-Vidigal, M.C. 2017. Common bean germplasm resistant to races 73 and 2047 of *Colletotrichum lindemuthianum*. African Journal of Biotechnology 16(19):1142-1149. doi: 10.5897/AJB2016.15751.

David, P., Chen, N.W., Pedrosa-Harand, A., Thareau, V., Sévignac, M., Cannon, S.B., Debouck, D., Langin, T., Geffroy, V. 2009. A nomadic subtelomeric disease resistance

gene cluster in common bean. *Plant Physiology* 151(3):1048-1065. doi: 10.1104/pp.109.142109.

David, S. 1999. Beans in the farming system and domestic economy of Uganda: a tale of two parishes. Network on Bean Research in Africa, Occasional Publications Series, No. 28, CIAT, Kampala, Uganda.

Davide, L.M.C. and Souza, E.A. 2009. Pathogenic variability within race 65 of *Colletotrichum lindemuthianum* and its implications for common bean breeding. *Brazilian Society of Plant Breeding. Crop Breeding and Applied Biotechnology* 9:23-30.

Day, P.R. 1973. Genetic variability in crops. *Annual Review of Phytopathology* 11:293-312.

Ddamulira, G., Mukankusi, C., Ochwo-Ssemakula, M., Edema, R., Sseruwagi, P. and Gepts, P. 2015. Gene Pyramiding improved resistance to angular leaf spot in common bean. *Australian Journal of Experimental Agriculture* 9(2):1-12. doi: 10.9734/AJEA/2015/17833.

De Lima Castro, S.A., Gonçalves-Vidigal, M.C., Gilio, T.A.S., Lacanallo, G.F., Valentini, G., Martins, V.S.R., Song, Q., Galván, M.Z., Hurtado-Gonzales, O.P. and Pastor-Corrales, M.A. 2017. Genetics and mapping of a new anthracnose resistance locus in Andean common bean Paloma. *Biomedical Central Genomics* 18:306. doi 10.1186/s12864-017-3685-7.

De Silva, D.D., Crous, P.W., Ades, P.K., Hyde, K.D. and Taylor, P.W.J. 2017. Life styles of *Colletotrichum* species and implications for plant biosecurity. *Elsevier, Fungal Biology Reviews* 31:155-168.

Del Rio, L. and Bradley, C. 2004. Anthracnose of dry beans. North Dakota State University Extension Service, North Dakota State University, Fargo, North Dakota. 58105.

Doyle, J.J. and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19:11-15.

Falconer, D.S. and Mackay, T.F.C. 1996. Introduction to quantitative genetics. 4th Edition. Benjamin Cummings, England, ISBN-10: 0582243025.

FAOSTAT, 2008. <http://faostat.fao.org/site/339/default.aspx>>

FAOSTAT, 2012. <http://faostat.fao.org/site/616/DesktopDefault.aspx?PageID=616#ancor>

FAOSTAT, 2015. World food and agriculture. <http://www.fao.org/3/a-i4691e.pdf>. Food and Agriculture Organization of the United Nations, Rome. ISBN 978-92-5-108802-9.

Ferreira, J.J., Campa, A., Perez-Vega, E., Rodriguez-Suarez, C. and Giraldez, R. 2012. Introgression and pyramiding into common bean market fabada of genes conferring resistance to anthracnose. *Theoretical and Applied Genetics* 124:777-788.

Fininsa, C. and Tefera, T. 2007. Multiple disease resistance in common bean genotypes and their agronomic performance in eastern Ethiopia. *International Journal of Pest Management* 52(4):291-296. doi.org/ 10.1080/ 09670870600811836.

Garzon, L.N., Ligarreto, G.A. Blair, M.W. 2008. Molecular marker-assisted backcrossing of anthracnose resistance into Andean climbing beans (*Phaseolus vulgaris* L.). *Crop Science* 48:562-570. doi: 10.2135/cropsci2007.08.0462.

Geffroy, V., Sicard, D., de Oliveira, J.C.F., Sévignac, M., Cohen, S., Gepts, P., Neema, C., Langin, T. and Dron, M. 1999. Identification of an ancestral resistance gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*. *Molecular Plant Microbe Interaction* 12(9):774-784.

Genchev, D., Christova, P., Kiryakov, I., Beleva, M. and Batchvarova, R. 2010. Breeding of common bean for resistance to the physiological races of anthracnose identified in Bulgaria, *Biotechnology and Biotechnological Equipment* 24(2):1814-1823. doi.org/10.2478/V10133-010-0047-X.

Gepts, P. 1998. Phaseolin as an evolutionary marker. In: Gepts, P. (Ed). *Genetics resources, domestication and evolution of phaseolus beans*. Dordrecht: Kluwer:215-241.

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

Gonzalves-Vidigal, M.C., Correa A.M., Destro, D., Ferreira, L.C. and Sobrinho T.A. 2003. Correlation and path analysis of common bean grain yield and its primary components. *Crop Breeding and Applied Biotechnology* 3(3):217-222.

Govindaraj, M., Selvi, B. and Kumar, I.S. 2011. Genetic diversity studies in indigenous pearl millet (*Pennisetum glauccum* (L) R. Br.) accessions based on biometrical and nutritional quality traits. *Indian Journal of Plant Genetic Resources* 24(2): 186-193.

Grace, J.B. and Bollen, K.A. 2005. Interpreting the results from multiple regression and structural equation models. *Bulletin of the Ecological Society of America* 15(5).

Greenway, P. 1945. The origin of some East African food plants, III. *East African Agricultural Journal* 10:177-180.

Griffiths, A.J.F., Miller, J.H., Suzuki, D.T., Lewontin, R.C. and Gelbart, W.M. 2000. *An introduction to genetic analysis*. 7th Edition. New York. W.H. Freeman. ISBN-10: 0-7167-3520-2.

- Guler, M., Adak, M.S. and Ulukan, H. 2001. Determining relationships among yield and some yield components using path coefficient analysis in chickpea (*Cicer arietinum* L). European Journal of Agronomy 14:161-166.
- Heil, M. and Baldwin, I.T. 2002. Fitness costs of induced resistance: emerging experimental support for a slippery concept. Trends in Plant Science 7:61-67.
- Heil, M. 2007. Trade-offs associated with induced resistance. In: Induced resistance for plant defence: a sustainable approach to crop protection. Edited by Dale Walters, Adrian Newton and Gary Lyon. Blackwell Publishing:171-192.
- Hubbeling, N. 1957. New aspects of breeding for disease resistance in beans (*Phaseolus vulgaris*). Euphytica 6:111-141.
- Hubberling, H. 1961. In Recent Advances in Botany. University of Toronto Press:438-443.
- Hubbeling, N. 1977. The new jota race of *Colletotrichum lindemuthianum*. Annual report for Bean Improvement Cooperative.
- Inglis, D.A., D.J. Hagedorn and Rand, R.E. 1988. Use of dry inoculum to evaluate beans for resistance to anthracnose and angular leaf spot. Plant Disease 72:771-774.
- Ishikawa, F.H., Souza, E.A., Shoji, J.Y., Connolly, L. 2012. Heterokaryon incompatibility is suppressed following conidial anastomosis tube fusion in a fungal plant pathogen. PLoS One 7: e31175. <http://dx.doi.org/10.1371/journal.pone.0031175>.
- ISSD. 2012. Uganda Seed Sector Assessment. Integrated Seed Sector Development Briefing note. Eds. Walter Simon de Boef, Gareth Borman and Elizabeth O'Keeffe. Centre for Development Innovation, Wageningen University and Research Centre, Wageningen, Netherlands:1-7.
- Johnson, H.W., Robinson, H. and Comstock, R. F. 1955. Estimates of genetic and environmental variability in soybean. Agronomy Journal 47:314-318.
- Joshi, K.R. and Nayak, S. 2010. Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. Biotechnology and Molecular Biology Review 5(3):51-60.
- Kalyebara, R. 2008. The impact of improved bush bean varieties in Uganda. Network on Bean Research in Africa. CIAT, Kampala, Uganda. Occasional Publication Series 43.
- Kaplan, L. 1965. Archeology and domestication in American Phaseolus (Beans). Economic Botany 19:358-368. doi:10.1007/BF02904806.
- Kelly J.D. and Vallejo, V.A. 2004. A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. HortScience 39:1196-1207.

Kelly, J.D. 2004. Advances in common bean improvement: some case histories with broader applications. In: XXVI International Horticultural Congress: Advances in Vegetable Breeding. International Society of Horticultural Science. Acta Horticulturae 637: DOI 10.17660/ActaHortic.2004.637.11.

Kelly, J.D., Afanador, L. and Cameroon, L.S. 1994. New races of *Colletotrichum lindemuthianum* in Michigan and implications in dry bean resistance breeding. Plant Disease 8:892-894.

Kelly, J.D., Afanador, L., Haley, S.S. 1995. Pyramiding genes resistance to bean common mosaic virus. Euphytica 82:207-212.

Kilimo Trust, 2012. Development of Inclusive Markets in Agriculture and Trade (DIMAT): The Nature and Markets of Bean Value Chains in Uganda.

Kivuru, A.N., 2013. Introgression of bean anthracnose resistance genes in common bean (*phaseolus vulgaris* L) lines with angular leafspot, common bacterial blight, and bean common mosaic virus disease resistances. MSc Thesis. Sokoine University of Agriculture, Tanzania.

Kjær, B., Jensen, H.P., Jensen, J. and Jørgensen, J.H. (1990). Associations between three ml-o powdery mildew resistance genes and agronomic traits in barley. Euphytica 46 (3):185-193.

Kolmer, J.A., Dyck, P.L. and Roelfs, A.P. 1991. An appraisal of stem and leaf rust resistance in North American hard red spring wheats and the probability of multiple mutations to virulence in populations of cereal rust fungi. Phytopathology 81(3):237-239.

Krüger, J., Hoffman, G.M. and Hubbelling, N. 1977. The Kappa race of *Colletotrichum lindemuthianum* and sources of resistance to anthracnose in Phaseolus beans. Euphytica 26:23-25.

Kumar, A., Sharma, P.N, Sharma, O.P, Tyagi, P.D. 1999. Epidemiology of bean anthracnose *Colletotrichum lindemuthianum* under sub-humid mid-hills zone of Himachal Pradesh. Indian Phytopathology 52:393-397.

Kute, N.K.S. and Kumar.A.R. 2013. Principles of plant breeding. First Edition. ISBN: 8192229025. New Delhi. 260p.

Lacanallo, G.F., Gonçalves-Vidigal, M.C., Vidigal Filho, P.S., Kami, J. and Gonela, A. 2010. Mapping of an Andean gene resistant to anthracnose in the land race Jalo Listras Pretas. Annual report of Bean Improvement Cooperative 53: 96-97. ISSN 0084-7747.

Latter, B.D.H., McIntosh, R.A., Ellison, F.W., Brennan, P.S., Fisher, J., Hollamby, G.J., Rathjen, A.J. and Wilson, R.E. 1998. Grain yields of near-isogenic lines with added genes for stem rust resistance. Proceedings of the 7th International Wheat Genetics Symposium: 1998 July

13–19; Cambridge, UK. Edited by Miller T.E, Koebner R.M.D. Cambridge, UK: Institute for Plant Science Research. 1988:901-906.

Le Gouis, J., Jestin, L., Ordon, F., de Froidmont, D., Béghin, D., Joseph, J.L. and Froidmont, F. 1999. Agronomic comparison of two sets of SSD barley lines differing for the *ym4* resistance gene against barley mosaic viruses. *Agronomie* 19:125-131.

Leaky, C.L.A. and Simbwa-Bunnya, M. 1972. Races of *Colletotrichum lindemuthianum* and implications for bean breeding in Uganda. *Annals of Applied Biology* 70: 25-34.

Liebenberg, M. M., Liebenberg, A. J. and Pretorius, Z. A. 2005. Breeding for rust resistance increases dry bean productivity in South Africa. *South African Journal of Plant and Soil* 22(2):129-139. doi: 10.1080/02571862.2005.10634695.

Mahuku, S. G and J. J. Riascos, 2004. Virulence and molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. *European Journal of Plant Pathology* 110:253–263.

Mahuku, S.G., Buruchara, R., Navia, M. and Otsyula, R. 2007. Development of PCR markers tightly linked to *Pyult1*, a gene that confers *Pythium* root rot resistance in the common bean genotype AND1062. *Phytopathology* 97:69-79.

McGrann, G.R.D., Stavrinides, A., Russell, J., Corbitt, M.M., Booth, A., Chartrain, L., Thomas, W.T.B., Brown, J.K.M. 2014. A trade-off between *mlo* resistance to powdery mildew and increased susceptibility of barley to a newly important disease, *Ramularia* leaf spot. *Journal of Experimental Botany* 65(4):1025-1037. doi.org/10.1093/jxb/ert452.

Melotto, M., Balardin, R.S. and Kelly, J.D. 2000. Host–pathogen interaction and variability of *Colletotrichum lindemuthianum*. In: Prusky D, Freeman S and Dickman MB (eds) *Colletotrichum* host specificity, pathology, and host–pathogen interaction APS Press:346-361.

Melotto, M. and Kelly, J.D. Fine mapping of the *Co-4* locus of common bean reveals a resistance gene candidate, *COK-4*, that encodes for a protein kinase. *Theoretical and Applied Genetics* 103:508–517.

Mendez de Vigo, B., Rodriguez, C., Paneda, A., Giraldez, R. and Ferreira, J.J. 2002. Development of a SCAR marker linked to *Co-9* gene in common bean. *Annual Report. Bean Improvement Cooperative* 45:116-117.

Méndez-Vigo, B., Rodríguez-Suárez, C., Pañeda, A., Ferreira, J.J. and Giraldez, R. 2005. Molecular markers and allelic relationships of anthracnose resistance gene cluster B4 in common bean. *Euphytica* 141:237-245.

Miklas, P.N, Fourie, D., Chaves, B. and Chiremba, C. 2017. Common bacterial blight resistance QTL BC420 and SU91 effect on seed yield, seed weight, and canning quality in dry bean. *Crop Science* 57:802–811.

- Miklas, P.N., Kelly, J.D., Beebe, S.E. and Blair, M.W. 2006. Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica* 147: 105–131.
- Miklas, P.N., W.C. Johnson, R. Delorme, R.H. Riley, and P. Gepts. 2001. QTL conditioning physiological resistance and avoidance to white mold in dry bean. *Crop Science* 41:309–315.
- Mohammed A (2013). An Overview of distribution, biology and the management of common Bean anthracnose. *Journal of Plant Pathology and Microbiology* 4(8):193. doi:10.4172/2157-7471.1000193.
- Mota, S.F., Barcelos, Q.L., Dias, M.A. and Souza, E.A. 2016. Variability of *Colletotrichum* spp in common bean. *Genetics and Molecular Research*. 15(2). doi.org/10.4238/gmr.15027176.
- Mahuku, S.G., Buruchara, R., Navia, M. and Otsyula, R. 2007. Development of PCR markers tightly linked to *Pyult1*, a gene that confers *Pythium* root rot resistance in the common bean genotype AND1062. *Phytopathology* 97:69-79.
- Mukankusi, C., Nkalubo, S. Kiryowa, M., Ddamulira, G., Obala, J., Sekamatte, A., Ochowo M., Tukamuhabwa, P., Gepts, P., Gibson, P. and Buruchara, R. 2016. Gene pyramiding resistance to major diseases of common bean (*Phaseolus vulgaris*). A poster presented at the 2016 Pan-African grain legume and World Cowpea conference. 28th February - 4th March 2016, Livingston, Zambia.
- Mukankusi, C.M., Male, A., Buruchara, R.A., Abang, M. M. 2012. Developing multiple disease resistant breeding parents through gene pyramiding with the aid of molecular markers. International Center for Tropical Agriculture (CIAT), Pan-Africa Bean Research Alliance – PABRA. Nairobi, KE, 1p. <https://hdl.handle.net/10568/92984>.
- Mulanya, M.M., Kimani, P.M and Narla, R.D. 2014. Selection for multiple disease resistance in bush snap bean lines developed in Kenya. University of Nairobi Library copyright 2002-2016.
- Munda, A., Radisek, S., Šuštar-Vozlic, J. and Javornik, B. 2009. Genetic variability of *Colletotrichum lindemuthianum* isolates from Slovenia and resistance of local *Phaseolus vulgaris* germplasm. *Journal of Plant Diseases and Protection* 116(1): 23-29. doi:10.1007/BF03356282.
- Mundt, C.C. 1990. Probability of mutation to multiple virulence and durability of resistance gene pyramids. *Phytopathology* 80(3):221-223.
- Mundt, C.C. 1991. Probability of mutation to multiple virulence and durability of resistance gene pyramids. *American Phytopathological Society* 81(3): 240-242.
- Mundt, C.C. 2014. Durable resistance: a key to sustainable management of pathogens and pests. *Infection Genetics and Evolution*:446-455. doi: 10.1016/j.meegid.2014.01.011.

Murrel, D. 2016. Global research and funding survey on pulse productivity and sustainability. A report for the Productivity and Sustainability Committee, Food and Agriculture Organization (FAO) International Year of Pulses, 2016.

Mwesigwa, J.B. 2008. Diversity of *Colletotrichum lindemuthianum* and reaction of common bean germplasm to anthracnose disease. MSc. Thesis. Makerere University. Uganda.

National Development Plan (2010). Growth, Employment and Socioeconomic Transformation. Government of Uganda.

Negash, K. 2006. Studies on genetic divergence in common bean (*Phaseolus vulgaris* L.) introductions of Ethiopia. MSc. Thesis. Addis Ababa University.

Njuguna, S.M. 2014. Marker assisted gamete selection for multiple disease resistance in Mesoamerican bean genotypes and race typing of angular leafspot pathogen in Kenya. Msc Thesis. University of Nairobi.

Nkalubo, S., Melis, R., Laing, M.D. and Opio, F. 2007. Yield loss associated with anthracnose disease on Ugandan market-class dry bean cultivars. African Crop Science Conference Proceedings 8:869-874.

Nkalubo, S.T. 2006. Physiological races of dry bean anthracnose (*Colletotrichum lindemuthianum*) in Uganda. PhD thesis. University of KwaZulu Natal, South Africa.

Nkalubo, S.T., Melis, R., Derera, J., Laing, M.D. and Opio, F. 2009. Genetic analysis of anthracnose resistance in common bean breeding source germplasm. Euphytica 167(3):303-312. doi.10.1007/s10681-008-9873-4.

Obala, J., Mukankusi, C., Rubaihayo, P.R., Gibson, P. and Edema, R. 2012. Improvement of resistance to Fusarium root rot through gene pyramiding in common bean. African Crop Science Journal 20(1):1-13.

Ogallo L.J. 1991. Pathogenic variation: its nature, origin and measurement. In: First Pan-African working group meeting on anthracnose of beans. Workshop proceedings. CIAT African Workshop Series 15.

Opio, F., Kyamanywa, M.A., David, S. and Mugisha-Mutetikka, M. 2001. In Joseph Mukiibi (ed) Agriculture in Uganda (Vol. 11). National Agriculture Research Organization (NARO).

Otsyula, R.M., Rubaihayo, P., Buruchara, R., Mahuku, G. and Kimani, P. 2005. Inheritance and genetic characterization of *Pythium* root rot-resistant bean varieties. 24-27 January 2005. Biotechnology, Breeding and Seed Systems for African Crops. Kenya Agricultural Research Institute (KARI), Nairobi, Kenya.

Pachico, D. 1993. The demand for bean technology: In G. Henry (ed) Trends in CIAT's commodities, CIAT Working Document 128: 60-73, Cali, Colombia.

Padder, B.A., Sharma P.N., Sharma O.P., Kapoor V. 2007. Genetic diversity and gene flow estimates among five populations of *Colletotrichum lindemuthianum* across Himachal Pradesh. *Physiological and Molecular Plant Pathology* 70: 8-12.

Pastor-Corrales, M.A. 1991. Estandarización de variedades diferenciales y de designación de razas de *Colletotrichum lindemuthianum*. *Phytopathology* 81:694.

Pastor-Corrales, M.A. and Tu, J.C. 1989. Anthracnose. In: H.F. Schwartz and M.A. Pastor-Corrales, (eds) Bean production problems in the Tropics. CIAT, Cali, Colombia:77-104.

Pastor-Corrales, M.A., Kelly, J.D., Steadman, J.R., Dale, Lindgren, T., Stavely, J.R. and Coyne, D.P. 2007. Registration of six great northern bean germplasm lines with enhanced resistance to rust and bean common mosaic and necrosis potyviruses. *Journal of Plant Registrations* 1:77-79. doi: 10.3198/jpr2005.12.0517crg.

Pastor-Corrales, M.A., Erazo, O.A., Estrada, E.I. and Singh, S.P. 1995. Inheritance of anthracnose resistance in common bean accession G2333. *Plant Disease* 78: 959-962.

Pastor-Corrales, M.A., Llano, G.A., Afanador, L., and Castellanos, G. 1985. Disease resistance mechanism in beans to the anthracnose pathogen. (Abstract) *Phytopathology* 75: 1176.

Pastor-Corrales, M.A., Otoya, M.M., Molina, A. and Singh, S.P. 1995. Resistance to *Colletotrichum lindemuthianum* isolates from Middle American and Andean South America in different common bean races. *Plant Disease* 79:63-67.

Peake, J.N., Copp, A.J. and Shawe, J. 2013. Knowledge and peri-conceptional use of folic acid for the prevention of neural tube defects in ethnic communities in the United Kingdom: systematic review and meta-analysis. *Birth Defects Research (Part A)* 97(7):444-451.

Pradhan, S.K., Nayak, D.K., Mohanty, S., Behera, L., Barik, S.R., Pandit, E., Lenka, S. and Anandan, A. 2015. Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deep water rice variety, Jalmagna. *Rice (N Y)* 8:19. doi:10.1186/s12284-015-0051-8.

Raffi, S.A. and Nath, U.K. 2004. Variability, heritability, genetic advance and relationships of yield and yield contributing characters in dry bean (*P. vulgaris* L.). *Journal of Biological Sciences* 4:157-159.

Ragagnin, V.A., De Souza, T.L.P.O, Sanglard, D.A., Arruda, K.M.A., Costa, M.R., Alzate-Marin, A.L. Carneiro, S., Moreira, M.A. and De Barrios, E.G. 2009. Development and agronomic performance of common bean lines simultaneously resistant to anthracnose, angular leaf spot and rust. *Plant Breeding* 128:156-163.

- Ramalho M.A.P., Santos, J. B., Zimmermman, M.J. *et al.* 1993. Quantitative genetics of autogamous plants: application to bean breeding. Science open research and publishing network, Boston:271.
- Rava, C.A., Molina, J., Kauffmann, M. and Briones, I. 1993. Determination of physiological races of *Colletotrichum lindemuthianum* in Nicaragua. Fitopatologia Brasileira 18:388-391.
- Rasel, M., Hassan, H., Hoque, M.I. and Saha S.R. 2018. Estimation of genetic variability, correlation and path coefficient analysis in local landraces of rice (*Oryza sativa* L.) for the improvement of salinity tolerance. Journal of Bangladesh Agricultural University 16(1): 41-46.
- REX Consortium. 2016. Combining Selective Pressures to Enhance the Durability of Disease Resistance Genes. Frontiers in Plant Science 7:1916. doi: 10.3389/fpls.2016.01916.
- Ribeiro, T., Esteves, J.A.F., Silva, D.A., Gonçalves, J.G.R., Carbonell, S.A.M. and Chiorato, A.F. 2016. Classification of *Colletotrichum lindemuthianum* races in differential cultivars of common bean. Acta Scientiarum. Agronomy 38(2):179-184.
- Roca, M.G., Davide, L.C., Mendes-Costa, M.C. and Wheals, A. 2003. Conidial anastomosis tubes in *Colletotrichum*. Fungal Genetics and Biology 40:138-145. doi.org/10.1016/S1087-1845(03)00088-4.
- Rocha, G.S., Pereira, L.P.L., Carneiro, P.C.S., Paula-Júnior, T.J., and de Souza Carneiro, J.E. 2012. Common bean breeding for resistance to anthracnose and angular leaf spot assisted by SCAR molecular markers. Crop Breeding and Applied Biotechnology 12: 34-42.
- Rodriguez and Barnado, 2014. Major constraints and trends for common bean production and commercialization; establishing priorities for future research. Agronomía Colombiana 32(3). 423-431, 2014. Doi: 10.15446/agron.colomb.v32n3.46052.
- Rodriguez, G.R. 1991. Identification of pathogenic races of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib. in the east of Durango through an internationally proposed system and response of drought-tolerant bean genotypes to pathogen breeds. M.Sc. Thesis. Universidad Autonoma Agraria Antonio Narro, Buenavista, Mexico.
- Roush, R.T. 2009. Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? Philosophical Transactions of the Royal Society of London 353:1777-1786. doi: 10.1098/rstb.1998.0330.
- Santa-Cruz, J.H., Kump, K.L., Arellano, C., Goodman, M.M., Krakowsky, M.D., Holland, J.B. and Balint-Kurti, P.J. 2014. Yield effects of two southern leaf blight resistance loci in maize. Hybrids Crop Science 54:882-894. doi: 10.2135/cropsci2013.08.0553.
- Schafer, J.F. and Roelfs, A.P. 1985. Estimated relation between numbers of urediniospores of *Puccinia graminis* f.sp. *tritici* and rates of occurrence of virulence. Phytopathology 75:749-750.

- Schnock, M. G., Hoffmann, G. M. and Krüger, J. 1975. A new physiological strain of *Colletotrichum lindemuthianum* infecting *Phaseolus vulgaris* L. Horticultural Science 10:140.
- Schreiber, N. 1932. Resistanz Zuchtung bei *Phaseolus vulgaris*. Phytopathology 4:415-454.
- Schwartz, H. and Pastor-Corrales, M.A. 1989. Bean production problems in the Tropics. Verlag Josef Margraf. ISBN-10: 382361147X.
- Schwartz, H.F., Steadman, J.R., Hall, R. and Foster, R.L. 2005. Compendium of bean diseases, 2nd edition. APS Press, St. Paul.
- Scully, B., Provvidenti, R., Benscher, D., Halseth, D.E., Miller, J.C. Jr. and Wallace, D.H. 1995. Five multiple-virus-resistant common bean breeding lines. Hortiscience 30(6):1320-1323.
- Servin, B., Martin, C.O., Mezard, M. and Hospital, F. 2004. Toward a theory of marker-assisted gene pyramiding. Genetics 168:513-523.
- Sharma, P.N., Kumar, A., Sharma, O.P. and Tyagi, P.D. 1999. Pathogenic variability in *Colletotrichum lindemuthianum* and evaluation of resistance in *Phaseolus vulgaris* in the North-Western Himalayan region of India. Journal of Phytopathology 147:41-45.
- Sharp, G.L., Martin, J.M., Lanning, S.P., Blake, N.K., Brey, C.W., Sivamani, E., Qu, R. and Talbert, L.E. 2002. Field evaluation of transgenic and classical sources of *wheat streak mosaic virus* resistance. Crop Science 42:105-110.
- Sicard, D., Buchet, S., Michalakakis, Y., Neema, C. 1997a. Genetic variability of *Colletotrichum lindemuthianum* in wild populations of common bean. Plant Pathology 46: 355-365.
- Silverio, L., Vidigal, M.C., Vidigal Filho, P.S., Barelli, M.A.A., Thomazell, C. and Nunes, W.M.C. 2002. Genetic resistance to *C. lindemuthianum* race 2047 in G2333. Annual Report. Bean Improvement Cooperative 45:74-75.
- Simms, E.L. and Fritz, R.S. 1990. The ecology and evolution of host-plant resistance to insects. Trends in Ecology and Evolution 5:356-360.
- Singh, B.D. 2009. Plant Breeding: Principles and methods, Edition 11. Kalyani publishers, New Delhi, India: 889.
- Singh, A., Avtar, R., Singh, D., Sangwan, O. and Balyan, P. 2013. Genetic variability, character association and path analysis for seed yield and component traits under two environments in Indian mustard. Journal of oilseed Brassica 4(1):43-48.
- Singh, D.N., Nandi, A. and Tripathy, P. 1994. Genetic variability and character association in French bean (*P. vulgaris*). Indian Journal of Agricultural Sciences 64:114-116.

- Singh, R.P. and Espino, J. 1997. Effect of leaf rust resistance gene *Lr34* on grain yield and agronomic traits of spring wheat. *Crop Science* 37:390-395.
- Singh, S. P., Schwartz, H. F., Terán, H., Viteri, D. and Otto, K. 2014. Pyramiding white mould resistance between and within common bean gene pools. *Canadian Journal of Plant Science* 94:947-954.
- Smedegaard-Petersen, V. and Tolstrup, K. 1985. The limiting effect of disease resistance on yield. *Annual Review of Phytopathology* 23:475-490.
- Sofkova, S., I. Poryazov, I. Kiryakov. 2010. Breeding green beans (*Phaseolus vulgaris* L.) for complex disease resistance. *Genetics and Breeding* 38(3):77-88.
- Souza, E.A.D., Camargo, O.A. and Pinto, J.M.A. 2010. Sexual recombination in *Colletotrichum lindemuthianum* occurs on a fine scale. *Genetics and Molecular Research* 9:1759-1769. doi.org/10.4238/vol9-3gmr863.
- Souza, T.L.P.O., Dessaune, S.N., Ragagnin, V.A., Carneiro, J.E.S., Moreira, M.A. and Barros, E.G. 2009. Rust resistance gene pyramiding in the common bean assisted by molecular markers. Plant and Animal Genome XVII Conference. Available at: www.intl-pag.org/17/abstracts/P03f_PAGXVII_185.html.
- Souza, T.L.P.O., Ragagnin, V.A., Dessaune, S.N., Sanglard, D.A., Carneiro, J.E.S., Moreira, M.A. and Barros, E.G. 2014. DNA marker-assisted selection to pyramid rust resistance genes in "carioca" seeded common bean lines. *Euphytica* 199: 303-316. doi:10.1007/s10681-014-1126-0.
- Suh, J.P., Cho, Y.C., Won, Y.J., Ahn, E.K., Baek, M.K., Kim, M.K., Kim, B.K., Jena, K.K. 2015. Development of resistant gene-pyramided Japonica rice for multiple biotic stresses using molecular marker-assisted selection. *Plant Breeding and Biotechnology* 3(4): 333-345. doi.org/10.9787/PBB.2015.3.4.333.
- Suh, J.P., Jeung, J.U., Noh, T.H., Cho, Y.C., Park, S.H., Park, H.S., Shin, M.S., Kim, C.K. and Jena, K.K. 2013. Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. *Rice (N Y)* 6(1):5. doi: 10.1186/1939-8433-6-5.
- Talamini, V., Souza, E.A., Pozza, E.A., Fernandes, F.R. 2006. Genetic divergence between and within *Colletotrichum lindemuthianum* breeds using RAPD. *Fitopatologia Brasileira* 31(6):545-550. doi.org/10.1590/S0100-41582006000600002.
- Tanweer, F.A., Rafii, M.Y., Sijam, K., Rahim, H.A., Ahmed, F., Ashkani, S. and Latif, MA. 2015. Introgression of blast resistance genes (Putative Pi-b and Pi-kh) into elite rice cultivar MR219 through marker assisted selection. *Frontiers in Plant Science* 6:1002. Doi:10.3389/fpls.2015.01002

Tera'n, H. and Singh, S. P. 2010. Gamete and recurrent selection for improving physiological resistance to white mold in common bean. *Canadian Journal of Plant Science* 90:153-162.

Thomas, W.T.B. Baird, E., Fuller, J.D, Lawrence, P., Young, G.R., Russell J, Ramsey L, Waugh R, Powell W. 1998. Identification of a QTL decreasing yield in barley linked to *Mlo* powdery mildew resistance. *Molecular Breeding* 4:381-393.

Thung, M. and Rao, I.M. 1999. Integrated management of abiotic stresses. In S.P Singh (ed) *Common bean improvement in the twenty first century*. Kluwer Academic Publishers. Dordrecht, Netherlands: 331-370.

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

Tu, J.C. and Aylesworth, J.W. 1980. An effective method of screening white (pea) bean seedlings (*Phaseolus vulgaris* L.) for resistance to *Colletotrichum lindemuthianum*. *Phytopathology* 99:131-139.

Tu, J.C. 1986. A detached leaf technique for screening beans (*Phaseolus vulgaris*) *in vitro* against anthracnose (*Colletotrichum lindemuthianum*). *Canadian Journal of Plant Science* 66:805-810.

Tu, J.C. 1992. *Colletotrichum lindemuthianum* on bean: population dynamics of the pathogen and breeding for resistance. In: *Colletotrichum: Biology, Pathology and Control* (Bailey, J.A. and Leger, M.J., eds). Wallingford, UK: CAB International. pp358-380.

UBOS and MAAIF, 2010. Uganda Census of Agriculture 2008/2009. Kampala: Uganda Bureau of Statistics and Ministry of Agriculture, Animal Industry and Fisheries.

Vallejo, V. and Kelly, J.D. 2001. Development of SCAR Markers Linked to the *Co-5* Locus in Common Bean. *Annual Report of the Bean Improvement Cooperative* 44:121-122.

Vallejo, V., and Kelly, J.D. 2009. New insights into the anthracnose resistance of common bean landrace G 2333. *The Open Horticulture Journal* 2:29-33.

Vidigal Filho, P. S., M. C. Goncalves-Vidigal, J. D. Kelly, and W. W. Kirk, 2007: Sources of resistance to anthracnose in traditional common bean cultivars from Parana, Brazil. *Journal of Phytopathology* 155:108-113.

Vieira, C. 1983. Diseases and pests of common bean. Federal University of Vicosa Press, Vicosa, MG, Brazil.

Wahome, S.W., Kimani, P.M, Muthomi, J.W., Narla, R.D. And Buruchara, R. 2011. Multiple disease resistance in snap bean genotypes in Kenya. *African Crop Science Journal* 19(4):289-302.

- Weher, C.R. and Moorthy, B.R. 1952. Heritable and non-heritable relationships and variability of oil content and agronomic characters in the F₂ generation of soybean crosses. *Agronomy Journal* 44:202-209.
- Wheeler, H. and Diachun, S. 1983. Mechanisms of pathogenesis. In: T. Kommedahl and P.H. Williams, eds, *Challenging problems in plant health*. American Phytopathological Society, St. Paul, Minnesota. pp324-333.
- Wortmann, C., Eledu, C. and David, S. 1999. Beans as a cash crop earner in Sub-Saharan Africa. *Annual Report. Bean Improvement Cooperative* 42:103-104.
- Wortmann, C.S. and Allen, D.J. 1994. African bean production environments, their characteristics and constraints. *Network on Bean Research in Africa. Occasional paper series*. No. 11, Dar-es-Salaam, Tanzania.
- Wricke, G. and Weber, W.E. 2010. *Quantitative genetics and selection in plant breeding*. Walter de Gruyter and Co. Berlin, Germany. Copyright year 1986. pp41-84.
- Ye, G. and Smith, F.K. 2008. Marker-assisted gene pyramiding for inbred line development: basic principles and practical guidelines. *International Journal of Plant Breeding* 2(1):1-10.
- Young, R.A. and Kelly, J.D. 1996. Characterization of the genetic resistance to *Colletotrichum lindemuthianum* in common bean differential cultivars. *Plant Disease* 80:650-654.
- Young, R.A., Melotto, M., Nodari, R.O. and Kelly, J.D. 1998. Marker assisted dissection of oligogenic anthracnose resistance in the common bean cultivar G2333. *Theoretical and Applied Genetics* 96:87-94.
- Yucel, O.D., Anlarsal, A.E., Yucel, C. 2006. Genetic variability, correlation and path analysis of yield, and yield components in chickpea (*Cicer arietinum* L.). *Turkish Journal of Agriculture and Forestry* 30:183-188.
- Zaumeyer, W.J, Thomas, H.R. 1957. A monographic study of bean diseases and methods for their control. *United States Department of Agricultural Technical Bulletin*. 868.