

**GENOTYPE – PATHOGEN CHARACTERIZATION AND MARKERS
IDENTIFICATION FOR ANGULAR LEAF SPOT DISEASE RESISTANCE IN
COMMON BEAN IN TANZANIA**

LUSEKO AMOS CHILAGANE

**A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF
AGRICULTURE. MOROGORO – TANZANIA**

2017

EXTENDED ABSTRACT

This work aimed at characterizing Angular Leaf Spot (ALS) and *Pseudocercospora griseola* in relation to its host and characterization of resistant genotypes amongst the locally adapted genotypes. A survey was conducted in nine regions where disease severity was established and samples of pathogen and genotypes collected for variability study based on molecular and pathogenicity characteristics. Resistant genotypes were characterized based on their response to ALS in field and screen house. The inheritance pattern, allelic relationship and maternal influence were elucidated and further, molecular markers linked to ALS disease resistance in the selected genotype were identified. The results showed that, ALS disease is distributed in all bean-growing regions with the highest severity of seven in Mbeya and Kagera, and lowest of four in Manyara and Rukwa. Molecular characterization showed the presence of 69.7% Andean and 30.3% Mesoamerican strains of *P. griseola* and 84.2% Andean and 15.8% Mesoamerican host genotypes where in both cases Andean gene pool out-numbered Mesoamerican gene pool with Andean genotypes being more susceptible to ALS as compared to Mesoamerican genotypes. In the identification of resistant genotypes, results indicated significant variations ($P \leq 0.01$) on the response of different genotypes to the disease. Beti-10 and line RJRILS135 were suggested as potential resistant parents with disease scores not different from the resistant controls Mexico 54 and CAL 143. In characterizing Beti-10, segregation results for F₂-Kablanketi/Beti-10 ($P = 0.806$) and F₂-Beti-10/Kablanketi ($P = 0.052$) indicates that, single dominant gene is responsible for resistance to ALS disease in Beti-10 with no maternal influence. Further there was no allelic relationship between Beti-10 and Mexico 54 which indicates independence of resistant genes in these genotypes. Among the SCAR markers used, PF13(*Phg109898*) and SN02(*Phg-2*) were found to be linked to the locus responsible for resistance in Beti-10 in repulsion and coupling phase respectively

and have 80% and 95% reliability for use in marker assisted selection. In this study, ALS pathogen is reported to be highly variable, genotypes that are resistant to ALS have been identified and characterized and markers linked to disease resistant loci identified of which their use will enhancing ALS resistance.

DECLARATION

I, LUSEKO AMOS CHILAGANE do here declare to the Senate of Sokoine University of Agriculture that, this thesis is my own original work and that it has neither been submitted nor concurrently submitted for a degree award in any other institution.

Luseko Amos Chilagane
(PhD Candidate)

Date

The above declaration is confirmed;

Prof. Susan Nchimbi-Msolla
(Supervisor)

Date

Prof. Paul Mbogo Kusolwa
(Supervisor)

Date

COPYRIGHT

No part of this thesis may be produced, stored in any retrievable system or transmitted in any form or by any means without a prior written permission of the author or Sokoine University of Agriculture in that behalf.

ACKNOWLEDGEMENTS

I am very grateful to all who extended their generous support in the accomplishment of this work as of to date put together as PhD thesis.

First I express my sincere thanks to my supervisors, Prof. Susan Nchimbi-Msolla and Prof. Paul Mbogo Kusolwa for the trust they put in me doing this work and of course for their scientific advises from the start of this work until this time putting together of these ideas and findings in writing. Whenever I was stuck and needed help, they were always there to guide and advice me. Special thanks also to Sokoine University of Agriculture for allowing me to take leave for my studies and the head of Department of Crop Science and Horticulture and all the members of staff in the department, you all in your position helped me in one way or another to accomplish this task.

I also acknowledge the Kirkhouse Trust for funding this research work, my sincere thanks to all the members of this project at Sokoine University of Agriculture and other members of the Kirkhouse Trust project in the region, the African Bean Consortium (ABC). Part of this research also was facilitated by USDA-ARS in Mayaguez, Puerto Rico and the University of Puerto Rico, and funded by the Feed-the-Future Grain Legumes project under the supervision of Dr. Tomothy G. Porch. I appreciate the contributions of all involved and look forward for more collaboration.

DEDICATION

To almighty God, my beloved wife Dorah Herman Bivugile and daughter Janelle Sehewa
Luseko and to all members of my family

TABLE OF CONTENTS

EXTENDED ABSTRACT	i
DECLARATION.....	iii
COPYRIGHT	iv
ACKNOWLEDGEMENTS	v
DEDICATION.....	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES.....	xii
ORGANISATION OF THE THESIS.....	xiv
CHAPTER ONE.....	1
1.0 GENERAL INTRODUCTION.....	1
1.1 Common Bean	1
1.2 Common Bean Production	1
1.3 Constraints to Common Bean Production	2
1.4 Angular Leaf Spot Disease	2
1.5 Disease Control.....	3
1.6 Genotype – Pathogen Characterization Studies.....	4
1.7 Justification and Objectives.....	5
1.9 References	6
CHAPTER TWO.....	10

2.0 ANGULAR LEAF SPOT DISEASE STATUS AND CHARACTERIZATION OF THE COMMON BEAN HOST AND PSEUDOCERCOSPORA GRISEOLA THE CAUSATIVE AGENT OF THE DISEASE IN TANZANIA.....	10
2.1 Abstract	10
2.2 Introduction	12
2.3 Materials and Methods.....	13
2.3.1 Sample collection and ALS severity assessment	13
2.3.2 Isolation of <i>P. griseola</i>	15
2.3.3 DNA Extraction and Amplification	15
2.3.4 Electrophoresis and Gel Documentation.....	16
2.3.5 DNA Sequencing and Phylogenetic Analysis of the <i>P. griseola</i> Isolates	16
2.3.6 Common Bean Phaseolin Protein Evaluation.....	17
2.3.7 Virulence of Selected Isolates	17
2.4 Results and Discussion.....	18
2.4.1 Angular Leaf Spot Disease Severity	18
2.4.2 Host genotype characterization.....	19
2.4.3 Pathogen Characterization.....	21
2.4.4 Relationships between Genotype and Pathogen	24
2.4.5 Virulence of <i>P. griseola</i> Isolates.....	25
2.5 Conclusions	27
2.6 References	28

CHAPTER THREE	32
3.0 LEVELS OF RESISTANCE TO ANGULAR LEAF SPOT DISEASE AMONG ADAPTED COMMON BEAN (<i>P. VULGARIS</i> L.) GENOTYPES IN TANZANIA	32
3.1 Abstract	32
3.2 Introduction	33
3.3 Materials and Methods	35
3.3.1 Source and description of the genotypes	35
3.3.2 Source of <i>P. griseola</i> isolate	36
3.3.3 Field evaluation for Angular leaf spot	36
3.3.4 Screen house evaluation for angular leaf spot	36
3.3.5 Disease assessment and selection of potential resistant parents	37
3.4 Results and Discussion	37
3.4.1 Evaluation of landraces for ALS	37
3.4.2 Evaluation of RIL's for ALS	40
3.5 Conclusion	42
3.6 References	43
CHAPTER FOUR	48
4.0 INHERITANCE, ALLELIC RELATIONSHIP AND IDENTIFICATION OF MOLECULAR MARKERS LINKED TO ANGULAR LEAF SPOT RESISTANCE IN THE COMMON BEAN LANDRACE BETI-10 IN TANZANIA	48
4.1 Abstract	48

4.2 Introduction	50
4.3 Materials and Methods	51
4.3.1 Genotypes and <i>P. griseola</i> isolates	51
4.3.2 Genetic characterization of Beti-10	52
4.3.4 SCAR markers co-segregation.....	52
4.4 Results and Discussion.....	53
4.4.1 Characterization of resistant gene from Beti-10	53
4.4.2 Co – segregation analysis	55
4.5 Conclusions	57
4.6 References	59
CHAPTER FIVE.....	63
5.0 GENERAL CONCLUSION AND RECOMMENDATIONS	63
5.1 General Conclusion.....	63
5.2 Recommendations.....	65

LIST OF TABLES

Table 2.1:	Distribution of Andean and Mesoamerican isolates, showing number and percent of isolates per location, of <i>P. griseola</i> as revealed by sequence analysis of the ITS region and the Actin gene.....	24
Table 2.2:	Pathotype designation, virulence phenotype and group, and the region of origin of <i>P. griseola</i> isolates used in this study.....	26
Table 3.1:	Levels of resistance to ALS among the bean landraces.....	38
Table 3.2:	Levels of resistance to ALS among the recombinant inbred lines (RIL's) population from CAL 143 and Rojo	41
Table 4.1:	Frequencies of Phenotypic classes for ALS resistance among different populations.....	55
Table 4.2:	Frequencies of Genotypic classes for ALS resistance among different populations screened with two molecular markers (SN02 and PF13).....	57

LIST OF FIGURES

- Figure 2.1:** A map of Tanzania showing the nine regions (shaded) where *P. griseola* isolates and seeds of common bean samples were collected..... 14
- Figure 1.2:** Angular Leaf Spot disease status in Tanzania..... 19
- Figure 2.3:** Polyacrylamide gel for Phaseolin protein marker where A = Andean, M = Mesoamerican; LD = molecular weight marker (50bp); Kab and Mex = controls (Kablanketi and Mexico 54); and Numbers 2 - 107 = genotype samples..... 20
- Figure 2.4:** Graph showing the frequency of Andean and Mesoamerican common bean genotypes across bean growing regions as determined by the Phaseolin marker..... 21
- Figure 2.5:** Consensus tree results from Bayesian analysis using concatenated ITS and Actin sequences showing the proportion of Tanzanian Andean and Mesoamerican ALS isolates as grouped with control sequences..... 23
- Figure 2.6:** Graph showing the number of strains of *P. griseola* from each gene pool collected from Mesoamerican or Andean common bean host genotype..... 25
- Figure 3.1:** Bean Production trends from 2010 to 2014 showing relationship between production and area harvested (FAOSTAT, 2014)..... 34
- Figure 3.2:** Response of (a) Mexico 54, (b) Kablanketi and (c) Beti-10 to Angular leaf spot under screen house conditions..... 39

Figure 4.1: PCR amplification products showing polymorphism for the SCAR markers SN02 and PF13; LD = 100kb ladder, 1 = Beti-10 (Resistant parent), 2 = Kablanketi (Susceptible parent), 3 = Resistant Bulk and 4 = Susceptible bulk..... 55

Figure 4.2: PCR amplification products for SN02 (Coupling phase) and PF13 (repulsion phase) markers showing results for selected individual F₂'s; LD = 100kb Ladder, 1 = Beti-10 (Resistant parent), 2 = Kablanketi (Susceptible parent), R = Resistant F₂'s and S = Susceptible F₂'s..... 56

ORGANISATION OF THE THESIS

This thesis is organized in the “Publishable manuscript format” and consists of five chapters as follows;

- a. Chapter one is the General Introduction
- b. Chapters two to Four are the three manuscripts out of each specific objective;
 - i. Angular leaf spot disease status and characterization of the common bean host and *Pseudocercospora griseola* the causative agent of the disease in Tanzania
 - ii. Levels of resistance to angular leaf spot disease among adapted common bean (*Phaseolus vulgaris* L.) genotypes in Tanzania
 - iii. Inheritance, allelic relationship and identification of molecular markers linked to angular leaf spot resistance in the common bean landrace beti-10 in Tanzania
- c. Chapter five is the General Conclusion and Recommendations

Part of chapter two has been published in the African Journal of Plant Science Vol.10 (11), pp. 238-245, November 2016 (DOI: 10.5897/AJPS2016.1427). This thesis is formatted following the guidelines of the targeted journal “The African Journal of Plant Science” as stipulated in “Regulations and guidelines for higher degrees, SUA, fifth edition, (2011) section 4.3.3.

LIST OF ABBREVIATIONS

ALS	Angular Leaf Spot
ANOVA	Analysis of Variance
TARS	Tropical Agriculture Research Station
BCMNV	Bean Common Mosaic Necrosis Virus
BCMV	Bean Common Mosaic Virus
CBB	Common Bacterial Blight
CIAT	Centro Internacional de Agricultura Tropical (International Centre for Tropical Agriculture)
CPC	Crop Protection Compendium
DNA	Deoxyribo Nucleic Acid
FAO	Food and Agriculture Organisation
HB	Halo Blight
hPAGE	Horizontal Poly Acrylamide Gel Electrophoresis
ITS	Internal Transcribed Spacer
MAS	Marker Assisted Selection
NCBI	National Centre for Biotechnological Information
PCR	Polymerase Chain Reaction
RIL	Recombinant Inbred Lines
SCAR	Sequence Characterized Amplified Regions
SUA	Sokoine University of Agriculture
USDA	United States Department of Agriculture

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Common Bean

Common bean (*Phaseolus vulgaris* L) is the most important food legume consumed worldwide (Miklas et al., 2006) and an important source of human dietary protein, calories, vitamins and minerals necessary for a healthy community. It has a great impact on food security of people in developing countries (Miklas et al., 2006).

In Tanzania, common bean is an important grain legume because it is a source of most dietary needs (Tryphone and Nchimbi-Msolla, 2010). Regular intake of common bean, as is the case for Tanzania, has medical benefits in lowering the risks of cancer, diabetes and heart diseases (Ocho-Anin Atchibri et al., 2010). Apart from being of dietary importance, the crop has also gained importance in Tanzania as a source of income where the surplus is being marketed as fresh bean or dry bean (Broughton et al., 2003).

1.2 Common Bean Production

In Tanzania, common bean is mainly grown from medium to high altitude areas; the major production areas are the Northern zone (Arusha, Manyara, Kilimanjaro and Tanga); the lake zone and the western regions (Kagera and Kigoma) and the southern highlands (Mbeya, Rukwa and Iringa). Tanzania is among the top ten largest producers of common bean in the world (FAOSTAT, 2014); production is estimated to be 1,114,500 metric tons per year. In Tanzania, the production of common bean has increased because of a rise in the total production area (FAOSTAT 2014) however per unit area production is still low, averaging at 500kg/ha compared with the production potential elsewhere (>1500kg/ha) (Hillocks et al., 2006) which necessitates more effort for increased production potential.

1.3 Constraints to Common Bean Production

Although some African countries including Tanzania, Kenya and Uganda, are among the top producers of common bean, the average production is still low. This low production is attributed mainly by many biotic and abiotic factors (Nzungize et al., 2011). Most important of these factors are poor seed quality, poor performance of the local landraces, low soil fertility and periodic water stress (Hillocks et al., 2006).

Bean productivity among other factors is severely constrained by diseases which are considered as the number one constraint (Hillocks et al., 2006; Muthomi et al., 2011). Of the large number of diseases that affect beans in the tropics, the most important in Tanzania are angular leaf spot (ALS), common bacterial blight (CBB), halo blight (HB), rust and Bean common mosaic virus disease (BCMV) (Hillocks et al., 2006). Among these diseases, ALS has the highest economic impact in African countries particularly Malawi, Ethiopia, Kenya, Uganda, Tanzania and the great lakes regions (Pastor-Corrales et al., 1998).

1.4 Angular Leaf Spot Disease

Angular leaf spot of common beans (*Phaseolus vulgaris*. L.) caused by the fungus *Pseudocercospora griseola* (Sacc) Crous and U. Braun (Crous et al., 2006) is an important disease both in the tropics and temperate regions where beans are grown. It is widely spread in tropical and subtropical countries of South America, Central America and East Africa (Miklas et al., 2006). In Tanzania, the disease is reported to be present from low to high altitude areas (Hillocks et al., 2006). The pathogen causes significant yield loss up to 80% of common bean in Africa and other parts of the world when susceptible varieties are grown (Muthomi et al., 2011). This disease primarily affects aerial parts of the plant and is more destructive in the warm, humid areas (Crous et al., 2006). It causes symptoms that consist of circular to elliptical red-brown lesions in pods and in foliage. The lesions start

as small brown or grey spots that become angular in shape and necrotic and these spots are being confined by the veins of the leaf (Correa-Victoria et al, 1989; CIAT, 1987). The disease causes premature defoliation (Correa-Victoria et al, 1989) and also causes the reduction of seed quality in case the disease invade seeds and thus affect the marketability of bean seeds across producing areas of the world (Pastor-Corrales et al, 1998).

1.5 Disease Control

The use of resistant cultivars is the most effective, economical and environmentally safe strategy for disease control especially for small-scale farmers (Mahuku et al., 2004; Miklas et al., 2006; Crous et al., 2006). The advantage of host plant resistance is that once the technology has been developed it is packaged in the seed which is easier to disseminate and deploy (Mahuku et al., 2009). It has been reported also that some methods of disease control such as the use of pathogen-free seeds, cultural practices and the use of chemicals (fungicides) can also be useful (Miklas et al., 2006). Breeding for resistance to ALS is rather complicated as the genetic resistance is mostly monogenic and race-specific (Miklas et al., 2006), while the causative pathogen is highly variable with many different races (Mahuku et al., 2002). Some resistant materials for breeding against the disease are known, in east Africa and Africa in general the donor Mexico 54 has been widely used which contains the gene *Phg-2* that confers resistance to the disease. In attempts to breed varieties resistant to most diseases including ALS, some molecular tools have been sought so as to facilitate easy selection (MAS) with some choice molecular markers to be used (Miklas, 2010). Despite these markers being used successfully in some areas, it has also been reported that in some backgrounds these markers are not polymorphic hence limit their use (Namayanja et al., 2009).

Due to the nature of *P. griseola* the causative of ALS being very variable there is a need to combine genes from diverse genetic backgrounds conferring resistance to different races of the pathogen for achieving durable resistance.

1.6 Genotype – Pathogen Characterization Studies

In the case of ALS, previous studies have revealed pathotypic and genetic variability (Mahuku et al., 2002) and that there is co-evolution between the ALS pathogen and the host gene pool (Guzmán et al., 1995). These studies have often being based on specific geographical regions and limited work has been done in Tanzania as a country considering its geographical location and the bean growing regions present.

The pathogen characterization studies have been done using morphological as well as molecular methods (Crous et al., 2006). It has been demonstrated that some morphological variations could not be used to group isolates (Crous et al., 2006), as these variations could be due to host cultivar interaction or interaction between the host gene pool and the pathogen origin. Studies in identification of decaying fungi have revealed that traditional methods of identification are difficult and culture methods are unable to differentiate closely related fungi species (Kim et al., 2005), thus, leading to reliance on molecular methods, which have increased sensitivity and selectivity (Ranjard et al., 2001). White et al. (1990) designed internal transcribed spacer (ITS) primer sets that are specific to fungi which have been utilized to characterize plant fungal pathogens (Crous et al., 2006; Nzungize et al., 2011 and Abd-elsalam et al., 2003). These primers work by amplification of the ribosomal RNA genes that are characteristics suitable for identifying pathogens at the species level (Abd-elsalam et al., 2003) due to their stability and the presence of a mosaic of conserved and variable regions within their genome.

1.7 Justification and Objectives

Despite genetic resistance being efficient, for ALS the challenge is the high variability of the causative pathogen (Mahuku et al., 2002), which implies that breeding for durable resistance is difficult to achieve (Pastor-Corrales et al., 1998). Knowledge on the nature of resistance in the donor genotypes and the races of the pathogen to be overcome is crucial when durable resistance is sought. Thus characterization of the resistant genotypes and monitoring of the pathogen changes is very important for durable resistance (Mahuku et al., 2002) and this is even more crucial when the pathogen shows some high levels of variability as the case for ALS pathogen. In Tanzania very little has been done to understand the levels of variability of this pathogen, the local available sources of resistance and the nature of the resistance in these donor materials which complicates breeding for durable resistance.

In this study, the overall objective was to increase the production potential of common bean by enhancing angular leaf spot disease resistance. In achieving this, the following were performed, (i) Characterization of *P. griseola* pathogen, the causative of Angular Leaf Spot disease of common bean in Tanzania, (ii) Determination of levels of resistance and susceptibility to Angular Leaf Spot disease among the locally adapted bean genotypes seeking to identify resistant donor parents for resistant genes and (iii) Identification of potential candidate markers linked to resistance genes to *P. griseola* in the selected local resistant genotype were accomplished.

The output of this research gives information on the ALS pathogen races that are present, providing suggestion on which resistant local genotypes to use in pyramiding genes for disease resistance, and identify molecular markers useful in the local parental bean lines.

References

- Abd-elsalam KA, Aly IN, Abdel-Satar MA, Khalil MS, Verreet JA (2003). PCR identification of *Fusarium* genus based on nuclear ribosomal-DNA sequence data. *African Journal of Biotechnology*. 2(4): 82–85.
- Broughton WJ, Hernandez G, Blair MW, Beebe SE, Gepts P, Vanderleyden J (2003). Beans (*Phaseolus spp.*) – model food legumes. *Plant and Soil*. 252: 55 – 128.
- CIAT (Centro Internacional de Agricultura Tropical) (1987). Standard system for the evaluation of bean germplasm. Van Schoonhoven, A. and Pastor-Corrales, M. A. (Compilers). Cali, Colombia. 54p.
- Correa-Victoria FJ, Pastor-Corrales MA, Saettler AW (1989). Angular leaf spot. In: *Bean Production Problems in the Tropics* (Schwartz HF, Pastor-Corrales MA, eds). Centro Internacional de Agricultura Tropical, Cali, Colombia: 59 – 75
- Crows PW, Liebenberg MM, Braun U, Groenewald JZ (2006). Re-evaluating the taxonomic status of *Phaeoisariopsis griseola*, the causal agent of angular leaf spot of bean. *Studies in mycology* 55: 163 – 173.
- FAOSTAT (2014). <http://www.fao.org/faostat/en/#data/QC> Site visited, Nov, 2017
- Guzmán P, Gilbertson RL, Nodari R, Johnson WC, Temple SR, Mandala D, Mkandawire ABC, Gepts P (1995). Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests co-evolution with the common bean (*Phaseolus vulgaris*). *Phytopathology* 85: 600 – 607.
- Hillocks RJ, Madata CS, Chirwa R, Minja EM, Msolla SN (2006). *Phaseolus* bean improvement in Tanzania, 1959 – 2005. *Euphytica* 150: 215 – 231.

- Kim GH, Lim YW, Song YS, King JJ (2005). Decay fungi from playground wood products in service using 28S rDNA sequence analysis. *Holzforschung*. 59(4): 459 – 466.
- Mahuku GS, Henríquez MA, Munõz J, Buruchara RA (2002). Molecular markers dispute the existence of the Afro-Andean group of the bean angular leaf spot pathogen, *Phaeoisariopsis griseola*. *Phytopathology* 92: 580 – 589.
- Mahuku GS, Iglesias MA, Jara C (2009). Genetics of Angular leaf spot resistance in the Andean Common bean accessions G5686 and identification of markers linked to the resistance genes. *Euphytica*. 167: 381 – 396.
- Mahuku G, Montoya C, Henríquez MA, Jara C, Teran H, Beebe S (2004). Inheritance and characterization of angular leaf spot resistance gene presence in common bean accession G 10474 and identification of an AFLP marker linked to the resistance gene. *Crop Science*. 44: 1817 – 1824.
- Michelmore RW, Paran I, Kesseli RV (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Science of the United States of America*. 88: 9828 – 9832.
- Miklas PN (2010). List of DNA SCAR markers linked with disease resistance traits in bean. [https://www.ars.usda.gov/ARSUserFiles/3848/PDF/Miklas_2011/SCAR%20Markers%202010.pdf]
- Miklas PN, Kelly JD, Beebe SE, Blair MW (2006). Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica*. 147: 105 – 131.

- Muthomi JW, Muimui KK, Kimani PM (2011). Inheritance of resistance to angular leaf spot in yellow beans. *African Crop Science Journal*. 19(4): 267 – 275.
- Namayanja A, Buruchara R, Mahuku G, Rubaihayo P, Kimani P, Mayanja S, Eyedu H (2006). Inheritance of resistance to angular leaf spot in common bean and validation of the utility of resistance linked markers for marker assisted selection outside the mapping population. *Euphytica*. 151: 361 – 369.
- Nzungize J, Gepts P, Buruchara R, Buah S, Ragama P, Busogoro JP (2011). Pathogenic and molecular characterization of *Pythium* species inducing root rot symptoms of common bean in Rwanda. *African Journal of Microbiology Research*. 5(10): 1169 – 1181.
- Ocho-Anin Atchibri AL, Broul KD, Kouakou TH, Kouadio YJ, Gnakri D (2010). Evaluation of bioactive components in seeds of *Phaseolus vulgaris* L. (fabaceae) cultivated in Côte d'Ivoire. *Journal of Applied Biosciences*. 31: 1928 – 1934
- Pastor-Corrales MA, Jara C, Singh S (1998). Pathogenic variation in, source of, and breeding for resistance to *Phaeoisariopsis griseola* causing angular leaf spot in common bean. *Euphytica*. 103: 161 – 171.
- Ranjard L, Poly F, Lata J, Mougel C, Thioulouse J, Nazaret S (2001). Characterization of Bacterial and Fungal Soil Communities by Automated Ribosomal Intergenic Spacer Analysis Fingerprints: Biological and Methodological Variability. *67(10): 4479 – 4487*.
- Tryphone GM, Nchimbi-Msolla S (2010). Diversity of common bean (*Phaseolus vulgaris* L.) genotypes in iron and zinc contents under greenhouse conditions. *African Journal of Agricultural Research*. 5(8): 738 – 747.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. (Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J eds). Academic Press, New York, USA. pp. 315 – 322.

CHAPTER TWO

2.0 ANGULAR LEAF SPOT DISEASE STATUS AND CHARACTERIZATION OF THE COMMON BEAN HOST AND PSEUDOCERCOSPORA GRISEOLA THE CAUSATIVE AGENT OF THE DISEASE IN TANZANIA

2.1 Abstract

Angular leaf spot (ALS), caused by the fungus *Pseudocercospora griseola*, is one of the most important diseases of common bean in Tanzania. Breeding for resistance to this disease is complicated by the variable nature of the pathogen. In Tanzania, a thorough analysis of the variability of this pathogen is lacking which limits the development of proper strategies for breeding for durable resistance. This work was aimed at characterizing *P. griseola* in relation to its host in Tanzania. A sample collection of both pathogen and host was conducted in the 2013 and 2014 growing seasons from nine bean growing regions and an assessment of disease severity in those regions was done. Single spore isolation was performed for *P. griseola* isolates followed by DNA extraction from both the *P. griseola* mycelium and leaves of the common bean plants from which the pathogen was collected. For characterization of the gene pool origin of the host, the Phaseolin protein marker was evaluated. For characterization of the pathogen, the Internal Transcribed Spacer (ITS) region and the Actin gene sequences were evaluated. Angular leaf spot disease is found to be distributed in all bean-growing regions with Mbeya and Kagera being the hot spots with severity scores of seven while Rukwa and Manyara had the least severity of four. Phylogenetic analysis showed the presence of 69.7% Andean and 30.3% Mesoamerican strains of *P. griseola* in Tanzania. The common bean host genotypes showed a similar distribution with 84.2% Andean and 15.8% Mesoamerican, as revealed by the Phaseolin marker. In both cases, Andean strains of the pathogen and Andean bean genotypes outnumbered Mesoamerican. In relation to the common bean genotypes,

Andean genotypes were more susceptible to ALS as compared to Mesoamerican genotypes. There were a few strains that were of Andean origin but were pathogenic on Mesoamerican common bean genotypes, a group that has previously been termed Afro-Andean. Geographically, most of the regions of Tanzania had only Andean strains except for Kagera where 60% of the strain were Mesoamerican, and in Arusha and Tanga, where 50% and 33% were Mesoamerican, respectively. Only three regions, including Kagera, Mbeya and Rukwa, were found to grow Mesoamerican beans with different proportions. The findings of this study are important in setting basic objectives for breeding for angular leaf spot disease in Tanzania.

2.2 Introduction

Angular leaf spot (ALS) of common bean (*Phaseolus vulgaris*. L.) caused by the fungus *Pseudocercospora griseola* (Sacc) Crous and U. Braun (Crous et al., 2006) is an important disease both in the tropics and in the sub-tropical regions where beans are grown. The pathogen causes significant yield losses of up to 80% of common bean in Africa and in other parts of the world (Muthomi et al., 2011). This disease primarily affects aerial parts of the plant and is more destructive in warm, humid production zones (Crous et al., 2006). The disease causes premature defoliation (Correa-Victoria et al., 1989) and it also causes the reduction of seed quality, in cases where the disease invades the seeds, and thus affecting the marketability of bean seed across production zones of the world (Pastor-Corrales et al., 1998).

ALS disease is best controlled by the use of resistant cultivars, but despite the existence of genetic resistance, the variable nature of the pathogen makes achieving durable resistance a challenge (Mahuku et al., 2002b; Pastor-Corrales et al., 1998). Therefore, knowledge about the races of the pathogen to be overcome in a region is crucial when durable resistance is sought, as well as the monitoring of the evolution of the pathogen over time (Mahuku et al., 2002b). The pathogen monitoring is even more important when the pathogen shows high levels of variability, as is the case for the ALS pathogen. Different methods have been applied in attempting to characterize the ALS pathogen including virulence testing, where isolates are classified according to the reaction they cause to a set of differential cultivars, and including molecular markers where the isolates are distinguished based on their genetic composition. The use of differential cultivars, however, has a major limitation due to variability in environmental conditions (Kolmer et al., 1995; Sebastian et al., 2006). The binomial characterization of the pathogen using the set of differentials can be supplemented with the molecular characterization of the

pathogen which detects the variability of the pathogen population regardless of its host and environment (Ddamulira et al., 2014). Different molecular methods have been used to characterize this pathogen including RAPD markers, ISSR markers, and Box primers (Abadio et al., 2012; Ddamulira et al., 2014). Sequences from the ITS and Actin genes were used in a similar study evaluating the taxonomic status of *P. griseola* (Crous et al., 2006). Studies on the variability of *P. griseola* isolates revealed the existence of two major groups of the pathogen, Andean and Mesoamerican, which correspond to and have co-evolved with the Andean and Mesoamerican gene pools of common bean (Guzman et al., 1995; Pastor-Corrales et al., 1998; Crous et al., 2006). Mesoamerican strains of this pathogen are considered more virulent as compared to Andean strains and they tend to affect both Mesoamerican and Andean beans while Andean strains are less virulent, affecting mostly Andean genotypes (Pastor Corrales et al., 1998). Apart from these two distinct sets of host and pathogen based on geographical origin, another group was found peculiar to Africa designated as Afro-Andean. This group has characteristics typical of the isolates of Andean origin but it was found to be pathogenic on Mesoamerican common bean, which is unusual (CIAT, 1997 and Mahuku et al., 2002a).

The objective of this work was to give an overview of ALS disease status in Tanzania and to characterize the diversity of *P. griseola* in nine regions of Tanzania using Internal Transcribed spacer region (ITS region) and Actin gene sequences of the fungus in relation to the gene pool origin of the common bean host and their distribution across the regions.

2.3 Materials and Methods

2.3.1 Sample collection and ALS severity assessment

P. griseola isolates and host tissue were collected from the nine common bean production regions of Tanzania (Fig. 2.1). The samples were collected from farmers' fields and some

were collected from Agriculture Research Stations including: Uyole in Mbeya, Seliani in Arusha, Maruku in Kagera and Sokoine University of Agriculture (SUA) in Morogoro.



Figure 2.1: A map of Tanzania showing the nine regions (shaded) where *P. griseola* isolates and seeds of common bean samples were collected.

Common bean infected leaves were collected from plants showing typical Angular Leaf Spot symptoms and were preserved in blotter paper and brought to SUA for isolation. Young common bean leaves were collected in eppendorf tubes at the same time as the pathogen was collected from the same plant.

Apart from sample collection, to establish an overview of ALS disease severity; in each region 5 fields with ALS disease were selected among the 10 visited for sample collection and two sites among the 5 fields and in each site within the field 5 plants were assessed for ALS disease infestation. Severity was assessed by using CIAT scale of 1 – 9 (Van Schoonhoven and Pastor-Corales, 1987) whereby the rating of 1 indicates no visible

disease symptoms (0% infection), 3 = plants with 5 – 10% leaf area having lesions, 5 = plants with 20% leaf area having lesions and sporulating, 7 = plants with up to 60% leaf area having lesions associated with chlorosis and necrosis, and 9 = plants with 90% leaf area having lesions associated with early leaf fall and plant death.

2.3.2 Isolation of *P. griseola*

Single spore isolation was completed in the pathology laboratory at Sokoine University of Agriculture following the CIAT guidelines (Castellanos et al., 2015). Following isolation, pure cultures were transferred to V8 medium without antibiotics and the cultures were incubated at 24°C for 15 to 20 days. After this incubation period, the mycelium was scraped directly from the media for DNA extraction. The isolates were then maintained following the CIAT guidelines (Castellanos et al., 2015).

2.3.3 DNA Extraction and Amplification

DNA from the *P. griseola* mycelia and from the common bean host were extracted using TES extraction buffer and a previously published protocol (Mahuku, 2004). Fungal PCR amplification was completed using two primer sets, the ITS 4 and ITS 5 primer pairs, to amplify the Internal Transcribed spacer (ITS) region (White et al., 1990), and the Actin gene with the ACT 512F and ACT 783R primers (Carbone and Kohn, 1999). The PCR conditions used to amplify the ITS region and the Actin gene were 94°C for 4 min for initial denaturation followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 sec, and elongation at 72°C for 1 min followed by another cycle of final elongation at 72°C for 4 min. DNA from the common bean samples were amplified using the phaseolin protein primers Phaseolin1-R and Phaseolin1-F and the PCR conditions were initial denaturation at 94°C for 4 min, followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 30 sec, and a final extension for 7 min at 72°C.

2.3.4 Electrophoresis and Gel Documentation

PCR products were separated by electrophoresis (110 volts for one hour and 30 minutes) and 3 μ L of PCR product were run on 1% agarose gel (Fisher Scientific, NJ) using 1X TBE buffer and 10 μ L of gel red (Biotum [®], Hayward CA). PCR products were visualized using a UV trans-illuminator and photographed using UVP[®] (Program version 6.5.2a 2007 program) (Upland, CA).

PCR products from common bean samples were separated using a 6% horizontal polyacrylamide gel (hPAGE technique) with electrophoresis at 120V for 3hours, post-stained with ethidium bromide, and using 1X TAE as the running buffer. The gel visualization and documentation was completed using a UV Trans-illuminator and the gel photo was captured with a Power Shot A650IS digital camera (Canon, USA).

2.3.5 DNA Sequencing and Phylogenetic Analysis of the *P. griseola* Isolates

PCR products for the ITS region and the Actin gene were purified, after confirming the expected band size (600-900bp for ITS; 200bp for Actin in an agarose gel), using a QIA quick PCR purification Kit (Qiagen, Valencia, CA). DNA quantification using a Nanodrop (ND-1000 UV/Vis Spectrophotometer, Wilmington, DE USA) was followed by dilution of the samples to 20–30ng/ul for Actin and 30-50ng/ul for ITS in preparation for sanger sequencing (Seq-Wright Genomic Services, Texas, USA).

Two sequences for both the Actin gene and the Ribosomal RNA gene (ITS), CPC 10468 and CPC 10463, representing the Andean and Mesoamerican gene pools of *P. griseola* (Crous et al., 2006), respectively, were obtained from the NCBI database and included in the analysis as controls. Also, two other sequences for *Passalora loranthi*, strain CBS122466 and *Passalora eucalypti* strain CBS111318, from the NCBI database were used as-out groups in the phylogenetic analysis to root the tree. These out groups (*P. loranthi* and *P.eucalypti*) are the sister clades to *P. griseola* (Crous et al., 2006)

Sequence data were first edited using Bio-Edit v7.2.5 and Sequencher to establish contigs (consensus sequence for each isolate) and the MEGA6 program was used to remove the primer sequence of each contig. The NCBI database was used to perform BLAST searches for each sequence to validate that the sequences belonged to *P. griseola*. Sequence for both the Actin gene and ITS region that were of high quality, were selected for phylogenetic analysis. The multiple sequence alignments were conducted with SATé (Kato and Standley 2013) using MAFFT for the alignment and RAxML for the tree estimator using 10 iterations. Four partitions were determined as input for Partition Finder V1.1.1 (Lanfear et al., 2014) to select the best molecular evolution model for each partition. The best molecular evolution model for each partition were K80+G model for 1st and 2nd codon position of the Actin gene and the ITS region and the HKY model for the 3d codon position of the Actin gene.

Bayesian Inference (BI) was performed to infer phylogeny. BI analysis was implemented in MrBayes 3.1 (Huelsenbeck and Ronquist 2001) with 4 runs and 4 chains for 25 million generations specified with the 2 molecular evolution models previously obtained, sampling every 10,000 generations. Convergence and stationarity were visualized in Tracer V.1.4 (Rambaut and Drummond 2007).

2.3.6 Common Bean Phaseolin Protein Evaluation

The common bean genotypes were evaluated for the Phaseolin marker by scoring the banding pattern on the gel. The data were used to group the genotypes as either Mesoamerican or Andean.

2.3.7 Virulence of Selected Isolates

From molecular characterization, eight isolates were selected randomly four from each Mesoamerican and Andean groups. Virulence of the isolates was determined using a set of 12 differential cultivars where four seeds of each cultivar were planted in pots in screen

house containing sterilized forest soil and inoculated with pure cultures of the isolates. The experiment was replicated three times for each isolate. Disease evaluation was done following the 1 – 9 CIAT scale (Van Schoonhoven and Pastor-Corrales, 1987) where average disease scores of 1 – 3 were considered resistant and above three susceptible. Pathotype designation was performed by adding the binary values designated for each differential cultivar following the procedures by Mahuku et al., (2002a).

2.4 Results and Discussion

2.4.1 Angular Leaf Spot Disease Severity

The result from this study indicates the presence of the disease in all the surveyed bean growing regions of Tanzania with varying severity. Kagera and Mbeya regions had the highest severity score of seven whereas Rukwa and Manyara had the lowest score of four (Fig. 2.2). This is not different from other reports that ALS disease is the major constraints of production in tropical and subtropical regions (Wortmann et al., 1998; Mahuku et al., 2002a; Alloreant and Savary, 2005).

This very high rate of the disease observed in this study may have been contributed by the practice of many farmers to use their own saved seeds that may have been contaminated and continued cultivating beans on their pieces of land year after year and not observing sanitation in their fields which are the practices that makes the disease persist more. This rate of disease severity is very high making this disease to cause high losses in Tanzania just as reported in other places where beans are grown (Wortmann et al., 1998; Stenglein et al., 2003; Muthomi et al., 2011).

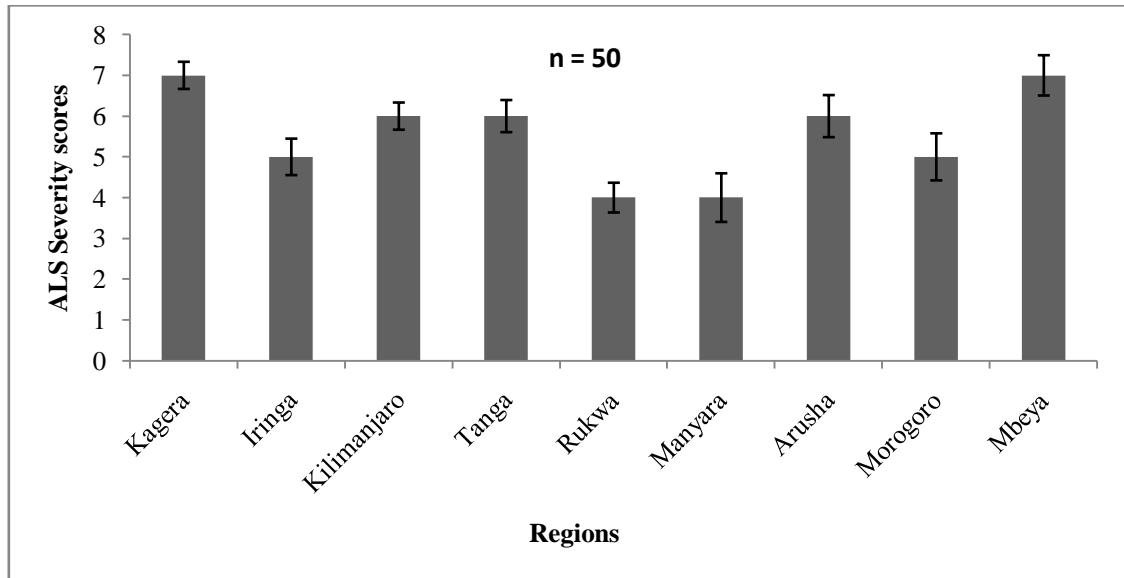


Figure 2.2: Angular Leaf Spot disease status in Tanzania

2.4.2 Host genotype characterization

The bean genotypes from which ALS isolates were collected were found to be of Mesoamerican and Andean origin although the gene pool composition differed from region to region of Tanzania. A total of 76 common bean genotypes were characterized using the phaseolin protein marker (Fig. 2.3) and of these 64 (84%) were of Andean origin and the other 12 (16%) were of Mesoamerican origin, thus, farmers prefer to cultivate Andean genotypes as opposed to Mesoamerican genotypes (Fig. 2.4).

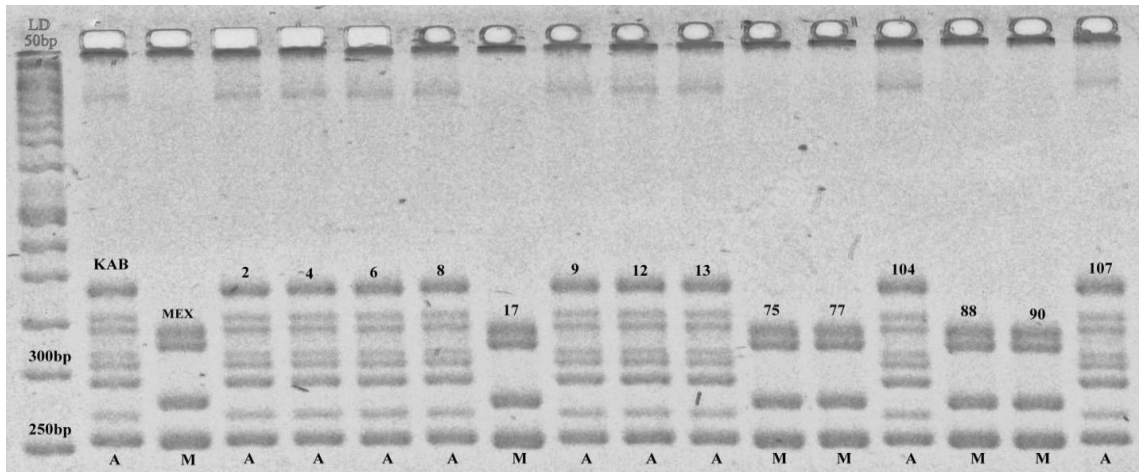


Figure 2.3: Polyacrylamide gel for Phaseolin protein marker where A = Andean, M = Mesoamerican; LD = molecular weight marker (50bp); Kab and Mex = controls (Kablanketi and Mex 54); and Numbers 2 - 107 = genotype samples.

These results suggest that most of the common bean genotypes that are cultivated in Tanzania are of Andean origin with few of Mesoamerican origin from this relatively small sample (Fig. 2.4). The distribution of these two gene pools across bean growing regions show that 75% of the total Mesoamerican bean samples were collected from the Kagera region, while very few were collected from Rukwa (17%) and Mbeya (8%) (Fig.2.4). These results from Tanzania confirm previous findings indicating that both Andean and Mesoamerican beans are grown within some African countries (Wortman et al., 1998; Mahuku et al., 2002a). In Tanzania, it has been found that farmers grow both Andean and Mesoamerican beans and in most cases they mix the two together in the same plot of land as a strategy of risk management since some cultivars fail and others are tolerant to biotic and abiotic factors (Blair et al., 2010).

In terms of regional preferences, the Kagera region of Tanzania leads in cultivation of the Mesoamerican bean type. The regional preference for small (Mesoamerican) or large (Andean) types may be associated with how the common beans are consumed, research need to be done to confirm this. In the lake zone, beans are mostly consumed when cooked with banana (Matoke) and in this dish the preference is for small seeded beans. In other

regions of Tanzania, beans are consumed mostly with rice and maize meal for which the preference is large seeded beans in these dishes. In studies in countries neighboring Tanzania, the proportion of Andean and Mesoamerican bean production in Uganda (Okii et al., 2014) is somewhat similar to Tanzania with Andean bean (51%) outnumbered Mesoamerican bean (49%) although this difference is very small to justify preference of Andean over Mesoamerican, while in Rwanda common beans of Mesoamerican origin (58.3%) outnumbered the Andean ones (37%) with the rest being as a result of introgression (Blair et al., 2010). The preference of Mesoamerican bean in Rwanda has been associated with increase in root rot diseases of beans of which Andean beans are susceptible and also the need for high yield considering scarcity of land where Mesoamerican are considered high yielding compared to Andean beans (Blair et al., 2010).

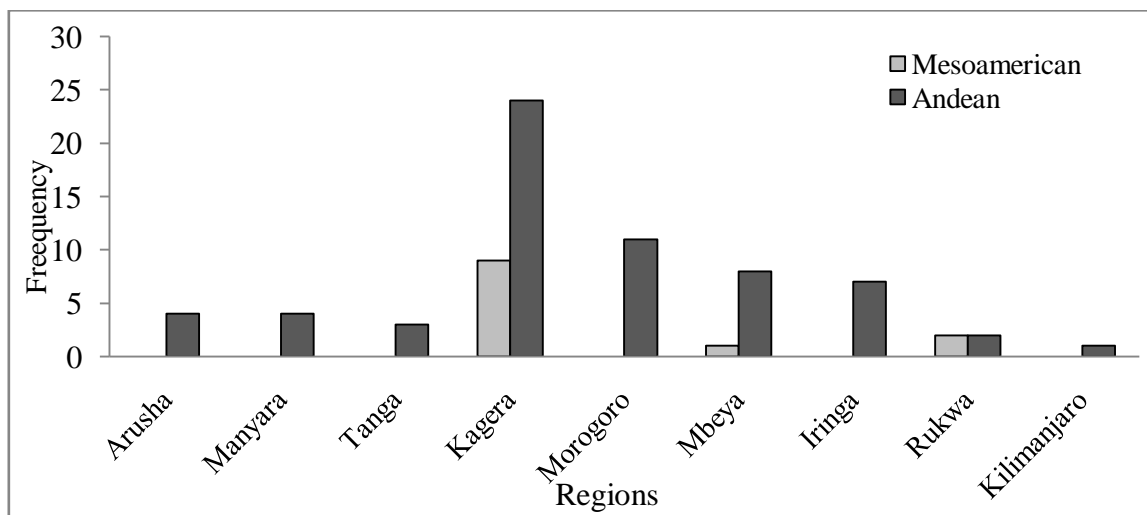


Figure 2.4: Graph showing the proportion of Andean and Mesoamerican common bean genotypes across bean growing regions as determined by the Phaseolin marker.

2.4.3 Pathogen Characterization

The results from the sequence analysis of the Actin gene and the ITS region showed that the ITS regions captures more sequence variation in the pathogen population as compared to the Actin gene. Phylogenetic analysis of the sequences from the Actin gene and the ITS

region depict the presence of two distinct clusters that group with the Andean and Mesoamerican control sequences (Fig. 2.5). These results were expected due to the presence of the two gene pools of the pathogen as reported by Pastor-Corrales et al., (1998). Furthermore, most of the isolates grouped with the Andean control isolate (60%) as compared to the Mesoamerican control isolate (40%). These results show correspondence between the occurrences of the common bean genotypes, as shown by the phaseolin marker results, with the pathogen strains of the same gene pool origin, thus correlating with the concept of co-evolution of this pathogen with its host (Guzman et al., 1995).

The distribution of isolates also showed the same trend as the distribution of the common bean genotypes where most of the Mesoamerican strains were collected from the Kagera region with very few from other regions of Tanzania. Thus, in the Kagera region 60.6% of the isolates were Mesoamerican while 39.4% were Andean. In Arusha, 50% were Andean and in Tanga 67% were Andean (Table. 2.1). This contrasts other findings reported in Uganda (Ddamulira et al., 2014) and in other common bean production regions (Sebastian et al., 2006; Sartorato, 2004) where gene pool grouping due to place of origin of the isolate was not observed.

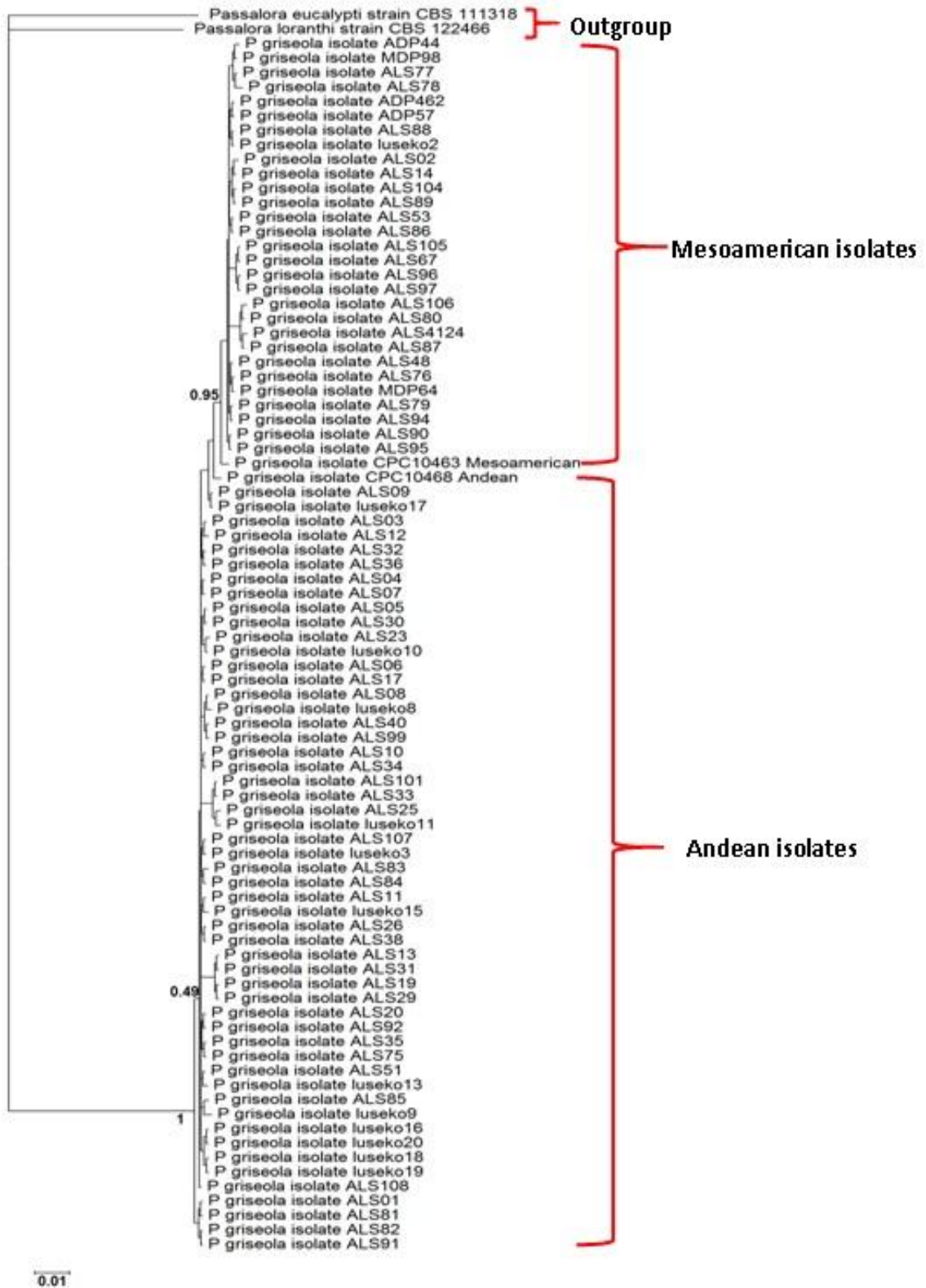


Figure 2.5: Consensus tree results from Bayesian analysis using concatenated ITS and Actin sequences showing the proportion of Tanzanian Andean and Mesoamerican ALS isolates as grouped with control sequences.

Table 2.1: Distribution of Andean and Mesoamerican isolates, showing number and percentage of isolates per location, of *P. griseola* as revealed by sequence analysis of the ITS region and the Actin gene.

S/N	Region	Andean (%)	Mesoamerican (%)	Total
1	Arusha	2 (50%)	2 (50%)	4
2	Kilimanjaro	1 (100%)	0 (0%)	1
3	Manyara	4 (100%)	0 (0%)	4
4	Morogoro	10 (100%)	0 (0%)	10
5	Tanga	2 (66.7%)	1 (33.3%)	3
6	Iringa	8 (100%)	0 (0%)	8
7	Mbeya	9 (100%)	0 (0%)	9
8	Rukwa	4 (100%)	0 (0%)	4
9	Kagera	13 (39.4%)	20 (60.6%)	33

2.4.4 Relationships between Genotype and Pathogen

The relationship between the pathogen and the genotypes on which the isolate was collected shows that Andean isolates infected mostly Andean genotypes (92%) and far less Mesoamerican genotypes (8%). On the other hand, Mesoamerican isolates infected mostly Andean bean genotypes (65%) and fewer Mesoamerican bean genotypes (35%). Generally, Andean genotypes are more susceptible to ALS as compared to Mesoamerican genotypes thus making Mesoamerican genotypes more resistant to both Andean and Mesoamerican isolates of *P. griseola*. These results may indicate that there are likely more genes for resistance to ALS in the Mesoamerican gene pool. Because we found some Andean isolates that were virulent on Mesoamerican genotypes, this suggests that in Tanzania, both common bean genetic backgrounds need to be improved so as to attain durable resistance through breeding for specific regions of Tanzania where specific seed classes are grown. Similar findings have been reported from pathogenic characterization using differential cultivars (Ddamulira et al., 2014), however in this study no differential cultivars were used and instead virulence grouping was employed as described by Mahuku et al., (2002a) in which the affected genotypes were characterized. Further, in this study,

there is interaction between Andean isolates with Mesoamerican genotypes where some Andean isolates showed to be virulent on Mesoamerican genotypes (Fig. 2.6). Using RAPD markers, the Afro-Andean group was included within Andean isolates (Mahuku et al., 2002a). This implies that, there is still within group evolution over time which indicates the continued need to evaluate pathogen variability over time in order to verify possible outbreaks of new strains.

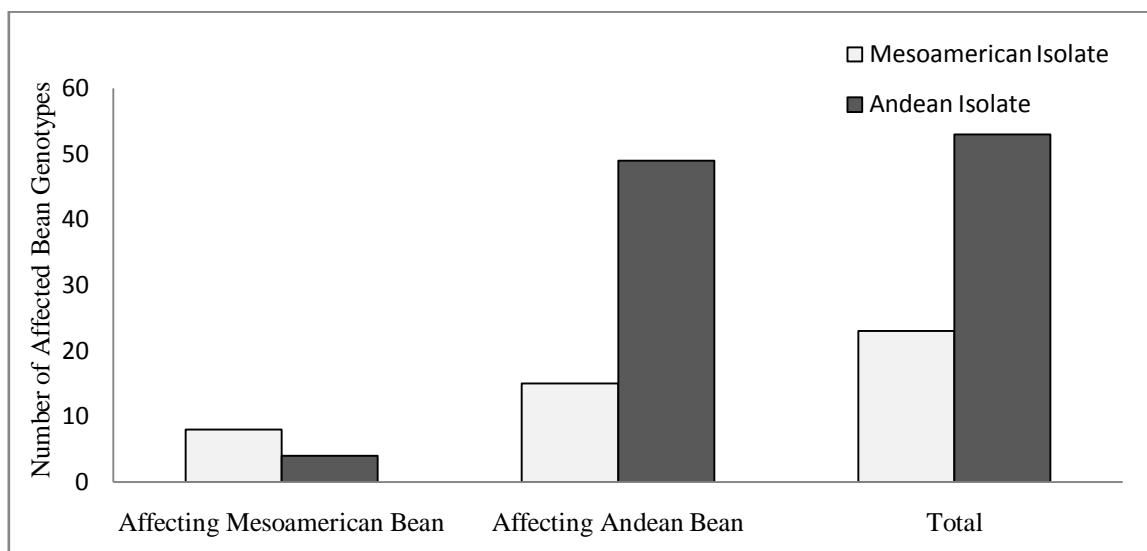


Figure 2.6: Graph showing the number of strains of *P. griseola* from each gene pool collected from Mesoamerican or Andean common bean host genotype.

2.4.5 Virulence of *P. griseola* Isolates

Among the eight selected isolates that were tested for virulence, six different reaction types (pathotypes) were discovered which indicates the existence of pathogenic variability of this fungus. Furthermore some isolates from different regions were of the same pathotypes, which indicates that the same pathotype can exist across different regions. Mexico 54 one of the differential varieties was found to be resistant to all the 8 isolates characterized in this study, which makes it a good source of resistance for ALS breeding in Tanzania (Table 2.2). In other studies in neighboring counties, the reports shows that there are many other isolates of this pathogen that affects Mexico 54 (Mahuku et al.,

2002a; Ddamulira et al., 2014), this could also be the case for Tanzania as only few isolates were tested for virulence using differential varieties in this study. This justifies the need to seek for more resistance sources to be used for breeding against this disease in order to attain durable resistance.

These results are in agreement with some previous findings that most of the Andean isolates affect mostly Andean and the Mesoamerican isolates affects both Mesoamerican and Andean (Mahuku et al., 2002b).

Table 2.2: Pathotype designation, virulence phenotype and group, and the region of origin of *P. griseola* isolates used in this study

Andean Group						Mesomeric Group						Pathotype ^a	Region (Genotype ^b) of collection
A	B	C	D	E	F	G	H	I	J	K	L	(number of isolates)	
1	2	4	8	16	32	1	2	4	8	16	32		
-	+	-	+	-	+	-	+	+	-	+	-	42:22 (1)	Morogoro (A)
+	+	+	+	+	+	-	+	-	-	+	+	63:50 (2)	Mbeya and Arusha(A,M)
-	-	+	-	-	-	-	+	+	-	-	-	4:6 (1)	Kagera (M)
-	-	-	-	+	-	-	+	-	-	+	+	16:50 (1)	Kagera (M)
-	+	+	+	+	+	+	+	-	-	-	-	62:3 (1)	Manyara (A)
+	+	+	+	+	-	-	-	-	-	-	-	31:0 (2)	Rukwa and Mbeya (A)

^aPathotype designation is based on susceptible (compatible) response to each isolate on a set of 12 differential varieties: Andean differential genotypes are A = Don Timoteo, B = G 11796, C = Bolon Bayo, D = Montcalm, E = Amendoim, and F = G5686; Mesoamerican differential genotypes are G = PAN 72, H = G2858, I = Flor de Mayo, J = Mexico 54, K = BAT 332, and L = Cornell 49242. ^bGenotype indicates the genotype from where the isolate was collected; A = Andean genotype and M = Mesoamerican genotype; + = Isolate virulent on cultivar; - = Isolate not virulent on cultivar and Numbers 1, 2, 4, 8, 16 and 32 are binary numbers to designate the Pathotypes

Two pathotypes, 63:50 and 31:0 were found to occur in different regions of which 63:50 is the most virulent pathotype affecting the entire Andean differential lines and half of the Mesoamerican differential lines and it was collected from both Andean genotypes and Mesoamerican genotypes. Pathotype 31:0 observed in this study was once observed in

previous studies to occur in high frequencies in most African countries including Tanzania (Mahuku et al., 2002a)

2.5 Conclusions

Angular leaf spot disease is widely distributed in the surveyed bean growing regions of Tanzania with different severity scores. Two regions, Mbeya and Kagera were found to be hot spots for this disease while Manyara and Rukwa were the regions with low severity. This study also reveals the presence of both Andean and Mesoamerican common bean genotypes in the nine major bean production regions of Tanzania and that Andean beans are the most preferred gene pool across all bean growing regions. This study also verified the presence of Andean and Mesoamerican strains of *P. griseola* and their distribution showing that Andean isolates of *P. griseola* are more prevalent than the Mesoamerican isolates. In the Kagera region (the Lake zone) as opposed to other regions, Mesoamerican isolates outnumbered Andean isolates. Andean isolates were found to affect most of the Andean common bean genotypes and less of the Mesoamerican common bean genotypes. Mesoamerican isolates were found to affect most of the Andean common bean genotypes and less of Mesoamerican common bean genotypes. Further, following virulence classification, it is clear that the pathogen is diverse with different pathotypes and other pathotypes were found to occur in different regions. The most virulent Pathotype among the ones characterized was found to be 63:50, it affects all the Andean differential lines and half of the Mesoamerican lines which makes it a good isolate so far to be used in screening resistant materials developed. The results from this study set a foundation for breeding for resistance to angular leaf spot disease in Tanzania.

References

- Abadio AKR, Lima SS, Santana MF, Salomão TMF, Sartorato A, Mizubuti ESG, de queiroz MV (2012). Genetic diversity analysis of isolates of the fungal bean pathogen *Pseudocercospora griseola* from central and southern Brazil. *Genet. Mol. Res.* 11: 1272 – 1279. <http://dx.doi.org/10.4238/2012.May.14.1>
- Allorent D, Savary S (2005). Epidemiology characteristics of angular leaf spot of bean: a systems analysis. *European Journal of Plant Pathology.* 113: 329 – 341.
- Blair MW, González LF, Kimani PM, Butare L (2010). Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from Central Africa. *Theoretical and Applied Genetics.* 121(2): 237 – 248. <http://doi.org/10.1007/s00122-010-1305-x>
- Carbone I, Kohn LM (1999). A method for designing primer set for speciation studies in filamentous ascomycetes. *Mycologia.* 91: 553 – 556.
- Castellanos G, Jara C, Mosquera G (2015). *Bean Pathogens: Practical Guide for Lab and Greenhouse Work.* (CIAT Publication No. 394), Centro Internacional de Agricultura Tropical (CIAT), 238p.
- CIAT (Centro Internacional de Agricultura Tropical). 1997. Bean Program. in: *Annual Report.* CIAT, Cali, Colombia. pp. 123 – 124.
- Correa-Victoria FJ, Pastor-Corrales MA, Saettler AW (1989). Angular leaf spot. In: *Bean Production Problems in the Tropics* (Schwartz HF, Pastor-Corrales MA, eds). Centro Internacional de Agricultura Tropical, Cali, Colombia. pp. 59–75

- Crous PW, Liebenberg MM, Braun U, Groenewald JZ (2006). Re-evaluating the taxonomic status of *Phaeoisariopsis griseola*, the causal agent of angular leaf spot of bean. *Stud. Mycol.* 55: 163 – 173.
- Ddamulira G, Mukankusi C, Ochwo-Ssemakula M, Edema R, Sseruwagi P, Gepts P (2014). Distribution and Variability of *Pseudocercospora griseola* in Uganda. *Journal of Agricultural Science.* 6(6): 16 – 29.
- Guzmán P, Gilbertson RL, Nodari R, Johnson WC, Temple SR, Mandala D, Gepts P (1995). Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests co-evolution with the common bean (*Phaseolus vulgaris*). *Phytopathology.* 85: 600 – 607. <http://dx.doi.org/10.1094/Phyto-85-600>
- Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics.* 17: 754 – 55.
- Katoh K, Standley DM (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* 30 (4): 772 – 780.
- Kolmer JA, Liu JQ, Sies M (1995). Virulence and molecular polymorphism in *Puccinia recondita* f.sp. *tritici* in Canada. *Phytopathology.* 85: 276 – 285. <http://dx.doi.org/10.1094/Phyto-85-276>
- Lanfear R, Calcott B, Kainer D, Mayer C, Stamatakis A (2014). Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology.* 14:82. <http://www.biomedcentral.com/1471-2148/14/82>
- Mahuku, GS, Iglesias AM, Jara, C (2009). Genetics of angular leaf spot resistance in the Andean common bean accession G5686 and identification of markers linked to

the resistance genes. *Euphytica*. 167: 381 – 396.

<http://dx.doi.org/10.1007/s10681-009-9897-4>

Mahuku GS, Henríquez, MA, Muñoz J, Buruchara RA (2002a). Molecular markers dispute the existence of the Afro-Andean group of the bean angular leaf spot pathogen, *Phaeoisariopsis griseola*. *Phytopathology*. 92: 580 – 589.

<http://dx.doi.org/10.1094/PHTO.2002.92.6.580>

Mahuku GS, Jara C, Cuasquer JB, Castellanos G (2002b). Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding of common bean. *Plant Pathology*. 51: 594 – 604.

Mahuku S (2004). A simple extraction method suitable for PCR-based analysis of plant fungi and bacterial DNA. *Plant molecular Biology Reporter*. 22: 71 – 81.

<http://dx.doi.org/10.1007/BF02773351>

Muthomi JW, Muimui KK, Kimani PM (2011). Inheritance of resistance to Angular Leaf Spot in yellow beans. *Afri. Crop Sci. J.* 19 (4): 267 – 275.

Okii D, Tukamuhabwa P, Kami J, Namayanja A, Paparu P, Ugen M, Gepts P (2014). The genetic diversity and population structure of common bean (*Phaseolus vulgaris* L) germplasm in Uganda. *African Journal of Biotechnology*. 13 (29): 2935 – 2949.

Pastor-Corrales, MA Jara C, Singh S (1998). Pathogenic variation in, source of, and breeding for resistance to *Phaeoisariopsis griseola* causing angular leaf spot in common bean. *Euphytica*. 103: 161 – 171.

<http://dx.doi.org/10.1023/A:1018350826591>

- Rambaut A, Drummond AJ (2007). Tracer v1.4, Retrieved 8 August 2015 from <http://beast.bio.ed.ac.uk/Tracer>
- Sartorato A (2004). Pathogenic Variability and Genetic Diversity of *Phaeoisariopsis griseola* isolates from two Counties in the State of Goias. Brazil J. Phytopathology. 152: 385 – 390. <http://dx.doi.org/10.1016/j.pmpp.2006.10.001>
- Sebastian A, Stenglein I, Pedro A, Balatti A (2006). Genetic diversity of *Phaeoisariopsis griseola* in Argentina as revealed by pathogenic and molecular markers. Physiological and Molecular Plant Pathology. 68: 158 – 167.
- Stenglein S, Ploper LD, Vizgarra O, Balatti P (2003). Angular leaf spot: A disease caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris on *Phaseolus vulgaris* L. Advances in Applied Microbiology. 52: 209 – 243.
- Van Schoonhoven A, Pastor-Corrales MA (1987). Standard system for the evaluation of bean germplasm. CIAT Cali, Colombia. 44p.
- Wortmann CS, Kiluby RA, Eledu CA, Arron DJ (1998). Atlas of Common Bean (*Phaseolus vulgaris* L.) Production in Africa. CIAT. Cali. Columbia. 133p.

CHAPTER THREE

3.0 LEVELS OF RESISTANCE TO ANGULAR LEAF SPOT DISEASE AMONG ADAPTED COMMON BEAN (*P. VULGARIS* L.) GENOTYPES IN TANZANIA

3.1 Abstract

Angular leaf spot of common bean (*Phaseolus vulgaris* L.) is the disease of major importance in Tanzania due to its wide distribution covering all bean growing regions and the nature of its causative pathogen being variable. This necessitates seeking durable resistance to the disease which can be achieved through pyramiding of genes for resistance into one background hence the need of constantly identifying new sources of resistance. This study aims at identifying sources of resistance to the disease by screening available locally adapted genotypes against this disease. Two populations, locally adapted genotypes (landraces and improved varieties) and RIL's from a cross between Rojo and CAL143 were evaluated in field and screen house for their response to ALS disease. Results from both experiments indicate that there was a significant difference ($P \leq 0.01$) on the response of different genotypes to the disease. Beti-10 and line RJRILS135 showed to be superior in both field and screen house with disease scores not different from the resistant control in the population of locally adapted genotypes and RIL's respectively. Results also indicated that small seeded beans were more resistant to the disease as compared to large seeded beans and that even most of the improved varieties succumb to the disease. In this study then, Beti-10 a small seeded landrace and RJRILS135 which is a medium sized seed line are recommended as the best breeding lines to be used in addition to the currently used parents for breeding against this disease which will cover genes from the Mesoamerican and Andean beans respectively as a safeguard against rapid resistance breakdown.

3.2 Introduction

Common bean (*Phaseolus vulgaris* L.) is the crop of major importance as grain legume and vegetable that is mostly consumed worldwide with its seeds and pods being edible (Heuze et al., 2015; Singh, 1999), and in some regions the bean leaves are also consumed as leafy vegetable. In the tropics, this legume is the principle food and cash crop with most of the production in developing countries (Tryphone et al., 2010; Hillocks et al., 2006). In terms of food security and nutrition, this legume is considered being one of the primary and inexpensive sources of calories, proteins, dietary fibers, minerals (iron and zinc) and vitamins (Hillocks et al., 2006; Guzman-Maldonado et al., 2000; Singh, 1999; Beebe et al., 2000) which makes it very important in the developing countries where the problem of food security and malnutrition exists. Common bean can also be used for their crop residues as fodder (Wortman, 2006) and being a legume it has an important role also in soil fertility management for their ability to fix atmospheric nitrogen into the soil (Herridge et al., 2008).

According to FAOSTAT (2014), the average global production of dry beans was approximately 26.5 million tons, of which 6.2 million tons were from Africa, 4.3 million tons from East Africa and about 1.1 million tons from Tanzania. Tanzania is the 7th largest producer of common bean, which makes it the first largest producer in Africa over the years. Looking at the trend of production and the area harvested it is clear that production has increased because mainly of increase in the area harvested which implies that there is very limited or no increase in potential production (Fig.3.1).

Different reports over the years have indicated that, there is an increasing trend in potential bean production worldwide, e.g. from 600 kg in 1996 to 750 kg/ha in 2008 (Margaret et al., 2014). This increase though is reported not to be significant in the resource poor regions as on most of the African countries where the average yields are still below 600kg/ha (Akibode and Maredia, 2011). Taking the FAO statistics for 2014, in Tanzania

the reported potential yield averages at about 938 kg/ha from the year 2011 to 2014 on which the yields have not fluctuated much.

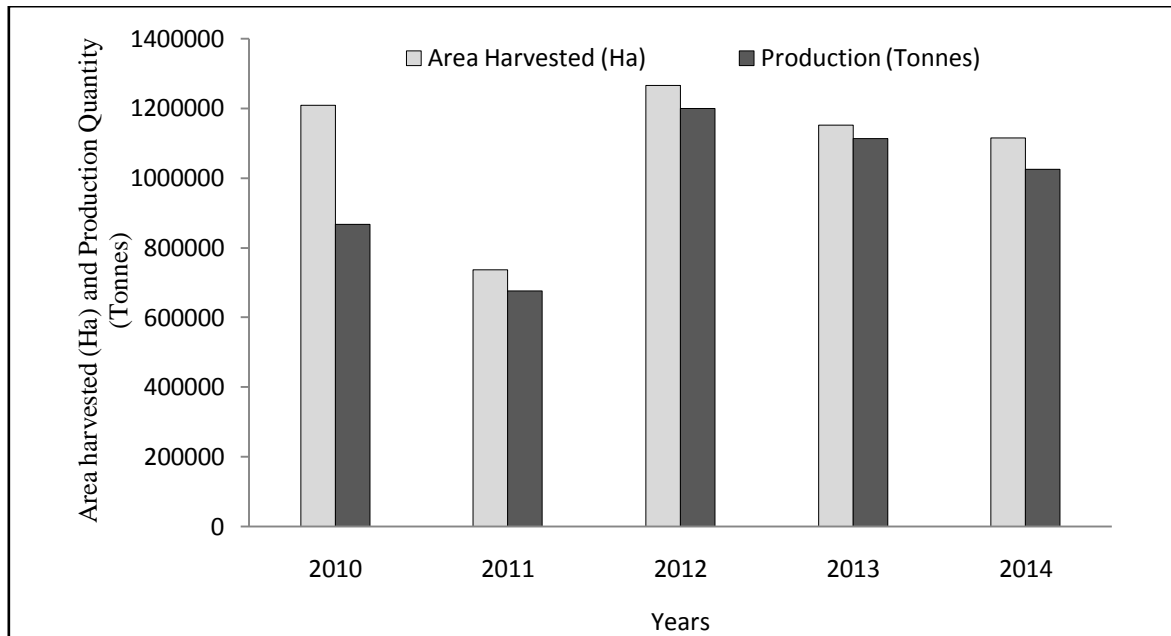


Figure 3.1: Bean Production trends from 2010 to 2014 showing relationship between production and area harvested (FAOSTAT 2014)

Though this figure seem to be a bit high, it is still below the reported potential (1,500 – 3000 kg/ha) that could be achieved under required bean production conditions (Hillocks et al., 2006).

The low level of realized potential of bean production is attributed by many factors, biotic and abiotic ones, most important of these are diseases, insect pests, low soil fertility, drought and poor crop management (Wortman et al., 2006; Beebe et al., 2013; Hillocks et al., 2006; Mwang'ombe et al., 2007). Among the factors attributing to low potential of bean production, bean diseases contribute significantly in the reduction of potential yields (Rodríguez De Luque and Creamer, 2014) and of which the common diseases are angular leaf spot, common bacterial blight, anthracnose, bean viruses (BCMV and BCMNV) and some diseases of the roots such as bean root rots (Rodríguez De Luque and Creamer, 2014; Hillocks et al., 2006 and Tryphone et al., 2012). Among these diseases Angular leaf

spot caused by *Pseudocercospora griseola* (Sacc) Crous and U. Braun (Crous et al., 2006) has been reported to be important in both tropics and temperate regions where bean is grown. The pathogen causes significant yield loss up to 80% (Muthomi et al., 2011) of common bean in Africa and other parts of the world. The reported best control measure is the use of resistant varieties (Crous et al., 2006) of which will best suit low income farmers in that, the farmers will not need any specialized handling of the technology than applying proper management after acquiring improved seed. It is also environmentally friendly compared to the use of fungicides which poses threats to the environment and also to the farmers if improperly handled. Different resistant parents have been identified which are in use for breeding for ALS and are effective against some isolates of the pathogen (Fereira et al., 2000; Stenglein et al., 2003), but there is no single variety that can offer complete resistance to all the races of the pathogen (Wagara et al., 2011). In Tanzania, bean line Mexico 54 has been widely used in bean breeding programs and it offers resistance to some isolates, although it offers some good resistance to be relied on it narrows the genetic base for resistance to the disease. Considering the high variability nature of *P. griseola* and the wide distribution of the disease in Tanzania, a single source of resistance is inadequate. Pyramiding several genes in one background is a way of achieving broad and durable resistance to many races of *P. griseola* (Mahuku et al., 2002). In that light, this work focuses in establishing the levels of resistance to ALS among the locally adapted bean genotypes as a strategy of identifying other potential sources of resistance to ALS from the already adapted common bean genotypes in Tanzania.

3.3 Materials and Methods

3.3.1 Source and description of the genotypes

Two populations of bean genotypes were used in this study. First is the population of 158 recombinant inbred lines (RIL's) developed from the cross between Rojo and CAL 143

and the parents making 160 genotypes. Rojo is an improved variety released at SUA and is susceptible to most races of *P. griseola* and CAL 143 is the variety that is resistant to some races of *P. griseola*. Second is the population of locally grown genotypes (landraces and improved varieties) collected from different bean growing regions of which a total of 104 genotypes including the resistant control Mexico 54 were evaluated.

3.3.2 Source of *P. griseola* isolate

For the screen house experiment, inoculation was done using an isolate that was chosen from a group of eight isolates that were originally characterized using ITS and Actin gene sequences. These eight isolates (four from Mesoamerican race and four from Andean race) were also originally characterized and the virulent isolate based on differential cultivars is the one isolate that was chosen for this study. This isolate was collected from Mesoamerican bean in Arusha and Andean bean in Mbeya regions and was characterized using molecular markers (ITS and Actin gene) and fell under Andean race of the pathogen.

3.3.3 Field evaluation for Angular leaf spot

In the field experiment, the two populations were planted in randomized blocks in three replications, each plot consisted of four rows of 2 meters each. For the CAL/Rojo RIL's, evaluation was done in two growing seasons (2015 and 2016) while for the Local genotypes evaluation was done only in one growing season (2016). The experiments were set in Mbeya region as is the hot spot for ALS disease and considered to have a mixture of races of the pathogen. Data were collected in terms of disease scores as per CIAT scale of 1 – 9 (Van schooven and Pastor-Corrales, 1987)

3.3.4 Screen house evaluation for angular leaf spot

For screen house evaluation, the two populations were screened once. Beans were planted in pots containing sterilized forest soil. The experiment was set in completely randomized design with three replications. Inoculation was done when the plants had attained 17 days

after planting and disease evaluation started from the first appearance of ALS symptoms. Data was taken four times at an interval of 4 days using the CIAT scale of 1 – 9.

3.3.5 Disease assessment and selection of potential resistant parents

Data were analyzed using Genstat 5th Edition software, where genotypes were categorized based on their average scores where a score of 1 – 3 were considered resistant; >3 – 6 moderately resistant and above 6 being susceptible. For the field experiment ANOVA was also used to infer the influence of season on ALS severity. Selection of potential genotypes was based on the disease scores where the genotype should have an average severity of less than or equal to the resistant parent used and also should perform best across experiments.

3.4 Results and Discussion

3.4.1 Evaluation of landraces for ALS

Field results indicated significant variations ($P \leq 0.01$) of resistance levels to angular leaf spot among the genotypes used. For the locally adapted genotypes, disease scores ranged from one for landrace Beti-10 which was also the score for the resistant check Mexico 54 to a score of 6 for the susceptible landrace and the susceptible check being Kablanketi which had a score of 5.5 (Table 3.1). In disease classification based on CIAT scale; the score of 5.5 is considered to be moderately resistant, for landrace Kablanketi this is not always the case when grown in the field and when conditions are perfect for the disease. This indicates that disease pressure in that season was not high which can be caused by the factors attributing to the development of the disease such as humidity and temperature. Other reports also indicate that disease pressure in different seasons can be different where wet season will fetch high scores as opposed to dry season (Parrella et al., 2013), this could have attributed to this observations in this season.

Beti-10, a Mesoamerican landrace showed to be superior among the other landraces as it had a score of 1 which is the same as Mexico 54 which is the known popular resistant parent used for breeding for angular leaf spot and Masusu (landrace) is the least with a score of 6. Apart from Beti-10, 44% of the landraces evaluated had scores ranging between 1 and 3 indicating resistance, and the remaining 56% between 3.1 and 6. This indicates that, some other landraces apart from Beti-10 could also have some resistance to some isolates of the pathogen.

For the screen house experiment, results indicated that the disease scores ranged from 1.5 to 8.7 which is the range encompassing all the three categories as resistant (1 – 3), moderately resistant (>3 – 6) and susceptible (>6 – 9). The resistant check had a score of 1.5 and susceptible check had a score of 8.3 (Table 3.1 and Figure 3.2).

Table 3.1: Levels of resistance to ALS among the bean landraces

	Field experiment 2016	Screen house experiment
Minimum disease score	1.0	1.5
Maximum disease score	6.0	8.7
Kablanketi	5.5	8.3
Mex 54	1.0	1.5
Beti-10	1.0	1.5
Mean disease score	3.4	6.0
LSD _(P=0.05)	1.5	1.5
S.E	1.0	0.9
CV (%)	28.2	14.9

Again landrace Beti-10 was superior among other landraces as it had the least score of 1.5 being equal to the resistant control. In this experiment only 2.9% of the landrace were resistant, 41.3% intermediate resistant and 55.8% susceptible.

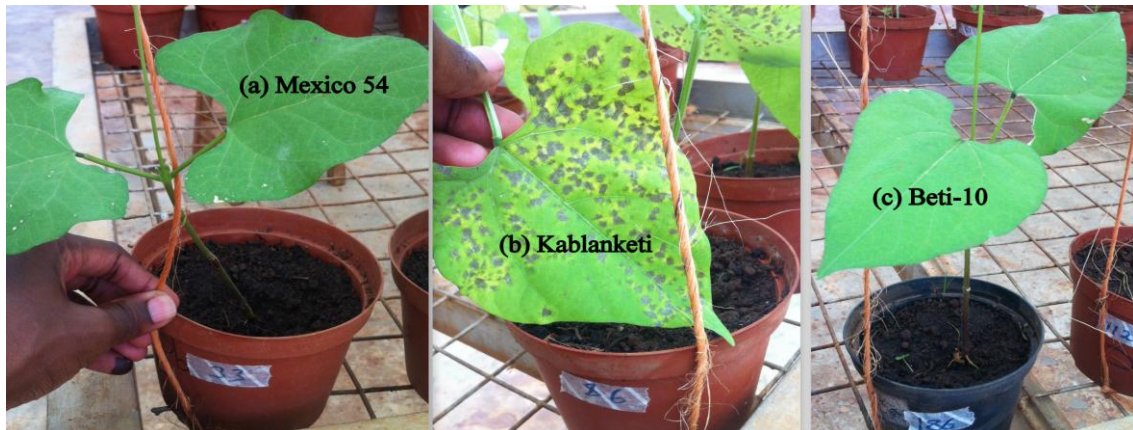


Figure 3.2: Response of (a) Mexico 54, (b) Kablanketi and (c) Beti-10 to Angular leaf spot under screen house conditions

These results indicate that, most of the landraces are susceptible to the disease when there are perfect conditions for disease development and when virulent isolates are present. Apart from Beti-10, only one other landrace Nkanamna was grouped as resistant having an average score of 2.3.

These results confirm the previous findings that Beti-10 and Nkanamna (landraces) have some levels of resistance to ALS (Fivawo and Msolla, 2006). In the same study, Nanka and Nanavala (landraces) were also possessing good levels of resistant of which both of them in this study had intermediate levels of resistance. All these landraces possessing good levels of resistance are small seeded bean (Mesoamerican) which insists that disease resistant levels are high among the Mesoamericans as compared to Andean beans. This observation is similar with other results from previous studies that most small seeded beans (Mesoamerican) have good resistance to diseases as compared to large seeded beans (Andean) when tested with different races of the pathogen in field and screen house conditions (Wagara et al., 2011; Pastor-Corrales et al., 1998; Liebenberg and Pretorius, 1997). Further in this study most of the Andean beans that are mostly preferred (market class) have low levels of resistance to the disease as the case for Kablanketi and Njano in this case with disease scores of 8.3 and 6.5 respectively. Far as much Mesoamerican beans have been reported to have good levels of resistance in Africa, for durable resistance there

is a need to pyramid resistance genes from both Andean and Mesoamerican bean due to the presence of Afro-andean races that are able to affect both Andean and Mesoamerican bean (Mahuku et al., 2002).

3.4.2 Evaluation of RIL's for ALS

This population was evaluated twice in the field covering two growing seasons 2015 and 2016. In all the seasons there were significant variations ($P \leq 0.01$) of response of these lines to angular leaf spot disease. The results for both 2015 and 2016 seasons and the combined results are presented in Table 3.2. In growing season 2015, none of the RILS was grouped as resistant (Score 1 – 3), 14.4% were moderately resistant and 85.6% were susceptible. The minimum and maximum disease scores were 3.7 and 8.7 respectively and the resistant control Mexico 54 had a score of 5 which falls under intermediate resistant. In that season, the disease pressure was very high that none of the even known sources of resistance had a score of 1 – 3 and only a few lines expressed intermediate resistance while majority were susceptible. In 2016 season, likewise in the evaluation of the locally adapted genotypes, the disease pressure was low and results indicated that only two groups of resistant (66.2%) and moderately resistant (33.8%) were found. This low disease pressure was possibly contributed by the prevalent dry conditions during that growing season as compared to the year 2015. The minimum and maximum disease scores were 1 and 6 respectively and the resistant control Mexico 54 had a score of 1. Combined analysis for 2015 and 2016 indicated significant contribution of seasons to ALS severity as it is observed that in the season 2015 the disease pressure was high compared to the season 2016 in the field. This also affirms the results from the study by Augustus et al., (2013) that season can significantly affect the severity of angular leaf spot disease. Results from the screen house indicated that 51% of the lines were resistant, 47% intermediate resistant

and 2% susceptible and there is a significant variation ($P \leq 0.01$) amongst the lines in their response to ALS disease.

Table 3.2: Levels of resistance to ALS among the recombinant inbred lines (RIL's) population from CAL 143 and Rojo

	Field experiment 2015	Field experiment 2016	Combined results (Field)*	Screen house experiment
Minimum disease score	3.7	1.0	2.6	1.0
Maximum disease score	8.7	6.0	7.2	7.0
RJRILS135	4.0	2.0	3.0	1.0
Rojo	7.0	4.0	5.5	3.0
CAL 143	5.3	2.0	3.4	2.0
Mexico 54	5.0	1.0	3.0	1.0
Mean disease score	7.0	2.9	4.9	3.3
LSD _(P=0.05)	1.5	1.3	1.0	1.0
S.E	0.93	0.8	0.9	0.6
CV (%)	13.3	28.0	17.7	19.5

*Combined results (2015/2016) for field screening experiment

Among many lines that showed to be resistant and moderately resistant to the disease in this population, line RJRILS135 showed to be superior. Selection of this line was based on comparing it to the controls for this case Mexico 54 which is the resistant donor currently in use and CAL 143 which is the donor parent for ALS in the test population. The differences in disease scores if any between this line and the controls showed not to be significant ($P \leq 0.001$). This line performed well in all the experiments (in the field for two seasons and in the screen house) showing that apart from just being resistant, it has broad range of resistance and also it is stable across seasons and different isolates. It is reported that, due to pathogenic variability of *P. griseola*, varieties that are resistant in one location or one season can easily be susceptible in another location or season (Mahuku et al., 2002). This attribute of RJRILS135 line to be stable in resistance across seasons and location gives it advantage of being used as a potential donor for resistance as one seeks durable resistance. More work needs to be done to look at it closely so as to identify other good traits it possess.

3.5 Conclusion

In this study, two genotypes Beti-10 (landrace) and RJRILS135 (line) were selected as potential best breeding parents for angular leaf spot disease. These genotypes, one from Andean and another from Mesoamerican if used together may compliment the currently used donor in most bean breeding programs in Tanzania and can safeguard the newly developed varieties from rapid disease resistance break down as suggested by Mahuku et al., (2009). In this study, most of the released improved varieties that were reported to be resistant to ALS (Kanyeka et al., 2007) are no longer resistant to the disease. Similar observations were made in Kenya where the newly released varieties were resistant to ALS and those that were released much earlier were susceptible. This suggests that even with the newly released varieties, resistance breakdown may occur over time (Ddamulira, 2014). This may be due to the changes in the virulence of the pathogen due to inherent evolution which over time allows new strains to develop that overcome resistance in these improved materials (Crous et al., 2006). Therefore, these genotypes identified when used will reduce the risk of resistance breakdown, as different alleles will be combined together into one genotype and hence increase the sustainability of the resistance.

References

- Akibode S, Maredia M (2011). Global and Regional Trends in Production, Trade and Consumption of Food Legume Crops, Report Submitted to SPIA. 83p
<http://impact.cgiar.org/sites/default/files/images/Legumetrendsv2.pdf>
- Beebe S, Skroch PW, Tohme J, Duque MC, Pedraza F, Nienhuis J (2000). Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. *Crop Science Journal*. 40: 264 – 273.
- Beebe SE, Rao IM, Blair MW, Acosta-Gallegos JA (2013). Phenotyping common beans for adaptation to drought. *Frontiers in Physiology*. 4: 35.
<http://doi.org/10.3389/fphys.2013.00035>
- Crous PW, Liebenberg MM, Braun U, Groenewald JZ (2006). Re – evaluating the taxonomic status of *Phaeoisariopsis griseola*, the causal agent of angular leaf spot of bean. *Studies in Mycology*. 55: 163 – 173.
<http://dx.doi.org/10.3114/sim.55.1.163>
- Ddamulira G, Mukankusi C, Edema R, Sseruwagi P, Gepts P (2014). Identification of new sources of resistance to angular leaf spot among Uganda common bean landraces. *Canadian Journal of Plant Breeding*. 2(2): 55 – 65.
- FAOSTAT (2014). FAOSTAT Data available at <http://www.fao.org/faostat/en/#data/QC>
(Page visited, Nov, 2017)
- Ferreira CF, Borém A, Carvalho GA, Nietzsche S, Paula TJ, Barros EG (2000). Inheritance of angular leaf spot resistance in common bean and identification of a RAPD marker linked to a resistance gene. *Crop Science*. 40: 1130 – 1133.

- Fivawo NC, Msolla SN (2006). Virulence of 6 isolates of *Phaeoisariopsis griseola* (sacc) [ferr] upon 41 common bean landraces in Tanzania. 150: 95 – 96.
- Guzman-Maldonado SH, Acosta-Gallegos J, Paredes-Lopez O (2000). Protein and mineral content of a novel collection of wild and weedy common bean (*Phaseolus vulgaris* L.). Journal of Science of Food and Agriculture. 80: 1874 – 1881.
- Herridge DF, Peoples MB, Boddey RM (2008). Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil. 311: 1 – 18. DOI 10.1007/s11104-008-9668-3
- Heuzé V, Tran G, Nozière P, Lebas F (2015). Common bean (*Phaseolus vulgaris*). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. <http://www.feedipedia.org/node/266>
- Hillocks RJ, Madata CS, Chirwa R, Minja EM, Msolla SN (2006). Phaseolus bean improvement in Tanzania, 1959 – 2005. Euphytica. 150: 215 – 231.
- Kanyeka E, Kamala R, Kasuga R (2007). Improved Agricultural research technologies recommended in Tanzania. Department of Research and Training, Ministry of Agriculture, Food Security and Cooperatives. Dar es salaam Tanzania. p 38 – 42.
- Liebenberg MM, Pretorius ZA (1997). A review of angular leaf spot of common bean (*Phaseolus vulgaris* L.). African Plant Protection. 3: 81 – 106.
- Mahuku GS, Iglesias AM, Jara C (2009). Genetics of angular leaf spot resistance in the Andean common bean accession G5686 and identification of markers linked to the resistance genes. Euphytica. 167: 381 – 396.

- Mahuku GS, Jara C, Cuasquer JB, Castellanos G (2002). Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding of common bean. *Plant Pathology*. 51: 594 – 604.
- Margaret N, Tenywa JS, Otabbong E, Mubiru DN, Ali T (2014). Development of common bean (*Phaseolus vulgaris* L.) production under low soil phosphorus and drought in Sub-Saharan Africa: A Review. *Journal of Sustainable Development*. 7(5): 128 – 139. <http://dx.doi.org/10.5539/jsd.v7n5p128>
- Muthomi JW, Muimui KK, Kimani PM (2011). Inheritance of resistance to angular leaf spot in yellow beans. *African Crop Science Journal*. 19(4): 267 – 275.
- Mwang'ombe AW, Wagara IN, Kimenju JW, Buruchara RA (2007). Occurrence and severity of Angular Leaf Spot of Common bean in Kenya as influenced by geographical location, altitude and agro ecological zones. *Plant Pathology*. 6(3): 235 – 241.
- Parrella RADC, Santos JBD, Parrella NNLD, Silva DVFE (2013). Evaluation efficiency of severity of angular leaf spot in common bean based on diseased and healthy leaf area. *Crop Breed. Appl. Biotechnol*. 13(3): 178 – 185.
- Pastor-Corrales MA, Jara C, Singh SP (1998). Pathogenic variation in, sources of, and breeding for resistance to *Phaeoisariopsis griseola* causing angular leaf spot in common bean. *Euphytica*. 103: 161 – 171.
- Rodríguez D, Jesús J, Creamer B (2014). Major constraints and trends for common bean production and commercialization; establishing priorities for future research. *Agronomía Colombiana*. 32(3): 423 – 431. <http://hdl.handle.net/10568/56998>

- Singh PS (eds.) (1999). Common Bean Improvement in the Twenty- First Century. Kluwer Academic Publishers, Netherlands. 405p.
- Stenglein SLD, Ploper O, Vizgarra P, Balatti P (2003). Angular leaf spot: A disease caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris on *Phaseolus vulgaris* L. Advances in Applied Microbiology. 52: 209 – 243. [http://dx.doi.org/10.1016/S0065-2164\(03\)01009-8](http://dx.doi.org/10.1016/S0065-2164(03)01009-8)
- Tryphone GM, Nchimbi-Msolla S (2010). Diversity of common bean (*Phaseolus vulgaris* L.) genotypes in iron and zinc contents under screenhouse conditions. African Journal of Agricultural Research. 5 (8): 738 – 747.
- Tryphone GM, Chilagane LA, Kusolwa PM, Nchimbi-Msolla S (2012). Inheritance of Angular leaf spot (*Phaeosariopsis griseola* (Sacc.) Ferr) resistance in common bean (*Phaseolus vulgaris* L.) population. Journal of Agriculture Science and Technology A. 2(7): 586 – 862.
- Van Schoonhoven A and Pastor-Corrales MA (1987) Standard system for evaluation of bean germplasm. CIAT, Cali. 53p.
- Wagara IN, Mwang'ombe AW, Kimenju JW, Buruchara RA, Kimani PM (2011). Reaction of selected common bean genotypes to physiological races of *Phaeoisariopsis griseola* occurring in kenya. African Crop Science Journal 19 (4): 343 – 355.
- Wortmann CS, Kiluby RA, Eledu CA, Arron DJ (1998). Atlas of Common Bean (*Phaseolus vulgaris* L.) Production in Africa. CIAT. Cali. Columbia. 133p.

Wortmann CS (2006). *Phaseolus vulgaris* L. (Common Bean). Record from PROTA4U.

Brink, M. and Belay, G. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. <http://www.feedipedia.org/node/266> Page visited Dec 2016.

CHAPTER FOUR

4.0 INHERITANCE, ALLELIC RELATIONSHIP AND IDENTIFICATION OF MOLECULAR MARKERS LINKED TO ANGULAR LEAF SPOT RESISTANCE IN THE COMMON BEAN LANDRACE BETI-10 IN TANZANIA

4.1 Abstract

Angular leaf spot caused by the fungus *Pseudocercospora griseola* (Sacc) Crous and U. Braun is an important disease of common bean in Tanzania due to its wide distribution, high severity and its pathogen being highly variable. In order to attain durable resistance there is a great need to pyramid genes for resistance from diverse backgrounds which needs to be characterized. The objective of this work was to characterize angular leaf spot resistance found in the local landrace Beti-10, elucidating the allelic relationship between it and the currently used donor for resistance Mexico 54 using a characterized virulent strain of the pathogen and identification of molecular markers linked to the resistance in Beti-10. Crosses were made between Kablanketi vs Beti-10 and Beti-10 vs Mexico 54 and their reciprocals and their F₂ used for studies of inheritance, allelic relationship and co-segregation analysis to find molecular markers linked to resistance in Beti-10 for use in breeding. Segregation results among the F₂'s for Kablanketi vs Beti-10 ($\chi^2 = 0.061$ and $P = 0.806$) and Beti-10 vs Kablanketi ($\chi^2 = 3.776$ and $P = 0.052$) indicate that there is a ratio of 3:1 for Resistant: Susceptible respectively. This implies that, single dominant gene is responsible for resistance to angular leaf spot disease in Beti-10 and no maternal influence to disease was observed. Further there was no allelic relationship between Beti-10 and Mexico 54 which suggests that the genes responsible for angular leaf spot disease resistance in these genotypes are different. Among the SCAR markers used in co-segregation analysis, SCAR markers PF13 and SN02 linked to the gene *Phg109898* and

Phg-2 respectively were found to be linked to the locus responsible for resistance in Beti-10 in repulsion and coupling phase respectively. From the recombination analysis, these markers PF13 and SN02 have 92% and 95% reliability for use in marker assisted selection. This study opens room for Beti-10 landrace in addition to Mexico 54 along with SCAR markers PF13 and SN02 to be used for breeding for resistance against ALS disease which will simplify the breeding procedures using MAS and increase the durability of the resistance in developed genotypes.

4.2 Introduction

Common bean (*Phaseolus vulgaris* L.) is an important grain legume grown worldwide. It is very important in Africa and Latin America for its nutritional value being a source of protein, vitamins, minerals (iron and Zinc) and dietary fiber (Petry et al., 2014; Beebe et al., 2000; Bennink, 2006; Widors, 2006). In Africa, the consumption is in diverse forms including fresh or dry grains, green leaves as leafy vegetable and green pods (Kimani et al., 2006). Its importance cannot be underestimated especially in Africa where other sources of protein are expensive which leaves the only constant supply of protein to depend on beans (Beebe et al., 2013). It is estimated that beans supply more than 50% of dietary proteins in sub Saharan Africa (Broughton et al., 2003).

Tanzania is among the ten largest producers of dry beans in the world and ranks first in Africa (FAOSTAT 2014). Despite Tanzania being the largest producer and some other East African countries being among the top ten producers, common bean production potential has not been realized. Different reports shows that production in African countries hardly exceeds 600kg/ha which is far much below the reported potential production of 1500-3000kg/ha under optimum conditions (Akibode and Maredia 2011; Hillocks et al., 2006). Based on FAO data overall production in Tanzania mainly has increased with increase in total area under beans cultivation and in the year 2011 to 2014, average production was around 938kg/ha (FAOSTAT, 2014) which is still below the potential production reported.

Among the many factors that contribute to this low productivity is angular leaf spot disease caused by the fungus *Pseudocercospora griseola* (Sacc) Crous and U. Braun (Crous et al., 2006). To facilitate the use of resistant varieties in breeding against this disease, some molecular markers linked to different genes that resist some strains of the pathogen have been developed (Miklas, 2010). Some of the discovered genes and

molecular markers are; *Phg-2* (SN02) (Nietsche et al., 2000), *Phg-1* (SH13) *Ouro Negro* dominant gene (SAA19, BA16, SM02) (Queiroz et al., 2004a), *G10474* dominant gene (E-ACA/M-CTT) (Mahuku et al., 2004) and *Phg_{G109098}* (OPE04, PF13 and PF9) (Mahuku et al., 2011). In Tanzania and Africa in general the donor parent Mexico 54 which has the locus *Phg-2* has widely been used as a source of ALS resistance in breeding programs which in turn have resulted into many developed varieties bred for ALS resistance to contain the same locus. Despite these markers being used successfully in some areas, it has also been reported that in some backgrounds these markers are not polymorphic hence limit their use (Namayanja et al., 2006) and also the causative pathogen of the disease has been reported to be highly variable (Mahuku et al., 2002) which threatens the durability of the resistance. The proposed solutions to achieve durable resistance is to have variety of polymorphic markers to be used linked to different loci for ALS resistance and of course other sources of resistance as opposed on relying on only one source/locus for resistance.

This work focuses on characterizing resistance to ALS in locally adapted landrace Beti-10 and ultimate identification of polymorphic markers that can be used in breeding. This will ultimately increase the number of usable loci responsible for ALS resistance from the local genotypes which will reduce the risk of low adaptability of foreign genotypes to Tanzanian environment and also facilitate easy adoption of the released varieties based on the fact that the used donor is already adapted and cultivated.

4.3 Materials and Methods

4.3.1 Genotypes and *P. griseola* isolates

Bean seeds of landrace Beti-10, Kablanketi and the currently used donor for ALS resistance, Mexico 54 were obtained from SUA bean collection. Beti-10 has shown to be resistance to most races of the pathogen when grown in hot spot regions and when artificially inoculated. Kablanketi is the most popular market-class bean in Tanzania

which is grown and preferred in all bean growing regions. Beti-10 and Mexico 54 are Mesoamerican while Kablanketi is an Andean bean. The isolate used in this study is a characterized race 63:50 of *P. griseola* which is very virulent to Kablanketi and does not affect Mexico 54 and Beti-10. It was collected from Mbeya and Arusha regions, one of the hot spot regions for the disease. The isolate was collected from Andean (Mbeya) and Mesoamerican (Arusha) beans and grouped as Andean isolate when characterized using ITS and Actin gene sequences (Chilagane et al., 2016) which means it affects both Andean and Mesoamerican bean (Afro-Andean race) (Mahuku et al., 2002).

4.3.2 Genetic characterization of Beti-10

Crosses were made between Kablanketi vs Beti-10 and Beti-10 vs Mexico 54 and their reciprocals to generate F_1 's which were advanced to F_2 that were used for inheritance and allelism test. A total of 393 and 205 F_2 plants were evaluated for inheritance and allelism test respectively using one isolate (race 63:50). F_2 plants for each of the crosses were planted in pots containing sterilized forest soil, inoculation was done following the procedure by CIAT (Castellanos et al., 2015) and disease assessment was conducted. The CIAT scale of 1 – 9 was used in evaluation where a score of 1 – 3 was considered resistant and the rest (>3 – 9) being susceptible. Segregation ratios for the F_2 population of each of the crosses were computed and chi-square test was used to determine the deviation of observed from the expected results. Recombination frequencies were calculated as a fraction of recombinants over total number of plants assessed and distance between the gene for disease resistance in Mexico 54 and that of Beti-10 were estimated from the recombination frequency.

4.3.4 SCAR markers co-segregation

A segregating population of F_2 from the previous section from a cross between Kablanketi and Beti-10 was used for this study. A total of 100 F_2 plants from this cross were

evaluated after inoculation with the race 63:50 of *P. griseola* as described in the previous section. DNA was extracted from resistant and susceptible plants separately using TES buffer extraction method (Mahuku, 2004) and two DNA bulks (5 plants each) were generated from resistant and susceptible individuals. SCAR markers for ALS (Miklas, 2010; Mahuku et al., 2011; Nietsche et al., 2000) were tested to determine the linkage with ALS resistance in Beti-10 landrace. PCR amplification was done following the conditions of each primer (Miklas, 2010; Mahuku et al., 2011; Nietsche et al., 2000), DNA fragments were separated using a 1.5% agarose gel run in TAE electrophoresis buffer at 100V for 2.5hrs.

4.4 Results and Discussion

4.4.1 Characterization of resistant gene from Beti-10

Segregation results from F₂ populations showed that the resistance of Beti-10 to angular leaf spot race 63:50 is controlled by single gene as shown in the data for disease reaction where the observed ratio for F₂ population was not significantly different from the expected for single dominant gene inheritance. In the analyzed population of Kablanketi x Beti-10, the chi square and probability was 0.061 and 0.806 and in the population from its reciprocal cross 3.776 and 0.052 respectively (Table 4.1). This finding is in agreement with other many different studies which indicate that angular leaf spot disease resistance in different parents is controlled by one, two or three dominant genes (Caixeta et al. 2005; Namayanja et al., 2006, and Mahuku et al., 2009). The most common type of inheritance reported being dominant and monogenic (Caixeta et al., 2003 and Namayanja et al., 2006). Another study using uncharacterized isolate of the pathogen collected in Morogoro region found that resistance to angular leaf spot in Beti-10, Nanka, Nkanamna and Nanavala landraces of Tanzania is controlled by single dominant gene (Tryphone et al., 2015). All these results supports the findings of this study that one dominant gene is responsible for

the resistance in Beti-10. This mode of inheritance makes Beti-10 easy to deploy as a breeding parent for the disease in Tanzania which will increase the chance of attaining durable resistance.

Further, when comparing the results from reciprocal crosses, there is no notable difference in the results from these populations ($X^2 = 3.84$ and $P = 0.28$). This suggests that, the results from reciprocal crosses are the same that rules out maternal influence to angular leaf spot disease resistance at least for this race of the pathogen and the parents used. Despite this study showing no maternal influence for ALS disease, for other bean diseases, Mukankusi et al., (2011) reported its existence when studying resistance to *Fusarium* root rot in common bean. Many breeding programs normally use the donor parent as the male parent which undermines the existence of maternal influence, therefore there is a need to research more on this aspect.

Allelism results showed that the genes (locus) responsible for angular leaf spot disease resistance in Beti-10 are different from that in Mexico 54. This is shown by the presence of recombinants (individual plants that are different from their parents in disease response) that occurred in F_2 individuals assessed for the disease for the cross between Mexico 54 x Beti-10 and Beti-10 x Mexico 54 (Table 4.1).

Further, the low frequency of occurrence of recombination suggests that, the genes in Mexico 54 and that in Beti-10 may not be very far apart from one another. This puts Beti-10 being valuable as it offers other locus for use in pyramiding genes for resistance to this disease which in turn increases the life of resistance in the developed materials.

Table 4.1: Frequencies of Phenotypic classes for ALS resistance among different populations

Parent/cross	Generation	Number of plants		Expected ratio	X ²	*P
		Resistant	Susceptible			
Mex 54	Parent	58	0	1:0	-	-
Beti-10	Parent	55	0	1:0	-	-
Kablanketi	Parent	0	57	0:1	-	-
Kabl x Beti-10	F ₂	150	48	3:1	0.061	0.806
Beti-10 x Kabl	F ₂	158	37	3:1	3.776	0.052
Mex 54 x Beti-10	F ₂	103	3	1:0	-	-
Beti-10 x Mex 54	F ₂	98	1	1:0	-	-

*P values greater than 0.05 indicates that the observed frequencies are not significantly different from the expected ones

4.4.2 Co – segregation analysis

Among the markers that were evaluated, SCAR markers PF13 (Mahuku et al., 2011) and SN02 (Nietsche et al., 2000) were found to be polymorphic between Beti-10 and Kablanketi and further were able to also be linked in repulsion and coupling phase respectively to the angular leaf spot disease resistance in the landrace Beti-10 (Fig 4.1 and Fig 4.2).

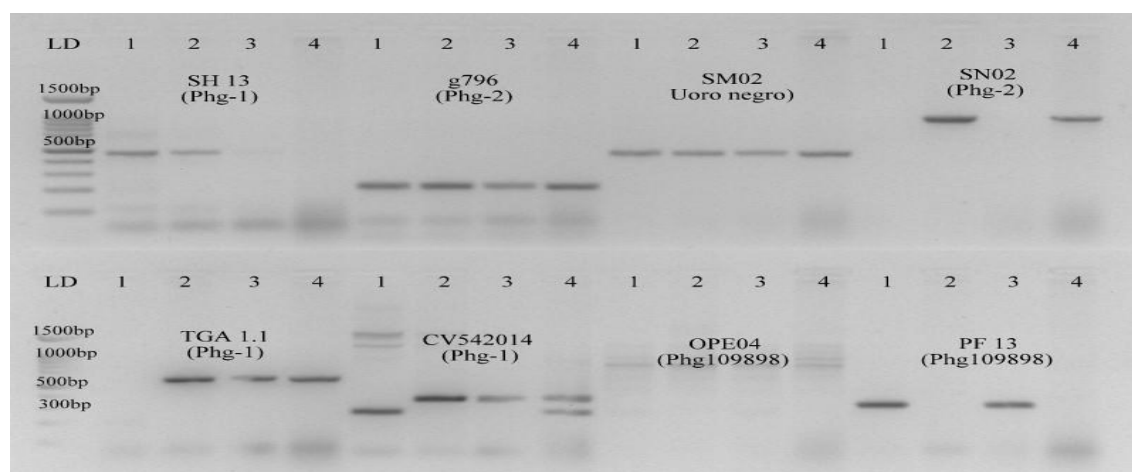


Figure 4.1: PCR amplification products showing polymorphism for the SCAR markers SN02 and PF13; LD = 100kb ladder, 1 = Beti-10 (Resistant parent), 2 = Kablanketi (Susceptible parent), 3 = Resistant Bulk and 4 = Susceptible bulk.

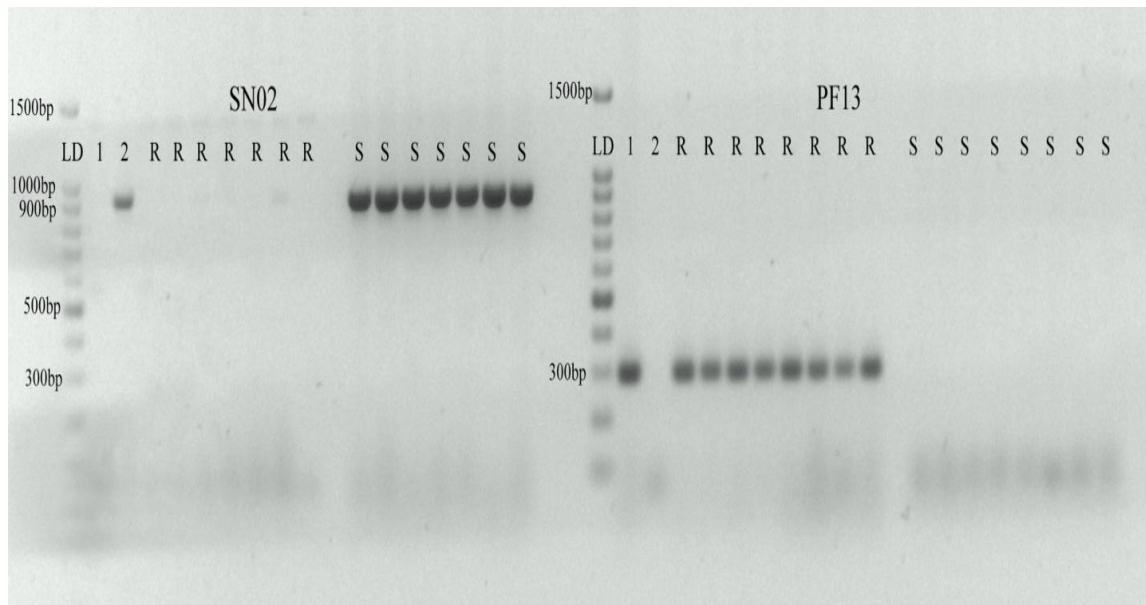


Figure 4.2: PCR amplification products for SN02 (Coupling phase) and PF13 (repulsion phase) markers showing results for selected individual F₂'s; LD = 100kb Ladder, 1 = Beti-10 (Resistant parent), 2 = Kablanketi (Susceptible parent), R = Resistant F₂'s and S = Susceptible F₂'s.

All the two markers have been reported to be in linkage group 8 of common bean and were further characterized to be linked to the genes *Phg_{G109098}* and *Phg-2* at a distance of 4.9cM and 3.2cM respectively (Mahuku et al., 2011; Nietsche et al., 2000). Recombination was observed for both markers where some samples gave unexpected results. Among 100 F₂ plants evaluated using the two markers, 8 plants and 5 plants gave either false positive or false negative when phenotypic results were compared with the molecular marker screening results for PF13 and SN02 markers respectively. Using marker PF13, the reliability of selection of the right genotype based on the level of recombination is 92% and for the marker SN02 is 95%. (Table 4.2).

Table 4.2: Frequencies of Genotypic classes for ALS resistance among different populations screened with two molecular markers (SN02 and PF13)

SCAR Markers	Generation	Phenotypic ratio		Genotypic ratio		Expected ratio	X ²	P*
		R	S	R	S			
PF13	P ₁ (Beti-10)	10	0	10	0	1:0	-	-
PF13	P ₂ (Kablanketi)	0	10	0	10	0:1	-	-
PF13	F ₂ (Kab x Beti-10)	77	23	69	31	3:1	1.92	0.166
SN02	P ₁ (Beti-10)	10	0	10	0	1:0	-	-
SN02	P ₂ (Kablanketi)	0	10	0	10	0:1	-	-
SN02	F ₂ (Kab x Beti-10)	77	23	72	28	3:1	0.48	0.488

*P values greater than 0.05 indicates that the observed frequencies are not significantly different from the expected ones

From the inheritance studies and allelism test in previous section, it is clear that the gene in Beti-10 landrace is not the same as the gene *Phg-2* that is present in Mexico 54 (Nietsche et al., 2000) but the genes were found to be close to one another considering the obtained few recombinants. Further this section confirms that these genes are all in chromosome 8 as these SCAR markers found to be linked to resistance in Beti-10 are all coming from chromosome 8 of common bean. Chromosome 8 of common bean has also been reported to contain other genes responsible for resistance to other common bean diseases such as anthracnose (Kelly et al., 2003), rust (Mienie et al., 2005) and common bacterial blight (Bai et al., 1997), therefore using these markers may probably not only assist in getting the only gene for angular leaf spot but also getting other genes as well present in this chromosome.

4.5 Conclusions

This study indicates that inheritance of angular leaf spot in Beti-10 landrace is controlled by single dominant gene at least for the most virulent isolate of the pathogen selected for this study. Results from allelism test also shows that, the gene present in Beti-10 that is

responsible for resistance is different from the gene *Phg-2* which is in accession Mexico 54 that is currently in use as the donor parent for resistance to the disease. This implies that, Beti-10 landrace can be a potential source of other locus for resistance to angular leaf spot that will in turn increase the duration of resistance in improved genotypes. Co-segregation results indicate that SCAR markers SN02 and PF13 are potential molecular markers that can be used to simplify selection process with high levels of precisions. Results from this study therefore will pave way into using Beti-10 as an alternative characterized source of resistance to angular leaf spot disease in addition to Mexico 54 which will in turn widens the genetic base of the resistance and increase durability of the resistance in the developed genotypes. Further, the SCAR markers identified in this study to be linked to resistance in Beti-10 will facilitate easy selection of resistant genotypes in breeding when using this parent as resistance donor.

References

- Akibode S, Maredia M (2011). Global and Regional Trends in Production, Trade and Consumption of Food Legume Crops. Report submitted to SPIA. 83pp.
<https://impact.cgiar.org/sites/default/files/images/Legumetrendsv2.pdf>
- Bai Y, Michaels TE, Pauls KP (1997). Identification of RAPD markers linked to common bacterial blight resistance genes in *Phaseolus vulgaris* L. Genome. 40: 544 – 551.
- Beebe S, Rao MI, Blair WM, Acosta-Gallegos AJ (2013). Phenotyping common beans for adaptation to drought. Africa Crop Science. 4(35): 1-20.
- Beebe S, Skroch P, Tohme J, Duque MC, Pedraza F, Nienhuis J (2000). Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. Crop Sci. 40: 264 – 273.
- Bennink M (2006). Eat beans for good health. Annual Report Bean Improvement Cooperative. 48: 1 – 5.
- Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J (2003). Beans (*Phaseolus spp.*) model food legumes. Plant Soil. 252: 55 – 128.
 doi:10.1023/A:1024146710611
- Caixeta EF, Borem A, Alzate-Marin AL, De Azevedo Fagundes S, De Moraes Silva MG, de Barros EG, Moreira MA (2005). Allelic relationships for genes that confer resistance to angular leaf spot in common bean. Euphytica. 145: 237 – 245.
- Castellanos G, Jara C, Mosquera G (2015). Bean Pathogens: Practical Guide for Lab and Greenhouse Work. (CIAT Publication No. 394), Centro Internacional de Agricultura Tropical (CIAT) 238p

- Chilagane LA, Nchimbi-Msolla S, Kusolwa PM, Porch TG, Serrato Diaz LM, Tryphone GM (2016). Characterization of the common bean host and *Pseudocercospora griseola*, the causative agent of angular leaf spot disease in Tanzania. *African Journal of Plant Science*. 10: 238 – 245.
- Crous PW, Liebenberg MM, Braun U, Groenewald JZ (2006). Re- evaluating the taxonomic status of *Phaeoisariopsis griseola*, the causal agent of angular leaf spot of bean. *Stud. Mycol*. 55: 163 – 173.
- FAOSTAT (2014). FAOSTAT Data available at <http://www.fao.org/faostat/en/#data> (Page visited, December 2016)
- Hillocks RJ, Madata SC, Chirwa R, Minja ME, Msolla SN (2006). Phaseolus bean improvement in Tanzania 1956 – 2005. *Euphytica*. 150: 215 – 231. <http://dx.doi.org/10.1007/s10681-006-9112-9>
- Kelly JD, Gepts P, Miklas PN, Coyn DP (2003). Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. *Field Crops Research*. 82: 135 – 154.
- Kimani P, Karuri ME, Mwaura S (2006). Iron, zinc and protein concentration in African bean cultivars. *Annual Report of the Bean Improvement Cooperative*. 49: 155 – 156.
- Mahuku GS (2004). A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. *Plant Molecular Biology Reporter*. 22: 71 – 81.
- Mahuku GS, Henriquez MA, Montoya C, Jara C, Teran H, Beebe S (2011). Inheritance and development of molecular markers linked to angular leaf spot resistance

- genes in the common bean accession G10909. *Mol. Breed.* 28: 57 – 71. doi: 10.1007/s11032-010-9461-x
- Mahuku GS, Iglesias AM, Jara C (2009). Genetics of angular leaf spot resistance in the Andean common bean accession G5686 and identification of markers linked to the resistance genes. *Euphytica*. 167: 381 – 396.
- Mahuku GS, Jara C, Cuasquer JB, Castellanos G (2002). Genetic variability within *Phaeoisariopsis griseola* from Central America and its implication for resistance breeding of common bean. *Plant Pathology*. 51: 594 – 604.
- Mienie CMS, Liebenberg MM, Pretorius ZA, Miklas PN (2005). SCAR markers linked to the common bean rust resistance gene Ur-13. *Theoretical and Applied Genetics*. 111: 972 – 979.
- Miklas PN (2010). SCAR markers linked with disease resistance traits in common bean (*Phaseolus vulgaris*). https://www.ars.usda.gov/ARSUserFiles/3848/PDF/Miklas_2011/SCAR%20Markers%202010.pdf
- Mukankusi C, Derera J, Melis R, Gibson PT, Buruchara R (2011). Genetic analysis of resistance to *Fusarium* root rot in common bean. *Euphytica*. 182: 11
- Muthomi JW, Muimui KK, Kimani PM (2011). Inheritance of resistance to Angular Leaf Spot in yellow beans. *Afr. Crop Sci. J.* 19(4): 267 – 275.
- Namayanja A, Buruchara R, Mahuku G, Rubaihayo P, Kimani P, Mayanja S, Eyedu H (2006). Inheritance of resistance to angular leaf spot in common bean and validation of the utility of resistance linked markers for marker assisted selection outside the mapping population. *Euphytica*. 151(3): 361 – 369.

- Nietsche S, Borem A, Carvalho GA, Rocha RC, Paula TJ, de Barros EG, Moreira MA (2000). RAPD and SCAR markers linked to a gene conferring resistance to angular leaf spot in common bean. *Journal of Phytopathology*. 148: 117 – 121.
- Petry N, Boy E, Wirth JP, Hurrell RF (2015). Review: the potential of the common bean (*Phaseolus vulgaris*) as a vehicle for iron biofortification. *Nutrients*. 7: 1144 – 1173.
- Queiroz VT, Souza CS, Costa MR, Sanglard DA, Arruda KMA, Souza TLO, Ragagnin VA, Barros EG, Moreira MA (2004). Development of SCAR markers linked to common bean angular leaf spot resistance genes. *Annual Report of the Bean Improvement Cooperative*. 47: 237 – 238.
- Tryphone GM, Chilagane LA, Nchimbi-Msolla S, Kusolwa PM (2015). Genetic characterization of angular leaf spot resistance in selected common bean landraces from Tanzania. *African Journal of Biotechnology*. 14: 2943 – 2948.
- Widers IE (2006). The beans for health alliance: A public-private sector partnership to support research on the nutritional and health attributes of beans. *Annual Report Bean Improvement Cooperative*. 49: 3 – 5.

CHAPTER FIVE

5.0 GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 General Conclusion

In this work, an attempt has been made towards improving common bean production by enhancing angular leaf spot disease resistance.

This work revealed that, Angular leaf spot disease is distributed in all bean growing regions of Tanzania. Mbeya and Kagera regions were found to be hot spots for this disease with severity scores of 7 while Manyara and Rukwa regions had the least scores of 4. This study also reveals the presence of both Andean and Mesoamerican gene pools for both common bean genotypes and pathogen isolates in Tanzania. Andean beans outnumbered Mesoamerican beans in all regions, hence the most preferred gene pool. Comparing regions, 75% of all the Mesoamerican beans were collected from Kagera region which makes this region to have some preference on small seeded beans as compared to other regions. Likewise in the case of pathogen (*P. griseola*) isolates, Andean isolates outnumbered the Mesoamerican isolates. Region wise, Kagera region (the Lake zone) as opposed to other regions, Mesoamerican isolates outnumbered Andean isolates which correlates with the findings that in this region also there are many Mesoamerican genotypes that suggests co-evolution of the pathogen with the host. Following virulence classification, it is clear that the pathogen is diverse with different pathotypes where other pathotypes were found to occur in different regions. The most virulent Pathotype among the ones characterized was found to be 63:50, which makes it a good, isolate so far to be used in screening resistant materials developed.

In an attempt to identify other sources of resistance to angular leaf spot from locally adapted genotypes, two genotypes Beti-10 which is a local landrace and RJRILS135 which is an inbred line from CAL 143 (ALS resistant foreign genotype) and Rojo (ALS

susceptible variety bred at SUA) were found to be highly resistant to ALS when tested in the field (Hot spot region) and in the screen house using the identified most virulent isolate of the pathogen. These genotypes, Beti-10 (Small seeded) and RJRILS135 (Medium sized seeded), performed well compared to the resistant controls that were used and there was no significant difference of disease scores from these genotypes when compared to the resistant controls (Mexico 54 and CAL 143) in all the experiments. These genotypes, if used together may compliment the currently used donor in most bean breeding programs in Tanzania (Mexico 54) and can safeguard the newly developed varieties from rapid disease resistance break down.

Characterization of Beti-10, one of the identified resistant local landrace indicates that inheritance of angular leaf spot resistance in Beti-10 to Pathotype 63:50 is controlled by single dominant gene. Allelism test shows that, the gene present in Beti-10 that is responsible for resistance is different from the gene *Phg-2* which is in accession Mexico 54 that is currently in use as the donor parent for resistance to the disease. This implies that, Beti-10 landrace can be a potential source of other locus for resistance to angular leaf spot that will in turn increase durability of resistance in improved genotypes. Co-segregation results indicate that SCAR markers SN02 and PF13 are potential molecular markers that can be used to simplify selection.

This study therefore, gives insight of the angular leaf spot disease distribution and severity, pathogen and genotype variability, distribution and interaction, identification and characterization of other sources of resistance to ALS and discovers markers that will assist in breeding for resistance using the newly identified and characterized source of resistance. All these aspects are very important in enhancing angular leaf spot resistance.

5.2 Recommendations

From this study, the following are recommended for enhancing angular leaf spot disease resistance in Tanzania

- Since ALS disease is distributed over all bean growing regions with both Andean and Mesoamerican gene pools of the pathogen, then breeding should focus on pyramiding resistant loci from diverse backgrounds (Andean and Mesoamerican gene pools) as a strategy of developing durable resistance.
- For durable resistance, Mbeya and Kagera regions are recommended as hot spots sites for ALS disease evaluation. This is because, in these regions the disease is severe and also the chance of getting Andean and Mesoamerican isolates of the disease which is good when broad resistance is sought.
- Breeders should not rely on only one source of resistance as this will narrow the genetic base of the disease which will make the developed materials to easily suffer resistance breakdown due to reliance on only one loci due to the fact that the pathogen also shows to be variable. Tanzania bean breeders should think of starting using the identified sources of resistance Beti-10 and RJRILS135 to compliment Mexico 54.
- In order to be able to pyramid genes on the same background, there is a need to identify more molecular markers, validate them and use them so as to make the process of selection easy as opposed to phenotypic screening which takes time and very difficult especially when having many pathotypes as the case for ALS.
- There should be identification and characterization of more resistant genotypes to be used as breeding parents and further discover more molecular markers to be used along with the identified genotypes.

- There should be continued variability study of the pathogen over time to highlights the occurrence of new races of the pathogen and further, more virulence test is needed with a large collection of isolates from all bean growing regions.
- The population developed from Beti-10 should be advanced and sequenced to further characterize the molecular bases of resistance in Beti-10 and develop more markers linked to ALS resistant genes.